UNIVERSITÀ DI MILANO
"CENTRO DINO FERRARI"
PER LA DIAGNOSI E LA TERAPIA DELLE MALATTIE
NEUROMUSCOLARI, NEURODEGENERATIVE E CEREBROVASCOLARI

FONDAZIONE I.R.C.C.S. CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO
ISTITUTO DI RICOVERO E CURA A CARATTERE
SCIENTIFICO DI NATURA PUBBLICA

COLLABORAZIONI NAZIONALI E INTERNAZIONALI

E

FRONTESPIZI

LAVORI SCIENTIFICI

2023

"CENTRO DINO FERRARI"

Sezione di Neuroscienze
Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti
Università degli Studi di Milano
Fondazione I.R.C.C.S. Ca' Granda - Ospedale Maggiore Policlinico

COORDINATORE SCIENTIFICO PROF. GIACOMO PIETRO COMI
Via F. Sforza, 35 - 20122 Milano – Tel. 02.5503.3809 - Fax 02.5503.3800
E-mail: giacomo.comi@unimi.it - centro.dinoferrari@policlinico.mi.it - www.centrodinoferrari.com

UNIVERSITÀ DI MILANO
"CENTRO DINO FERRARI"
PER LA DIAGNOSI E LA TERAPIA DELLE MALATTIE
NEUROMUSCOLARI, NEURODEGENERATIVE E CEREBROVASCOLARI

FONDAZIONE I.R.C.C.S. CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO
ISTITUTO DI RICOVERO E CURA A CARATTERE
SCIENTIFICO DI NATURA PUBBLICA

Istituti di ricerca del "Centro Dino Ferrari":

- **IRCCS FONDAZIONE CA' GRANDA OSPEDALE MAGGIORE POLICLINICO UNIVERSITA' DEGLI STUDI DI MILANO**
  - Laboratorio di biochimica, Genetica e Colture Cellulari
  - Laboratorio di Cellule Staminali Neurali
  - S.S.D. Malattie Neuromuscolari e Rare
  - S.S.D. Malattie Neurodegenerative – Unità Valutativa Alzheimer (U.V.A.)
  - Centro Sclerosi Multipla
  - Laboratorio Parkinson e altri Disturbi del Movimento
  - Laboratorio di Cellule Staminali
  - S.S. Stroke Unit
- **IRCCS ISTITUTO AUXOLOGICO ITALIANO – UNIVERSITA' DEGLI STUDI DI MILANO**
  - U.O. Neurologia - Stoke Unit
  - Laboratorio di Neuroscienze
- **IRCCS ISTITUTO SCIENTIFICO E. MEDEA BOSISIO PARINI (LC)**
  - Laboratorio di Biologia Molecolare, Citogenetica, Analisi Biochimico- Cliniche Bioinformatiche

COORDINATORE SCIENTIFICO PROF. GIACOMO PIETRO COMI
Via F. Sforza, 35 - 20122 Milano – Tel. 02.5503.3809 - Fax 02.5503.3800
E-mail: giacomo.comi@unimi.it - centro.dinoferrari@policlinico.mi.it - www.centrodinoferrari.com

Centri Nazionali di Ricerca che collaborano con il "Centro Dino Ferrari"

- Prof. Valerio Carelli, **Università di Bologna**
- Prof. Daniele Ghezzi, **Istituto Neurologico Carlo Besta Milano**
- Dott. Lorenzo Maggi, **Istituto Neurologico Carlo Besta Milano**
- Prof. Enrico Silvio Bertini, **Ospedale Pediatrico Bambin Gesù Roma**
- Prof. Antonio Toscano, **Università di Messina**
- Prof. Michelangelo Mancuso, **Università di Pisa**
- Prof. Gabriele Siciliano, **Università di Pisa**
- Prof. Carlo Minetti, **Università di Genova**
- Prof. Paola Tonin, **Università di Verona**
- Prof. Serenella Servidei, **Università Cattolica del Sacro Cuore**
- Prof. S. Barabino**, Università degli Studi di Milano-Bicocca.**
- Dr. Bernasconi e Dr. Marcuzzo**, Istituto Neurologico Besta.**
- Dr. Di Schiavi**, CNR di Napoli.**
- Prof. Antonia Ratti, **Department of Neurology - Stroke Unit and Laboratory of Neuroscience, Istituto Auxologico Italiano, IRCCS, Milano.**
- Prof. Valentina Bollati, **Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano, Milano.**
- Dr. Simona Lodato, **Humanitas Research Center, Milano.**
- MANAVA Plus**, Milano, Italia.**
- Italfarmaco**, Milano, Italia.**
- Prof. Marco Onorati**, Universita' di Pisa, Pisa.**
- Prof. Luciano Conti**, Universita' di Trento, Trento.**
- Dr. Enrico Bertini**, Ospedale Pediatrico Bambino Gesù di Roma.**
- Prof. Alessandro Usiello**, Universita' della Campania-Vanvitelli, Caserta.**
- Prof. Luigi Zeni**, Universita' della Campania-Vanvitelli, Caserta.**
- Prof. Grazia Daniela Raffa**, Universita' La Sapienza, Roma.**
- Prof. Carlo Viscomi**, Universita' di Padova.**
- l'Associazione Mitofusina 2 **Rignano Sull'Arno FI**

- Stefano Ferrero Bogetto, **direttore UOC Anatomia Patologica, Fondazione IRCCS Ca' Granda** Ospedale Maggiore Policlinico di Milano, Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano
- Elena Cattaneo**, Department of Biosciences and Centre for Stem cell Research, Università degli Studi di Milano**
- Prof. Lorenza Lazzari, **Department of Regenerative Medicine, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico di Milano**
- Dr. Massimo Aureli, **Università Degli Studi di Milano, Ospedale san Raffaele, Milano**
- Dr. Franco Taroni – **IRCCS Istituto C. Besta, Milano**
- Prof. Fabio Triulzi, Dott. Alessandro Sillani, Dott.ssa Clara Sina, Dott. Giorgio Conte, Ing. Valeria Contarino, **IRCCS Ca' Granda – Ospedale Maggiore Policlinico, Milano**
- Dott. Rosanna Asselta - **Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Milano, Italy.**
- Prof. Jenny Sassone **Pagano, Università Vita-Salute San Raffaele**
- Prof. Elisa Greggio**, Università degli Studi di Padova**
- Prof. Mario Bortolozzi**, Veneto Institute of Molecular Medicine**
- Prof. Fabio Moda**, Fondazione IRCCS Istituto Neurologico Carlo Besta**
- Prof. Arianna Bellucci**, Università degli Studi di Brescia**
- Prof. A. Maggi, **Centro di Biotecnologie Farmacologiche, Dipartimento di Scienze Farmacologiche, Università di Milano**
- Dott. Gianluigi Forloni, **Istituto di Ricerche Farmacologiche Mario Negri, Milano**
- Prof. Stefano Cappa, **Università di Pavia**
- Proff. Alessandro Padovani e Barbara Borroni, **Università di Brescia**
- Dott.ssa Claudia Verderio CNR instituite of Neuroscience, **Università Milano-Bicocca**
- Prof. Marco Bozzali, **Università di Torino**
- Dott.ssa Carmen Giordano, **Politecnico di Milano**
- Humberto Cerrel Bazo, **direttore Dipartimento Medicina riabilitativa AUSL Piacenza**
- Maurilio Sampaolesi, **Stem Cell Research Institute, University Hospital Gasthuisberg, Leuven, Belgium, Human Anatomy Section, University of Pavia, Pavia, Italy, Interuniversity Institute of Myology (IIM), Italy**
- Giuseppe Perale**, I.B.I. S/A, Svizzera, Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta" Sezione Chimica Fisica Applicata, Politecnico di Milano, Milano**

- Roberto Maggi, **Professore universitario di seconda fascia Fisiologia, Facoltà di Farmacia, Università degli Studi di Milano**
- Mario Pellegrino, **Prof. Associato presso il Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa**
- Daniele Cusi**, Professore di Nefrologia, Università degli Studi di Milano**
- **Cristina Barlassina**, Dipartimento di Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano
- Anna Spada, **U.O. di Endocrinologia e Diabetologia, Dipartimento di Scienze Mediche, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico**
- Irene Cettin, **Direttore UO Complessa di Ostetricia e Ginecologia, Direttore Centro di Ricerche Fetali Giorgio Pardi, Università degli Studi di Milano - Polo Universitario Ospedale L. Sacco di Milano**
- Paola Rossi, **Professore universitario per il settore scientifico disciplinare BIOO9 (Fisiologia Generale) presso il Dipartimento di Scienze Fisiologiche e Farmacologiche cellulari e molecolari- Sezione di Fisiologia dell'Università di Pavia.**
- Angelo Poletti, **Biologia Applicata, Università degli Studi di Milano, Facoltà di Farmacia, Università degli tudi di Milano**
- Silvio Bicciato**, bioinformatics unit, Faculty of Biosciences and Biotechnologies, University of Modena and Reggio Emilia**
- Enrico Tagliafico, **clinical Biochemistry, University of Modena and Reggio Emilia**
- Sergio Abrignani, **direttore del National Institute of Molecular Genetics (INGM), Milan, Italy**
- Silvano Bosari**, direttore UOC Anatomia Patologica, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano**
- Carlo Agostoni**, Direttore della Clinica Pediatrica II dell'Università degli Studi, IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano**
- Laura Porretti**, referente del Servizio di Citofluorimetria e Core Facility di Citofluorimetria e Cell Sorting del Laboratorio Analisi, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico di Milano**
- Agostino Cortelezzi, **direttore UOC Ematologia I e Centro Trapainti Midollo, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico**
- Giuseppe D'Antona**, Department of Molecular Medicine, University of Pavia, Pavia, Italy LUSAMMR, Laboratory for Motor Activities in Rare Diseases, Sport Medicine, Centre Voghera, Voghera, Italy**
- Enzo Nisoli, **Center for Study and Research on Obesity, Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy;**

- Dario Parazzoli, **Imaging Facility IFOM Foundation – The FIRC Institute of Molecular Oncology Foundation, Milan, Italy**
- Stefano Campaner**, Center for Genomic Science of IIT@SEMM; Istituto Italiano di Tecnologia (IIT); Milan, Italy**
- Francesco Nicassio, **Department of Experimental Oncology, European Institute of Oncology, IFOM-IEO Campus**
- Alessandro Quattrone**, Director of CiBio, University of Trento**
- Giovanna Cantarella**, Dirigente Medico Otorinolaringoiatra e Foniatra, Padiglione Monteggia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano**
- Mauro Pluderi e Nadia Grimoldi, **UO Neurochirurgia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano**
- Paolo Vezzoni, **Dirigente di Ricerca e Responsabile dell'Unità Operativa di Supporto (UOS) dell'Istituto di Ricerca Genetica e Biomedica (IRGB) del CNR.**
- Marina Bouchè, **Unit of Histology, and IIM, Sapienza University, DAHFMO, Rome, Italy**
- Davide Gabellini, **Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, Milan**
- Franco Rustichelli**, Dipartimento di Scienze Cliniche e Odontostomatologiche, Sezione di Biochimica, Biologia e Fisica, Università Politecnica delle Marche, Ancona, Italy**
- Silvia Della Bella, **Lab of Clinical and Experimental Immunology, Humanitas Clinical and Research Center, Rozzano (MI), Italy, Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy**
- Aldo Pagano, **Department of Experimental Medicine, University of Genoa, Italy, IRCCS Azienda Ospedaliera Universitaria San Martino-IST, Genova, Italy**
- Francesco Meinardi**, Professore di Fisica della Materia, l'Università di Milano Bicocca**
- Jose F Rodriguez-Matas-, **Associate professor, LabS (www.labsmech.polimi.it) Chemistry, Materials and Chemical Engineering Department "Giulio Natta" Politecnico di Milano, Italy**
- Giorgio Roberto Merlo**, Dipartimento di Biotecnologie Molecolari e Scienze per la salute- Università di Torino**
- Giorgio Pajardi, **Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano, Direttore U.O.C. di Chirurgia e Riabilitazione della Mano Ospedale S. Giuseppe Milano, Gruppo MultiMedica IRCCS**
- Prof. Stefano Biressi – **Centro di Biologia Integrata – CIBIO, Università degli Studi di Trento**
- Prof. Lorenzo Bello - **Neurochirurgia Oncologica, Humanitas, Milano**
- Prof. Alberto Priori - **U.O.C. Neurologia, Ospedale San Paolo, Milano**

- Prof. Pierluigi Mauri – **Istituto di Tecnologie Biomediche, Consiglio Nazionale delle Ricerche (CNR-ITB), Milano**
- Prof.ssa M.G. Bruzzone – **UOC Neuroradiologia, Istituto Neurologico Besta, Milan, Italy**
- Prof. Simone Guglielmetti - **Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano, Milano**
- Prof. Umberto Galderisi – **Dipartimento di Medicina Sperimentale, Università degli Studi della Campania "Luigi Vanvitelli"**
- D.ssa Barbara Cassani – **Instituto di Genetica e Biomedicina (IRGB), National Research Council (CNR) Milano**
- Massimiliano Pagani- **Molecular Oncology and Immunology lab- IFOM, Università degli Studi di Milano**
- Federica Facciotti- **Istituto Europeo di Oncologia (IEO)-Università di Milano-Bicocca**
- Flavio Caprioli- **U-O. di Gastroenterologia ed Endoscopia Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico di Milano; Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti dell'Università degli Studi di Milano**
- Prof. Giacomo Comi, Prof. Stefania Corti, Dott.ssa Daniela Galimberti, Dott. Prof. Elio Scarpini, Dott. Alessio di Fonzo **"Centro Dino Ferrari" IRCCS Ca' Granda – Ospedale Maggiore Policlinico, Milano**
- Dott.ssa Cinzia Gellera, Prof. Giuseppe Lauria Pinter, Dott.ssa Giacomina Rossi, Dott. FrabrizioTagliavini, Dott. Pietro Tiraboschi, **PADOVANO IRCCS Istituto C. Besta, Milano**
- Prof.ssa Valeria Sansone **Centro Clinico Nemo, Milano**
- Prof.ssa Carolina Lombardi **Centro Sonno Istituto Auxologico Italiano IRCCS, Milano**
- Prof. Luca Persani **Dipartimento di Endocrinologia IRCCS Istituto Auxologico Italiano, Milano**
- Prof.ssa Palma Finelli, Dott.ssa Daniela Giardino, **Laboratorio di Citogenetica, IRCCS Istituto Auxologico Italiano, Milano**
- Dott. Luigi Sironi, Dott. A.E. Rigamonti, **Dipartimento di Farmacologia Università di Milano - CEND**
- Prof. Massimo Filippi, Prof.ssa Federica Agosta, **Neuroimaging Research Unit and Department of Neurology, Institute of Experimental Neurology, Milano**
- Prof. Andrea Falini, **Division of Neuroscience and Department of Neuroradiology, Vita-Salute University and San Raffaele Scientific Institute, Milan**
- Dott. Emanuele Buratti, Dott. Marco Baralle **Laboratory of Molecular PathologyInternational Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste**
- Dott.ssa Valeria Crippa **Dipartimento di Scienze Farmacologiche Biomolecolari, CEND, Università degli Studi di Milano**

- Dott. Francesco Bifari, **Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano**
- Dott. Marco Feligioni, **Centro EBRI, Roma**
- Prof. Adriano Chiò, Prof. Andrea Calvo, **Università degli Studi di Torino**
- Dott.ssa R. Ghidoni, Dott.ssa L. Benussi, **IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia**
- Dott. FM Santorelli- **Fondazione IRCCS Stella Maris, Pisa**
- Prof Luca De Gioia - **Department of Biotechnology and Biosciences, University of Milan-Bicocca, 20126 Milan, Italy.**
- Prof. Mario Clerici -**Department of Physiopathology and Transplantation, University of Milan, 20090 Milan, Italy. and Don C. Gnocchi Foundation ONLUS, IRCCS, 20148 Milan, Italy.**
- Prof Giuseppe Bianchi - **Nephrology and Dialysis Unit, San Raffaele Scientific Institute, University Vita Salute San Raffaele, Milan, Ita**
- Dott. Franca Guerini - **Don C. Gnocchi Foundation ONLUS, IRCCS, 20100 Milan, Italy**
- Dott. Mara Biasin - **Department of Biomedical and Clinical Sciences, University of Milan, 20157 Milan, Italy**
- Prof. Roberto de Franchis - **IBD Unit, Chair of Gastroenterology, Luigi Sacco University Hospital, 20157 Milan**
- Dott. Sergio Lo Caputo - **S. Maria Annunziata Hospital, 50122 Florence, Italy**
- Dott. Matteo Cereda - **Department of Experimental Oncology, European Institute of Oncology (IEO), 20139 Milan, Italy**

Centri Internazionali di Ricerca che collaborano con il "Centro Dino Ferrari"

- Prof. Carsten G. Bönnemann, **NIH, Bethesda, USA**
- Prof. Michio Hirano, **Columbia University, New York, USA**
- Prof. Connie Bezzina, **Amsterdam UMC, Amsterdam, Olanda**
- Prof. S. Przedborski, director of the Center, and Prof. D. Re.**Columbia University, NY, USA,**
- Prof. Kathrin Mayer, Ph.D, Professore Associato, **The Research Institute at Nationwide Children's Hospital/ The Ohio State University, Center for Gene Therapy, Columbus, Ohio, USA.**
- Prof. H. Moulton **Oregon University.**
- Prof. Jeroen Pasterkamp, **Department of Translational Neuroscience, UMC Utrecht.**
- Prof. Michela Deleidi, **Institut Imagine, Parigi, Francia**
- Prof. Philip Van Damme, **University of Leuven Belgio**
- Prof. Stefano Stifani, **Montreal Neurological Institute of McGill University, Canada.**
- Prof. Francesco Lotti, **PhD, Assistant Professor, Center for Motor Neuron Biology and Disease, Columbia University Medical Center, New York, NY 10032, USA.**
- Dr. Piera Smeriglio, **Centre De Recherche En Myologie, Parigi, Francia.**
- **SLAGEN consortium**.
- **"EURALS Consortium**.
- Prof. Glenda Halliday, **University of Sydney, Australia**
- Prof. Catarina Quinzii, PhD, **Columbia University, New York, N.Y., USA**
- NYU Movement Disorders, **Fresco Institute for Parkinson's Disease, New York University – USA**
- Prof. Elena Moro, **Department of Psychiatry and Neurology, University Hospital Center of Grenoble, FRANCE**
- Prof. Ari Zimran - **Gaucher Unit, Shaare Zedek Medical Center, Jerusalem, Israel; Faculty of Medicine, Hebrew University, Jerusalem, Israel.**
- Prof. Mia Horowitz - **Schmunis School of Biomedicine and Cancer Research, Tel Aviv University, Tel Aviv, Israel**.
- Dr. Michael Zech - **Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany**.
- Prof. Marie Vidailhet - **Department of Neurology, Salpetriere Hospital, Sorbonne University, University Pierre and Marie Curie, ICM Research Centre**.

- Prof. Enrico Glaab - **Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg**.
- Dr. Manuel Schroeter - **ETH Zurich, Department of Biosystems Science and Engineering in Basel, Switzerland**.
- Prof. Jens Schwamborn**, University of Luxembourg**
- Prof. Nicolas Tritsch**, New York University Langone**
- Prof. Thomas Wisniewsky**, New York University Langone**
- Dr. Robert P. Lisak, **Dip. di Neurologia, Detroit (USA**)
- Prof. Philip Scheltens, Prof. Yolande Pijnenburg**, Dept. of Neurology, VU University Medical Center, Amsterdam, The Nertherlands**
- Prof. Janine Diehl**, Univerity of Munich, Germany**
- Prof. Glenda Halliday, **University of Sydney, Australia**
- Prof. Simon Ducharme, **McGill University, Vancouver, Canada**
- Dr. Anne Cross, **University of Saint Louis, USA**
- Prof. Jean Charles Lambert**, Lille, France**
- Dr. Jonathan Rohrer**, UCL, London, UK**
- Prof. An Goris, **Leuven, Belgium**
- Dr. Marta Alarcon, **Genyo, Granada, Spain**
- Prof. Robert H. Brown, Prof. John Landers, **University of Massachussetts Medical School, Department of Neurology Worcester, MA, USA**
- Prof.ssa Claudia Fallini, **Ryan Institute for Neuroscience University of Rhode Island Kingston Rhode Island, RI, USA**
- Prof. Albert Ludolph, **Dipartimento di Neurologia Università di Ulm, Germania**
- Prof. Markus Otto, **Dipartimento di Neurologia, Università Martin Luther di Halle-Wittenberg, Halle (Saale), Germania.**
- Prof. Ammar Al-Chalabi, Prof. Christopher Shaw, **Dipartimento di Neurologia King's College, London**
- Prof. Leonard Petrucelli, **Department of Neurology Mayo Clinic, Florida, USA**
- Prof. Markus Weber, **Dipartimento di Neurologia Università di St. Gallen, Svizzera**
- Dott. Damian Wollny, **Max Planck Institute, Leipzig, Germania**
- Prof. Hiroshi Mitsumoto, **Department of Neurology Eleanor and Lou Gehrig MDA/ALS Research Center Columbia University Medical Center New York – USA**
- Prof. Merit E. Cudkowicz, **Neuromuscular Division Neurology Massachusetts General Hospital Boston – USA**

- Prof. Stanley H. Appel **Department of Neurology Methodist Neurological Institute Chair Houston -USA**
- Prof. Sharon Abrahams, **Euan Mac Donald Centre for Motor Neurone Disease Research University of Edinburgh – UK**
- E.I Rugali **University of Cologne Joseph-Stelzmann-Str. 26 50931 Köln Germany**
- Edward J Hollox **- Department of Genetics, University of Leicester, Leicester LE2 1TE, UK**
- Nasser M. Al-Daghri - **Biomarker research program, Biochemistry Department, College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia (KSA) and Prince Mutaib Chair for Biomarkers of Osteoporosis, Biochemistry Department, College of science, King Saud University, Riyadh, KSA**
- Dott. Juan Antonio Pineda - **Infectious Diseases and Microbiology Clinical Unit. Valme Hospital, Seville, Spain**
- Dott. Antonio Rivero-Juarez - **Maimonides Institut for Biomedical Research (IMIBIC)- Reina Sofia Universitary Hospital-University of Cordoba, Spain**
- Dott. Antonio Caruz **- Immunogenetics Unit, Department of Experimental Biology, University of Jaen, Jaen, Spain**
- Dott. Manuel Comabella - **Hospital Universitari Vall d´ Hebron (HUVH). Barcelona, Spain**
- Dott. Matteo Fumagalli - **UCL Genetics Institute, Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, United Kingdom**

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 26 September 2023
DOI 10.3389/fneur.2023.1249429

OPEN ACCESS

EDITED BY
Corrado Italo Angelini,
University of Padua, Italy

REVIEWED BY
Mauro Ceroni,
Neurological Institute Foundation Casimiro Mondino (IRCCS), Italy
Gianni Sorarù,
University of Padua, Italy
Zorica Dragisa Stevic,
University of Belgrade, Serbia

*CORRESPONDENCE
Nicola Ticozzi
✉ n.ticozzi@auxologico.it

RECEIVED 29 June 2023
ACCEPTED 13 September 2023
PUBLISHED 26 September 2023

CITATION
Colombo E, Gentile F, Maranzano A, Doretti A, Verde F, Olivero M, Gagliardi D, Faré M, Meneri M, Poletti B, Maderna L, Corti S, Corbo M, Morelli C, Silani V and Ticozzi N (2023) The impact of upper motor neuron involvement on clinical features, disease progression and prognosis in amyotrophic lateral sclerosis. *Front. Neurol.* 14:1249429. doi: 10.3389/fneur.2023.1249429

The impact of upper motor neuron involvement on clinical features, disease progression and prognosis in amyotrophic lateral sclerosis

Eleonora Colombo[1], Francesco Gentile[2], Alessio Maranzano[1], Alberto Doretti[1], Federico Verde[1,3], Marco Olivero[2], Delia Gagliardi[3,4], Matteo Faré[5,6], Megi Meneri[3,4], Barbara Poletti[1], Luca Maderna[1], Stefania Corti[3,4], Massimo Corbo[7], Claudia Morelli[1], Vincenzo Silani[1,3] and Nicola Ticozzi[1,3]*

[1]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [2]Neurology Residency Program, Università degli Studi di Milano, Milan, Italy, [3]"Dino Ferrari" Center, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy, [4]Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [5]Department of Neurology, San Gerardo Hospital ASST, Monza, Italy, [6]School of Medicine and Surgery, Università degli Studi di Milano-Bicocca, Milan, Italy, [7]Department of Neurorehabilitation Sciences, Casa di Cura Igea (CCI), Milan, Italy

Objectives: In amyotrophic lateral sclerosis (ALS) both upper (UMNs) and lower motor neurons (LMNs) are involved in the process of neurodegeneration, accounting for the great disease heterogeneity. We evaluated the associations of the burden of UMN impairment, assessed through the Penn Upper Motor Neuron Score (PUMNS), with demographic and clinical features of ALS patients to define the independent role of UMN involvement in generating disease heterogeneity, predicting disease progression and prognosis.

Methods: We collected the following clinical parameters on a cohort of 875 ALS patients: age and site of onset, survival, MRC scale, lower motor neuron score (LMNS), PUMNS, ALSFRS-R, change in ALSFRS-R over time (DFS), MITOS and King's staging systems (KSS). Transcranial magnetic stimulation was performed on a subgroup of patients and central motor conduction time (CMCT) and cortical silent period (CSP) were calculated.

Results: We observed that patients with an earlier age at onset and bulbar onset had higher PUMNS values. Higher values were also associated to lower ALSFRS-R and to higher DFS scores, as well as to higher MITOS and KSS, indicating that a greater UMN burden correlates with disease severity. Conversely, we did not appreciate any association between UMN involvement and survival or markers of LMN impairment. Moreover, PUMNS values showed a positive association with CMCT and a negative one with CSP values.

Interpretation: Our results suggest that the burden of UMN pathology, assessed through PUMNS, has an important independent role in defining clinical characteristics, functional disability, disease progression and prognosis in ALS patients. We also support the role of TMS in defining severity of UMN involvement.

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 30 January 2023
DOI 10.3389/fneur.2023.1095121

OPEN ACCESS

EDITED BY
Rosanna Cardani,
IRCCS San Donato Polyclinic, Italy

REVIEWED BY
David Bendahan,
UMR7339 Centre de Résonance Magnétique Biologique et Médicale (CRMBM), France
Yi Dai,
Peking Union Medical College Hospital (CAMS), China
Susan T. Iannaccone,
University of Texas Southwestern Medical Center, United States

*CORRESPONDENCE
Giacomo P. Comi
giacomo.comi@unimi.it

SPECIALTY SECTION
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

RECEIVED 10 November 2022
ACCEPTED 09 January 2023
PUBLISHED 30 January 2023

CITATION
Comi GP, Niks EH, Vandenborne K, Cinnante CM, Kan HE, Willcocks RJ, Velardo D, Magri F, Ripolone M, van Benthem JJ, van de Velde NM, Nava S, Ambrosoli L, Cazzaniga S and Bettica PU (2023) Givinostat for Becker muscular dystrophy: A randomized, placebo-controlled, double-blind study. *Front. Neurol.* 14:1095121. doi: 10.3389/fneur.2023.1095121

Givinostat for Becker muscular dystrophy: A randomized, placebo-controlled, double-blind study

Giacomo P. Comi [1,2]*, Erik H. Niks [3,4], Krista Vandenborne[5], Claudia M. Cinnante [6], Hermien E. Kan [4,7], Rebecca J. Willcocks[5], Daniele Velardo[1], Francesca Magri[8], Michela Ripolone [1], Jules J. van Benthem[9], Nienke M. van de Velde[3,4], Simone Nava[6], Laura Ambrosoli[10], Sara Cazzaniga[11] and Paolo U. Bettica[11]

[1]Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [2]Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, Milan, Italy, [3]Department of Neurology, Leiden University Medical Center, Leiden, Netherlands, [4]Duchenne Center Netherlands, Netherlands, [5]ImagingDMD, University of Florida, Gainesville, FL, United States, [6]Radiology Department, Istituto Auxologico Italiano, IRCCS, Milan, Italy, [7]Department of Radiology, C.J. Gorter MRI Center, Leiden University Medical Center, Leiden, Netherlands, [8]Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [9]Department of Orthopedics, Rehabilitation and Physiotherapy, Leiden University Medical Center, Leiden, Netherlands, [10]OPIS srl, Milan, Italy, [11]Italfarmaco SpA, Milan, Italy

Objective: No treatments are approved for Becker muscular dystrophy (BMD). This study investigated the efficacy and safety of givinostat, a histone deacetylase pan-inhibitor, in adults with BMD.

Methods: Males aged 18–65 years with a diagnosis of BMD confirmed by genetic testing were randomized 2:1 to 12 months treatment with givinostat or placebo. The primary objective was to demonstrate statistical superiority of givinostat over placebo for mean change from baseline in total fibrosis after 12 months. Secondary efficacy endpoints included other histological parameters, magnetic resonance imaging and spectroscopy (MRI and MRS) measures, and functional evaluations.

Results: Of 51 patients enrolled, 44 completed treatment. At baseline, there was greater disease involvement in the placebo group than givinostat, based on total fibrosis (mean 30.8 vs. 22.8%) and functional endpoints. Mean total fibrosis did not change from baseline in either group, and the two groups did not differ at Month 12 (least squares mean [LSM] difference 1.04%; $p = 0.8282$). Secondary histology parameters, MRS, and functional evaluations were consistent with the primary. MRI fat fraction in whole thigh and quadriceps did not change from baseline in the givinostat group, but values increased with placebo, with LSM givinostat–placebo differences at Month 12 of −1.35% ($p = 0.0149$) and −1.96% ($p = 0.0022$), respectively. Adverse events, most mild or moderate, were reported by 88.2% and 52.9% patients receiving givinostat and placebo.

Conclusion: The study failed to achieve the primary endpoint. However, there was a potential signal from the MRI assessments suggesting givinostat could prevent (or slow down) BMD disease progression.

KEYWORDS

Becker muscular dystrophy, therapy, disease progression, fibrosis, magnetic resonance imaging (MRI)

International Journal of
Molecular Sciences

MDPI

Review

Advancing Stroke Research on Cerebral Thrombi with Omic Technologies

Gianluca Costamagna [1,2,*], Sara Bonato [2], Stefania Corti [1,2] and Megi Meneri [1,2]

[1] Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Via Francesco Sforza 35, 20122 Milan, Italy
[2] Stroke Unit, Neurology Unit, Neuroscience and Mental Health Department, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
* Correspondence: gianluca.costamagna@unimi.it

Abstract: Cerebrovascular diseases represent a leading cause of disability, morbidity, and death worldwide. In the last decade, the advances in endovascular procedures have not only improved acute ischemic stroke care but also conceded a thorough analysis of patients' thrombi. Although early anatomopathological and immunohistochemical analyses have provided valuable insights into thrombus composition and its correlation with radiological features, response to reperfusion therapies, and stroke etiology, these results have been inconclusive so far. Recent studies applied single- or multi-omic approaches—such as proteomics, metabolomics, transcriptomics, or a combination of these—to investigate clot composition and stroke mechanisms, showing high predictive power. Particularly, one pilot studies showed that combined deep phenotyping of stroke thrombi may be superior to classic clinical predictors in defining stroke mechanisms. Small sample sizes, varying methodologies, and lack of adjustments for potential confounders still represent roadblocks to generalizing these findings. However, these techniques hold the potential to better investigate stroke-related thrombogenesis and select secondary prevention strategies, and to prompt the discovery of novel biomarkers and therapeutic targets. In this review, we summarize the most recent findings, overview current strengths and limitations, and present future perspectives in the field.

Keywords: ischemic stroke; thrombi; clots; proteomics; metabolomics; transcriptomics; multiomic; large vessel occlusion; thrombectomy

Citation: Costamagna, G.; Bonato, S.; Corti, S.; Meneri, M. Advancing Stroke Research on Cerebral Thrombi with Omic Technologies. *Int. J. Mol. Sci.* **2023**, *24*, 3419. https://doi.org/10.3390/ijms24043419

Academic Editor: Adria Arboix

Received: 30 December 2022
Revised: 5 February 2023
Accepted: 7 February 2023
Published: 8 February 2023

1. Introduction

The etiological diagnosis of acute ischemic stroke (AIS) subtypes is paramount to drive accurate secondary prevention strategies (such as anticoagulation in cardioembolic stroke—CE, associated with atrial fibrillation or antiplatelets in large artery atherosclerosis stroke—LAA), and to avoid recurrences. Undetermined stroke accounts for at least one-third of stroke patients [1], and up to 50% in certain subpopulations (e.g., cancer patients) [2], posing several challenges regarding secondary prevention.

In the last decade, the number of endovascular thrombectomy (EVT) interventions in patients with stroke and large vessel occlusions (LVOs) has dramatically increased following positive findings from crucial clinical trials [3], enabling histological, biochemical, and structural analysis of retrieved thrombi [4]. These analyses have correlated thrombi composition with histological and immunohistochemical methods with EVT recanalization rates, response to intravenous thrombolysis (IVT), radiological features, stroke severity, and functional outcomes. In addition, the cellular and molecular characteristics of cerebral thrombi are heterogeneous and provide information about their etiology [4]. Early studies investigated mainly red blood cells (RBCs), fibrin, and platelets [4,5], while more recent reports measured other components such as leukocytes, von Willebrand factor (VWF), and neutrophil extracellular traps (NETs) [6–8]. Particularly, two studies found that CE thrombi

genes

MDPI

Article

Using Cluster Analysis to Overcome the Limits of Traditional Phenotype–Genotype Correlations: The Example of *RYR1*-Related Myopathies

Claudia Dosi [1,†], Anna Rubegni [1], Jacopo Baldacci [2], Daniele Galatolo [1], Stefano Doccini [1], Guja Astrea [1], Angela Berardinelli [3], Claudio Bruno [4,5], Giorgia Bruno [6], Giacomo Pietro Comi [7,8], Maria Alice Donati [9], Maria Teresa Dotti [10], Massimiliano Filosto [11], Chiara Fiorillo [5], Fabio Giannini [10], Gian Luigi Gigli [12,13], Marina Grandis [5,14], Diego Lopergolo [10], Francesca Magri [8], Maria Antonietta Maioli [15], Alessandro Malandrini [10], Roberto Massa [16], Sabrina Matà [17], Federico Melani [18], Sonia Messina [19], Andrea Mignarri [10], Maurizio Moggio [20], Elena Maria Pennisi [21], Elena Pegoraro [22], Giulia Ricci [23], Michele Sacchini [9], Angelo Schenone [5,14], Simone Sampaolo [6], Monica Sciacco [20], Gabriele Siciliano [23], Giorgio Tasca [24,25], Paola Tonin [26], Rossella Tupler [27,28], Mariarosaria Valente [12,13], Nila Volpi [10], Denise Cassandrini [1] and Filippo Maria Santorelli [1,*]

[1] IRCCS Fondazione Stella Maris, 56128 Pisa, Italy
[2] Kode Data Analysis s.r.l., 56128 Pisa, Italy
[3] IRCCS C. Mondino Foundation, 27100 Pavia, Italy
[4] Center of Translational and Experimental Myology, IRCCS Istituto Giannina Gaslini, 16147 Genova, Italy
[5] Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health—DINOGMI, University of Genova, 16147 Genova, Italy
[6] Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", 81100 Naples, Italy
[7] Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy
[8] Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, 20122 Milan, Italy
[9] Metabolic Disease Unit, AOU Meyer Children Hospital, 50139 Florence, Italy
[10] Unit of Neurology and Neurometabolic Diseases, Department of Medical, Surgical and Neurological Sciences, University of Siena, Viale Bracci 2, 53100 Siena, Italy
[11] Department of Clinical and Experimental Sciences, University of Brescia, NeMO-Brescia Clinical Center for Neuromuscular Diseases, 25064 Brescia, Italy
[12] Neurology Unit, Department of Neurosciences, University Hospital of Udine, 33100 Udine, Italy
[13] Department of Medicine, University of Udine, 33100 Udine, Italy
[14] IRCCS Ospedale Policlinico San Martino, 16132 Genova, Italy
[15] Centro Sclerosi Multipla, ASL Cagliari, 09047 Cagliari, Italy
[16] Neuromuscular Diseases Unit, Department of Systems Medicine, Tor Vergata University of Rome, 00133 Rome, Italy
[17] Careggi University Hospital, Neurology Unit, 50134 Florence, Italy
[18] Pediatric Neurology, AOU Meyer Children Hospital, 50139 Florence, Italy
[19] Unit of Neurology and Neuromuscular Disorders, Department of Clinical and Experimental Medicine, University of Messina, 98122 Messina, Italy
[20] Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuromuscular and Rare Diseases Unit, Department of Neuroscience, 20122 Milan, Italy
[21] Neuromuscular Diseases Center, Neurology Unit, San Filippo Neri Hospital, 00135 Rome, Italy
[22] Department of Neurosciences, University of Padova, 35122 Padova, Italy
[23] Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy
[24] Unit of Neurology, Fondazione Policlinico Universitario A. Gemelli IRCSS, 00168 Rome, Italy
[25] John Walton Muscular Dystrophy Research Centre, Newcastle University and Newcastle Hospitals NHS Foundation Trusts, Newcastle upon Tyne NE1 3BZ, UK
[26] Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, 37129 Verona, Italy
[27] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41121 Modena, Italy
[28] Department of Molecular Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01655, USA
* Correspondence: filippo3364@gmail.com; Tel.: +39-050886275; Fax: +39-050886247
† Present Address: UOC Developmental Neurology, IRCCS C. Besta, 20133 Milan, Italy.

Citation: Dosi, C.; Rubegni, A.; Baldacci, J.; Galatolo, D.; Doccini, S.; Astrea, G.; Berardinelli, A.; Bruno, C.; Bruno, G.; Comi, G.P.; et al. Using Cluster Analysis to Overcome the Limits of Traditional Phenotype–Genotype Correlations: The Example of *RYR1*-Related Myopathies. *Genes* **2023**, *14*, 298. https://doi.org/10.3390/genes14020298

Academic Editor: Allison D. Ebert

Received: 25 November 2022
Revised: 14 January 2023
Accepted: 17 January 2023
Published: 23 January 2023

brain sciences

MDPI

Case Report

Lafora Disease: A Case Report and Evolving Treatment Advancements

Carola Rita Ferrari Aggradi [1,†], Martina Rimoldi [2,3,†], Gloria Romagnoli [1], Daniele Velardo [2], Megi Meneri [1,4], Davide Iacobucci [5], Michela Ripolone [2], Laura Napoli [2], Patrizia Ciscato [2], Maurizio Moggio [2], Giacomo Pietro Comi [1,5], Dario Ronchi [1,5], Stefania Corti [1,2,*,†] and Elena Abati [1,*,†]

[1] Dino Ferrari Centre, Department of Pathophysiology and Transplantation (DEPT), University of Milan, 20122 Milan, Italy; carola.ferrari@unimi.it (C.R.F.A.); gloria.romagnoli@unimi.it (G.R.); megi.meneri@unimi.it (M.M.); giacomo.comi@unimi.it (G.P.C.); dario.ronchi@unimi.it (D.R.)
[2] Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy; martina.rimoldi@policlinico.mi.it (M.R.); daniele.velardo@policlinico.mi.it (D.V.); michela.ripolone@policlinico.mi.it (M.R.); patrizia.ciscato@policlinico.mi.it (P.C.); maurizio.moggio@policlinico.mi.it (M.M.)
[3] Medical Genetics Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[4] Stroke Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[5] Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
* Correspondence: stefania.corti@unimi.it (S.C.); elena.abati@unimi.it (E.A.)
† These authors contributed equally to this work.

Abstract: Lafora disease is a rare genetic disorder characterized by a disruption in glycogen metabolism. It manifests as progressive myoclonus epilepsy and cognitive decline during adolescence. Pathognomonic is the presence of abnormal glycogen aggregates that, over time, produce large inclusions (Lafora bodies) in various tissues. This study aims to describe the clinical and histopathological aspects of a novel Lafora disease patient, and to provide an update on the therapeutical advancements for this disorder. A 20-year-old Libyan boy presented with generalized tonic–clonic seizures, sporadic muscular jerks, eyelid spasms, and mental impairment. Electroencephalography showed multiple discharges across both brain hemispheres. Brain magnetic resonance imaging was unremarkable. Muscle biopsy showed increased lipid content and a very mild increase of intermyofibrillar glycogen, without the polyglucosan accumulation typically observed in Lafora bodies. Despite undergoing three lines of antiepileptic treatment, the patient's condition showed minimal to no improvement. We identified the homozygous variant c.137G>A, p.(Cys46Tyr), in the *EPM2B/NHLRC1* gene, confirming the diagnosis of Lafora disease. To our knowledge, the presence of lipid aggregates without Lafora bodies is atypical. Lafora disease should be considered during the differential diagnosis of progressive, myoclonic, and refractory epilepsies in both children and young adults, especially when accompanied by cognitive decline. Although there are no effective therapies yet, the development of promising new strategies prompts the need for an early and accurate diagnosis.

Keywords: Lafora disease; therapeutic strategies; EPM2B; EPM2A; tonic–clonic seizures; Lafora bodies; laforin; malin

Citation: Ferrari Aggradi, C.R.; Rimoldi, M.; Romagnoli, G.; Velardo, D.; Meneri, M.; Iacobucci, D.; Ripolone, M.; Napoli, L.; Ciscato, P.; Moggio, M.; et al. Lafora Disease: A Case Report and Evolving Treatment Advancements. *Brain Sci.* **2023**, *13*, 1679. https://doi.org/10.3390/brainsci13121679

Academic Editor: Moussa Antoine Chalah

Received: 18 October 2023
Revised: 20 November 2023
Accepted: 3 December 2023
Published: 6 December 2023

1. Introduction

Lafora disease (LD, OMIM #254780) is a rare, autosomal, recessive, neurodegenerative disorder, belonging to a group of epilepsies defined as progressive myoclonus epilepsies (PMEs) [1]. It has an estimated prevalence of approximately four cases per one million individuals and it occurs most frequently in Mediterranean countries, South India, North Africa, and the Middle East [2]. Early LD symptoms may appear during late childhood or adolescence and typically include myoclonus, visual seizures, hallucinations, generalized tonic–clonic seizures, muscle wasting, behavioral changes, dysarthria, depression, and cognitive decline [3–5]. The clinical phenotype invariably worsens over time, resulting in a fatal outcome within 10 years of symptom onset [6].

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 17 May 2023
DOI 10.3389/fneur.2023.1169689

OPEN ACCESS

EDITED BY
Marka van Blitterswijk,
Mayo Clinic Florida, United States

REVIEWED BY
Melissa Nel,
University of Cape Town, South Africa
Philippe Corcia,
Université de Tours, France

*CORRESPONDENCE
Stefania Corti
✉ stefania.corti@unimi.it

†These authors have contributed equally to this work

RECEIVED 19 February 2023
ACCEPTED 19 April 2023
PUBLISHED 17 May 2023

CITATION
Gagliardi D, Ripellino P, Meneri M, Del Bo R, Antognozzi S, Comi GP, Gobbi C, Ratti A, Ticozzi N, Silani V, Ronchi D and Corti S (2023) Clinical and molecular features of patients with amyotrophic lateral sclerosis and *SOD1* mutations: a monocentric study.
Front. Neurol. 14:1169689.
doi: 10.3389/fneur.2023.1169689

Clinical and molecular features of patients with amyotrophic lateral sclerosis and *SOD1* mutations: a monocentric study

Delia Gagliardi[1,2†], Paolo Ripellino[3†], Megi Meneri[1,2], Roberto Del Bo[1], Sara Antognozzi[1], Giacomo Pietro Comi[1,4], Claudio Gobbi[3,5], Antonia Ratti[6,7], Nicola Ticozzi[1,6], Vincenzo Silani[1,6], Dario Ronchi[1] and Stefania Corti[1,2*]

[1]Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), Dino Ferrari Centre, University of Milan, Milan, Italy, [2]Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [3]Department of Neurology, Neurocenter of Southern Switzerland EOC, Lugano, Switzerland, [4]Neuromuscular and Rare Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [5]Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano, Switzerland, [6]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [7]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy

Introduction: *SOD1* was the first gene associated with both familial and sporadic forms of amyotrophic lateral sclerosis (ALS) and is the second most mutated gene in Caucasian ALS patients. Given their high clinical and molecular heterogeneity, a detailed characterization of *SOD1*-ALS patients could improve knowledge about the natural history of this disease. Here, the authors aimed to provide a clinical and molecular description of a monocentric cohort of *SOD1*-ALS patients.

Methods: Amyotrophic lateral sclerosis (ALS) patients referring to the neurology unit of our center between 2008 and 2021 were clinically assessed and underwent molecular testing for *SOD1*. Segregation studies in available family members and *in silico* analysis were performed to sustain the pathogenicity of the identified *SOD1* variants.

Results: Among the 576 patients in our cohort, we identified 19 individuals harboring a mutation in *SOD1* (3.3%), including 15 (78.9%) with a familial and four (21.1%) with a sporadic form. The spinal onset of the disease was observed in all patients, and survival was extremely variable, ranging from 8months to over 30years. Twelve different *SOD1* missense variants were identified in our cohort, including one novel mutation (p.Pro67Leu).

Discussion: In the present series, we provided the first description of an Italian monocentric cohort of *SOD1*-ALS patients, and we expanded the repertoire of *SOD1* mutations. Our cohort presents several remarkable features, including variable expressivity in the same family, atypical presentation (ataxia, cognitive impairment, and other extra-motor symptoms), and different modes of inheritance of a given mutation in the same family. Given the recent authorization of *SOD1*-directed antisense oligonucleotide for use in *SOD1*-ALS patients, we recommend prompt screening for *SOD1* mutations in novel ALS patients with familiar or sporadic presentations.

KEYWORDS

amyotrophic lateral sclerosis, superoxide dismutase, *SOD1*-ALS, cohort, *SOD1* variants

genes

MDPI

Article

NGS-Based Genetic Analysis in a Cohort of Italian Patients with Suspected Inherited Myopathies and/or HyperCKemia

Federica Invernizzi [1], Rossella Izzo [1], Isabel Colangelo [1], Andrea Legati [1], Nadia Zanetti [1], Barbara Garavaglia [1], Eleonora Lamantea [1], Lorenzo Peverelli [1], Anna Ardissone [2], Isabella Moroni [2], Lorenzo Maggi [3], Silvia Bonanno [3], Laura Fiori [4], Daniele Velardo [5], Francesca Magri [6], Giacomo P. Comi [5,7], Dario Ronchi [6,7], Daniele Ghezzi [1,8,*] and Costanza Lamperti [1]

1 Medical Genetics and Neurogenetics Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20126 Milan, Italy; federica.invernizzi@istituto-besta.it (F.I.); rossella.izzo@istituto-besta.it (R.I.); andrea.legati@istituto-besta.it (A.L.); nadia.zanetti@istituto-besta.it (N.Z.); barbara.garavaglia@istituto-besta.it (B.G.); eleonora.lamantea@istituto-besta.it (E.L.); lorenzo.peverelli84@gmail.com (L.P.); costanza.lamperti@istituto-besta.it (C.L.)
2 Child Neurology Unit—Department of Pediatric Neuroscience, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy; anna.ardissone@istituto-besta.it (A.A.); isabella.moroni@istituto-besta.it (I.M.)
3 Department of Neuroimmunology and Neuromuscular Diseases, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy; lorenzo.maggi@istituto-besta.it (L.M.); silvia.bonanno@istituto-besta.it (S.B.)
4 UOS di Malattie Metaboliche e Nutrizione, Ospedale dei Bambini Vittore Buzzi, 20154 Milan, Italy; laurafiori69@gmail.com
5 Neuromuscular and Rare Disease Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy; daniele.velardo@policlinico.mi.it (D.V.); giacomo.comi@policlinico.mi.it (G.P.C.)
6 Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy; francesca.magri@policlinico.mi.it (F.M.); dario.ronchi@unimi.it (D.R.)
7 Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, 20122 Milan, Italy
8 Lab of Neurogenetics and Mitochondrial Disorders, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy
* Correspondence: daniele.ghezzi@istituto-besta.it

Abstract: Introduction/Aims HyperCKemia is considered a hallmark of neuromuscular diseases. It can be either isolated or associated with cramps, myalgia, weakness, myoglobinuria, or rhabdomyolysis, suggesting a metabolic myopathy. The aim of this work was to investigate possible genetic causes in order to help diagnose patients with recurrent hyperCKemia or clinical suspicion of inherited metabolic myopathy. Methods A cohort of 139 patients (90 adults and 49 children) was analyzed using a custom panel containing 54 genes associated with hyperCKemia. Results A definite genetic diagnosis was obtained in 15.1% of cases, while candidate variants or variants of uncertain significance were found in a further 39.5%. Similar percentages were obtained in patients with infantile or adult onset, with some different causative genes. *RYR1* was the gene most frequently identified, either with single or compound heterozygous variants, while *ETFDH* variants were the most common cause for recessive cases. In one patient, mRNA analysis allowed identifying a large *LPIN1* deletion missed by DNA sequencing, leading to a certain diagnosis. Conclusion These data confirm the high genetic heterogeneity of hyperCKemia and metabolic myopathies. The reduced diagnostic yield suggests the existence of additional genes associated with this condition but also allows speculation that a significant number of cases presenting with hyperCKemia or muscle symptoms are due to extrinsic, not genetic, factors.

Keywords: hyperCKemia; creatine kinase; rhabdomyolysis; skeletal muscle damage; Next Generation Sequencing (NGS); myoglobinuria

Citation: Invernizzi, F.; Izzo, R.; Colangelo, I.; Legati, A.; Zanetti, N.; Garavaglia, B.; Lamantea, E.; Peverelli, L.; Ardissone, A.; Moroni, I.; et al. NGS-Based Genetic Analysis in a Cohort of Italian Patients with Suspected Inherited Myopathies and/or HyperCKemia. *Genes* **2023**, *14*, 1393. https://doi.org/10.3390/genes14071393

Academic Editors: Raymond L. Rosales and Satish V Khadilkar

Received: 26 May 2023
Revised: 26 June 2023
Accepted: 28 June 2023
Published: 2 July 2023

scientific reports

Check for updates

OPEN

NOTCH2NLC GGC repeats are not expanded in Italian amyotrophic lateral sclerosis patients

Arianna Manini[1], Delia Gagliardi[1], Megi Meneri[1,2], Sara Antognozzi[1], Roberto Del Bo[1], Giacomo Pietro Comi[1,3], Stefania Corti[1,2] & Dario Ronchi[1]✉

Repeat expansions in genes other than *C9orf72* and *ATXN2* have been recently associated with Amyotrophic Lateral Sclerosis (ALS). Indeed, an abnormal number of GGC repeats in *NOTCH2NLC* has been recently reported in 0.7% of sporadic ALS patients from mainland China. This finding was not confirmed in an ALS cohort of subjects from Taiwan. As the involvement of expanded *NOTCH2NLC* alleles in ALS is debated, we addressed this point by evaluating *NOTCH2NLC* repeat expansions in an Italian cohort of ALS patients. A screening analysis of *NOTCH2NLC* GGC repeats was performed by repeat-primed polymerase chain reaction (RP-PCR) in a cohort of 385 probable/definite ALS Italian patients. Mean age at onset was 60.5 years (SD 13.7), and 60.9% were males. Sporadic cases were 357 (92.7%), and most patients had a spinal onset (71.8%). None of our patients showed the typical sawtooth tail pattern on RP-PCR, thus excluding abnormal repeat expansion in *NOTCH2NLC*. Overall, we suggest that *NOTCH2NLC* expanded alleles might be absent or at least extremely rare in ALS Italian patients. Further investigations in larger cohorts with different ethnic backgrounds are required to support the involvement of *NOTCH2NLC* in ALS.

The Notch 2 N-terminal like C gene (*NOTCH2NLC*), located at chromosome 1q21, differs from the other two human *NOTCH2* paralogs (*NOTCH2NLA* and *NOTCH2NLB*) for the presence of a repeat sequence (GGC)9(GGA)2(GGC)2 in the 5′ untranslated region (UTR), and for its enhanced expression in brain, especially in the prefrontal cortex[1,2]. Starting from 2019, *NOTCH2NLC* GGC repeat expansions in the 5′-UTR were found in patients affected by neuronal intranuclear inclusion disease (NIID), a neurodegenerative disorder characterized by eosinophilic, p62 and ubiquitin-positive intranuclear inclusions diffuse to different tissues, including the central and peripheral nervous systems[3–8]. NIID is a heterogeneous disorder characterized by a variety of neurological signs and symptoms, including cognitive impairment, parkinsonism, tremor, cerebellar ataxia, epilepsy, peripheral neuropathy, and autonomic dysfunction[2,5]. NIID is traditionally classified in three main types based on the predominant neurological features, namely muscle weakness-dominant, parkinsonism-dominant, and dementia-dominant[5]. *NOTCH2NLC* GGC repeat expansions have been reported in all these three forms, with a higher repeat size in the muscle weakness-dominant type[5]. An almost pathognomonic magnetic resonance imaging (MRI) marker of NIID is represented by a curvilinear hyperintensity at the corticomedullary junction at diffusion weighted imaging (DWI) sequences. However, its sensitivity is limited[2].

By employing long-read sequencing (LRS), repeat-primed polymerase chain reaction (RP-PCR) and GC-rich PCR, the screening of *NOTCH2NLC* GGC repeat expansions has been rapidly extended to a variety of neurological disorders, including oculopharyngodistal myopathy (OPDM)[9,10], Parkinson's disease (PD)[11–16], essential tremor (ET)[14,17–22], multiple system atrophy (MSA)[14,23,24], spinocerebellar ataxia (SCA)[5,14], dementia [i.e., Alzheimer disease (AD), frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), vascular dementia (VaD)][5,25,26], hereditary spastic paraplegia (HSP)[27], peripheral neuropathy[5,28–30], adult leukoencephalopathy[31–34], and specifically cerebral small vessel disease[35]. However, the results of these studies have been spurious, so that the pathogenic role of *NOTCH2NLC* in neurological disorders beyond NIID is still debated.

[1]Neuroscience Section, Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, Milan, Italy. [2]Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. [3]Neuromuscular and Rare Disease Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. ✉email: dario.ronchi@unimi.it

biomedicines

MDPI

Review

Identification of Novel Biomarkers of Spinal Muscular Atrophy and Therapeutic Response by Proteomic and Metabolomic Profiling of Human Biological Fluid Samples

Megi Meneri [1,2], Elena Abati [1,3], Delia Gagliardi [1,3], Irene Faravelli [1,3], Valeria Parente [3], Antonia Ratti [4,5], Federico Verde [1,4], Nicola Ticozzi [1,4], Giacomo P. Comi [1,3,†], Linda Ottoboni [1,†] and Stefania Corti [1,6,*,†]

1 Dino Ferrari Centre, Department of Pathophysiology and Transplantation (DEPT), University of Milan, 20122 Milan, Italy
2 Stroke Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
3 Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
4 Laboratory of Neuroscience, Department of Neurology, IRCCS Istituto Auxologico Italiano, 20095 Milan, Italy
5 Department Medical Biotechnology and Translational Medicine, University of Milan, 20100 Milan, Italy
6 Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
* Correspondence: stefania.corti@unimi.it or stefania.corti@policlinico.mi.it
† These authors contributed equally to this work.

Abstract: Spinal muscular atrophy (SMA) is a neuromuscular disease resulting from mutations or deletions in *SMN1* that lead to progressive death of alpha motor neurons, ultimately leading to severe muscle weakness and atrophy, as well as premature death in the absence of treatment. Recent approval of SMN-increasing medications as SMA therapy has altered the natural course of the disease. Thus, accurate biomarkers are needed to predict SMA severity, prognosis, drug response, and overall treatment efficacy. This article reviews novel non-targeted omics strategies that could become useful clinical tools for patients with SMA. Proteomics and metabolomics can provide insights into molecular events underlying disease progression and treatment response. High-throughput omics data have shown that untreated SMA patients have different profiles than controls. In addition, patients who clinically improved after treatment have a different profile than those who did not. These results provide a glimpse on potential markers that could assist in identifying therapy responders, in tracing the course of the disease, and in predicting its outcome. These studies have been restricted by the limited number of patients, but the approaches are feasible and can unravel severity-specific neuro-proteomic and metabolic SMA signatures.

Keywords: antisense oligonucleotide; cerebrospinal fluid; proteome; metabolome; nusinersen; spinal muscular atrophy

Citation: Meneri, M.; Abati, E.; Gagliardi, D.; Faravelli, I.; Parente, V.; Ratti, A.; Verde, F.; Ticozzi, N.; Comi, G.P.; Ottoboni, L.; et al. Identification of Novel Biomarkers of Spinal Muscular Atrophy and Therapeutic Response by Proteomic and Metabolomic Profiling of Human Biological Fluid Samples. *Biomedicines* **2023**, *11*, 1254. https://doi.org/10.3390/biomedicines11051254

Academic Editor: Kuen-Jer Tsai

Received: 16 January 2023
Revised: 16 April 2023
Accepted: 20 April 2023
Published: 23 April 2023

1. Introduction

Spinal muscular atrophy (SMA) is a neurodegenerative disorder caused by mutations in *SMN1* that determine a reduction in SMN protein [1,2] and a resulting loss of alpha motor neurons (MNs) in the brainstem and spinal cord, followed by progressive muscle weakness and atrophy, as well as early death [1,2]. Three types of pediatric SMA are recognized (types 1 to 3), as well as two less frequent types, prenatal (type 0) and adult (type 4) [1,2].

The full-length *SMN* mRNA is translated into a ubiquitously expressed 38-kDa protein [1,2]. The SMN protein is located inside the cytoplasm of various types of cells and in nuclear punctuated structures within the nucleus called gems. In fibroblasts from SMA patients, more gems are detected, less severe is the disease [1,2]. In neurons, SMN is present in axonal granules and moves bidirectionally at a rapid rate [1,2]. Recent studies have provided further insight into the role of SMN in cellular compartments and its association with disease progression in SMA patients [1,2].

Neurological Sciences
https://doi.org/10.1007/s10072-023-07169-x

ORIGINAL ARTICLE

Cognitive abnormalities in Becker muscular dystrophy: a mysterious link between dystrophin deficiency and executive functions

Laura Pezzoni[1] · Roberta Brusa[2] · Teresa Difonzo[1] · Francesca Magri[1] · Daniele Velardo[3] · Stefania Corti[3,4] · Giacomo Pietro Comi[1,4] · Maria Cristina Saetti[1,4]

Received: 13 June 2023 / Accepted: 28 October 2023

Abstract

Background Distrophinopathies are a heterogeneous group of neuromuscular disorders due to mutations in the *DMD* gene. Different isoforms of dystrophin are also expressed in the cerebral cortex and Purkinje cells. Despite cognitive abnormalities in Duchenne muscular dystrophy subjects that have been described in the literature, little is known about a comprehensive cognitive profile in Becker muscular dystrophy patients.

Aim The aim of this study was to assess cognitive functioning in Becker muscular dystrophy patients by using an extensive neuropsychological battery. Our hypothesis is that the most impaired functions are the highly intentional and conscious ones, such as working memory functions, which require a prolonged state of cellular activation.

Methods We performed an extensive neuropsychological assessment on 28 Becker muscular dystrophy patients from 18 to 65 years old. As control subjects, we selected 20 patients with limb-girdle muscular dystrophy, whose clinical picture was similar except for cognitive integrity. The evaluation, although extended to all areas, was focused on prefrontal control skills, with a distinction between inhibitory processes of selective attention and activating processes of working memory.

Results and conclusions Significant underperformances were found exclusively in the Dual Task and PASAT tests, to demonstrate a selective impairment of working memory that, while not causing intellectual disability, reduces the intellectual potential of patients with Becker muscular dystrophy.

Keywords Becker muscular dystrophy · Cognition · Neuropsychological tests · Executive functions

Introduction

Dystrophinopathies are related to the absence (Duchenne muscular dystrophy, DMD) or to the partial deficiency (Becker muscular dystrophy, BMD) of the dystrophin protein, encoded by the *DMD* gene on chromosome X. Although dystrophin is mainly expressed in the skeletal muscle, different isoforms are also expressed in other tissues, including the brain. The massive gene of dystrophin contains in fact a set of tightly regulated promoters that generate eight cell-specific protein isoforms, which all share the same C-terminal domain but start from different N-terminal domains [1]. In neural cells, no less than five isoforms of dystrophin are expressed: two full-length isoforms, cerebellar dystrophin and cortical dystrophin, and three short-form isoforms: Dp140, Dp116, and Dp71, which are the most abundant in the brain [2]. The function of all dystrophin isoforms in the brain is not entirely understood yet, but they appear to be involved in myelination during neural development [3], synaptic modulation [4], and neuronal differentiation through neuritis growth [5] as well as in cellular energy metabolism [6]. Their complete or partial loss in DMD and BMD seems to underlie the great variability of cognitive deficits observed in these individuals. Many studies in fact show a correlation between the risk of cognitive impairment in both DMD and BMD and cumulative loss of functional

✉ Maria Cristina Saetti
cristina.saetti@unimi.it

1 Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy

2 ASST Ovest Milanese, Ospedale Di Legnano, Neurology Unit, Legnano, Milan, Italy

3 Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuromuscular and Rare Diseases Unit, Milan, Italy

4 Department of Pathophysiology and Transplants, Dino Ferrari Center, University of Milan, Milan, Italy

Clinical Phenotype of Pediatric and Adult Patients With Spinal Muscular Atrophy With Four *SMN2* Copies: Are They Really All Stable?

Martina Ricci, MD,[1,2†] Gianpaolo Cicala, MD,[1,2†] Anna Capasso, MD,[1,2†] Giorgia Coratti, PhD,[1,2] Stefania Fiori, MLT,[3] Costanza Cutrona, MD,[1] Adele D'Amico, PhD,[4] Valeria A. Sansone, PhD,[5] Claudio Bruno, PhD,[6] Sonia Messina, PhD,[7] Tiziana Mongini, PhD,[8] Michela Coccia, MD,[9] Gabriele Siciliano, PhD,[10] Elena Pegoraro, PhD,[11] Riccardo Masson, MD,[12] Massimiliano Filosto, PhD,[13] Giacomo P. Comi, PhD,[14,15] Stefania Corti, PhD,[14,15] Dario Ronchi, PhD,[14,15] Lorenzo Maggi, MD,[16] Maria G. D'Angelo, PhD,[17] Veria Vacchiano, MD,[18] Chiara Ticci, MD,[19] Lucia Ruggiero, PhD,[20] Lorenzo Verriello, MD,[21] Federica S. Ricci, MD,[8] Angela L. Berardinelli, MD,[22] Maria Antonietta Maioli, PhD,[23] Matteo Garibaldi, PhD,[24] Vincenzo Nigro, MD,[25,26] Stefano C. Previtali, PhD,[27] Maria Carmela Pera, PhD,[1,2] Eduardo Tizzano, MD,[28] Marika Pane, PhD,[1,2] Francesco Danilo Tiziano, PhD,[3,29‡] and Eugenio Mercuri, PhD,[1,2‡] on behalf of ITASMAC Working Group

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26788

Received Jul 26, 2023, and in revised form Aug 29, 2023. Accepted for publication Sep 5, 2023.

Address correspondence to Eugenio Mercuri, Pediatric Neurology, Università Cattolica del Sacro Cuore, Rome, Italy.
E-mail: eugeniomaria.mercuri@unicatt.it

[†]These authors contributed equally as co-first authors.

[‡]Both of these authors should be considered senior authors.

From the [1]Pediatric Neurology, Università Cattolica del Sacro Cuore, Rome, Italy; [2]Centro Clinico Nemo, Fondazione Agostino Gemelli IRCCS, Rome, Italy; [3]Department of Life Sciences and Public Health, Section of Genomic Medicine, Università Cattolica del Sacro Cuore, Rome, Italy; [4]Department of Neurosciences, Unit of Neuromuscular and Neurodegenerative Disorders, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [5]The NEMO Center in Milan, Neurorehabilitation Unit, University of Milan, ASST Niguarda Hospital, Milan, Italy; [6]Center of Translational and Experimental Myology, and Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genova, IRCCS Istituto Giannina Gaslini, Genova, Italy; [7]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; [8]AOU Città della Salute e della Scienza di Torino, presidio Molinette e OIRM (SS Malattie neuromuscolari e SC Neuropsichiatria Infantile), Turin, Italy; [9]Department of Neurological Sciences, AOU Ospedali Riuniti di Ancona, Torrette, Ancona, Italy; [10]AOU Pisana (Department of Clinical and Experimental Medicine), Neurology Unit, Pisa, Italy; [11]Neurology Unit, Azienda Ospedale Padova, Padua, Italy; [12]Developmental Neurology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; [13]Department of Clinical and Experimental Sciences, University of Brescia (Italy), NeMO-Brescia Clinical Center for Neuromuscular Diseases, Brescia, Italy; [14]Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [15]Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy; [16]Fondazione IRCCS Istituto Neurologico Carlo Besta Developmental Neurology Unit, Milan, Italy;

frontiers | Frontiers in Genetics

TYPE Brief Research Report
PUBLISHED 30 November 2023
DOI 10.3389/fgene.2023.1278572

OPEN ACCESS

EDITED BY
Catarina M. Quinzii,
Columbia University, United States

REVIEWED BY
Edoardo Malfatti,
Hôpitaux Universitaires Henri Mondor, France
Valentina Emmanuele,
Columbia University, United States

*CORRESPONDENCE
Dario Ronchi,
dario.ronchi@unimi.it

RECEIVED 16 August 2023
ACCEPTED 20 November 2023
PUBLISHED 30 November 2023

CITATION
Rimoldi M, Magri F, Antognozzi S, Ripolone M, Salani S, Piga D, Bertolasi L, Zanotti S, Ciscato P, Fortunato F, Moggio M, Corti S, Comi GP and Ronchi D (2023), Prominent muscle involvement in a familial form of mitochondrial disease due to a *COA8* variant.
Front. Genet. 14:1278572.
doi: 10.3389/fgene.2023.1278572

Prominent muscle involvement in a familial form of mitochondrial disease due to a *COA8* variant

Martina Rimoldi[1], Francesca Magri[2], Sara Antognozzi[2], Michela Ripolone[1], Sabrina Salani[2], Daniela Piga[2], Letizia Bertolasi[2], Simona Zanotti[1], Patrizia Ciscato[1], Francesco Fortunato[2], Maurizio Moggio[1], Stefania Corti[1,3], Giacomo Pietro Comi[2,3] and Dario Ronchi[2,3]*

[1]Neuromuscular and Rare Diseases Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [2]Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [3]Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

Isolated mitochondrial respiratory chain Complex IV (Cytochrome c Oxidase or COX) deficiency is the second most frequent isolated respiratory chain defect. Causative mutations are mainly identified in structural COX subunits or in proteins involved in the maturation and assembly of the COX holocomplex. We describe an Italian familial case of mitochondrial myopathy due to a variant in the COX assembly factor 8 gene (*COA8*). Patient 1 is a 52-year-old woman who presented generalized epilepsy and retinitis pigmentosa at 10 years of age. From her early adulthood she complained about cramps and myalgia after exercise, and bilateral hearing loss emerged. Last neurological examination (52 years of age) showed bilateral ptosis, muscle weakness, peripheral neuropathy, mild dysarthria and dysphonia, cognitive impairment. Muscle biopsy had shown the presence of ragged-red fibers. Patient 2 (Patient 1's sister) is a 53-year-old woman presenting fatigability, myalgia, and hearing loss. Neurological examination showed ptosis and muscle weakness. Muscle biopsy displayed a diffuse reduction of COX activity staining and ragged-red fibers. Both sisters presented secondary amenorrhea. After ruling out mtDNA mutations, Whole Exome Sequencing analysis identified the novel homozygous *COA8* defect c.170_173dupGACC, p.(Pro59fs) in the probands. Loss-of-function *COA8* mutations have been associated with cavitating leukoencephalopathy with COX deficiency in 9 reported individuals. Disease course shows an early-onset rapid clinical deterioration, affecting both cognitive and motor functions over months, followed by stabilization and slow improvement over several years. Our findings expand the clinical spectrum of *COA8*-related disease. We confirm the benign course of this rare disorder, highlighting its (intrafamilial) clinical variability.

KEYWORDS

mitochondrial myopathy, cytochrome c oxidase deficiency, COA8, mitochondrial encefalomyopathies, whole exome sequencing

ESHG

ARTICLE

A biallelic variant in *COX18* cause isolated Complex IV deficiency associated with neonatal encephalo-cardio-myopathy and axonal sensory neuropathy

Dario Ronchi[1,2], Manuela Garbellini[2], Francesca Magri[2], Francesca Menni[3], Megi Meneri[1,2], Maria Francesca Bedeschi[4], Robertino Dilena[5], Valeria Cecchetti[6], Irene Picciolli[6], Francesca Furlan[3], Valentina Polimeni[6], Sabrina Salani[2], Laura Pezzoli[7], Francesco Fortunato[1], Matteo Bellini[7], Daniela Piga[2], Michela Ripolone[8], Simona Zanotti[8], Laura Napoli[8], Patrizia Ciscato[8], Monica Sciacco[8], Giovanna Mangili[9], Fabio Mosca[6,10], Stefania Corti[1,8], Maria Iascone[7] and Giacomo Pietro Comi[1,2]✉

Pathogenic variants impacting upon assembly of mitochondrial respiratory chain Complex IV (Cytochrome c Oxidase or COX) predominantly result in early onset mitochondrial disorders often leading to CNS, skeletal and cardiac muscle manifestations. The aim of this study is to describe a molecular defect in the COX assembly factor gene *COX18* as the likely cause of a neonatal form of mitochondrial encephalo-cardio-myopathy and axonal sensory neuropathy. The proband is a 19-months old female displaying hypertrophic cardiomyopathy at birth and myopathy with axonal sensory neuropathy and failure to thrive developing in the first months of life. Serum lactate was consistently increased. Whole exome sequencing allowed the prioritization of the unreported homozygous substitution NM_001297732.2:c.667 G > C p.(Asp223His) in *COX18*. Patient's muscle biopsy revealed severe and diffuse COX deficiency and striking mitochondrial abnormalities. Biochemical and enzymatic studies in patient's myoblasts and in HEK293 cells after *COX18* silencing showed a severe impairment of both COX activity and assembly. The biochemical defect was partially rescued by delivery of wild-type *COX18* cDNA into patient's myoblasts. Our study identifies a novel defect of COX assembly and expands the number of nuclear genes involved in a mitochondrial disorder due to isolated COX deficiency.

European Journal of Human Genetics (2023) 31:1414–1420; https://doi.org/10.1038/s41431-023-01433-6

INTRODUCTION

Cytochrome c oxidase (COX or Complex IV) is the terminal enzyme in the mitochondrial respiratory chain. Mammalian COX is a multisubunit enzyme composed of 14 subunits: the three core subunits MT-CO1, MT-CO2 and MT-CO3 are encoded by mitochondrial DNA (mtDNA), while the remaining structural subunits are imported into mitochondria after the cytosolic translation of the respective nuclear genes [1].

COX deficiency (MIM#220110) is a primary mitochondrial presentation associated with severe isolated reduction of Complex IV activity leading to impaired OXPHOS metabolism in the affected tissues. Molecular defects are mainly identified in nuclear or mtDNA genes encoding for structural COX subunits or in nuclear genes encoding for proteins involved in the maturation and assembly of the COX holocomplex. Pathogenic variants in several COX assembly genes mainly result in neonatal or childhood onset disorders featuring severe COX deficiency in muscle, brain and, rarely, liver [2]. Hypertrophic cardiomyopathy has been also frequently observed in these disorders (Supplementary Table 1).

COX18 encodes for a mitochondrial protein proposed to play a role in the maturation of MT-CO2 (COX-II) subunit [3, 4]. Genetic inactivation of *COX18* in eukaryotic models abolished COX assembly and activity [4], but no *COX18* variants have been detected in patients with isolated COX deficiency so far [5].

Here we describe the first association between a molecular defect in *COX18* and a mitochondrial disorder characterized by neonatal hypertrophic cardiomyopathy followed by signs of infantile myopathy and axonal polyneuropathy with predominant affection of sensory fibers.

METHODS

The subject underwent several pediatric metabolic evaluations, neurological examinations, cardiological assessments, brain MRI, and neurophysiological studies. Blood samples and a muscle biopsy were collected. The Ethics Committee of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) approved the study. Written informed consent for publication of clinical details and images were obtained from patient's parents.

[1]Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy. [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy. [3]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Regional Clinical Center for expanded newborn screening, Milan, Italy. [4]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Medical Genetics Unit, Milan, Italy. [5]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, UO Neurofisiopatologia, Milan, Italy. [6]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neonatal Intensive Care Unit, Milan, Italy. [7]ASST Papa Giovanni XXIII, Laboratorio di Genetica Medica, Bergamo, Italy. [8]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuromuscular and Rare Disease Unit, Milan, Italy. [9]ASST Papa Giovanni XXIII, Neonatology and NICU, Bergamo, Italy. [10]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy. ✉email: giacomo.comi@unimi.it

Received: 22 February 2023 Revised: 3 July 2023 Accepted: 10 July 2023
Published online: 19 July 2023

BMC Neurology

CASE REPORT

Open Access

Ischemic optic neuropathy as first presentation in patient with m.3243 A > G MELAS classic mutation

Simone Scarcella[1,2†], Laura Dell'Arti[3†], Delia Gagliardi[1,2], Francesca Magri[4], Alessandra Govoni[1], Daniele Velardo[1], Claudia Mainetti[4], Valeria Minorini[3], Dario Ronchi[1,4], Daniela Piga[2], Giacomo Pietro Comi[1,4], Stefania Corti[1,2] and Megi Meneri[1,2*]

Abstract

Background Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a systemic disorder in which multi-organ dysfunction may occur from mitochondrial metabolism failure. Maternally inherited mutations in the MT-TL1 gene are the most frequent causes for this disorder. Clinical manifestations may include stroke-like episodes, epilepsy, dementia, headache and myopathy. Among these, acute visual failure, usually in association with cortical blindness, can occur because of stroke-like episodes affecting the occipital cortex or the visual pathways. Vision loss due to optic neuropathy is otherwise considered a typical manifestation of other mitochondrial diseases such as Leber hereditary optic neuropathy (LHON).

Case presentation Here we describe a 55-year-old woman, sister of a previously described patient with MELAS harbouring the m.3243A > G (p.0, MT-TL1) mutation, with otherwise unremarkable medical history, that presented with subacute, painful visual impairment of one eye, accompanied by proximal muscular pain and headache. Over the next weeks, she developed severe and progressive vision loss limited to one eye. Ocular examination confirmed unilateral swelling of the optic nerve head; fluorescein angiography showed segmental perfusion delay in the optic disc and papillary leakage. Neuroimaging, blood and CSF examination and temporal artery biopsy ruled out neuroinflammatory disorders and giant cell arteritis (GCA). Mitochondrial sequencing analysis confirmed the m.3243A > G transition, and excluded the three most common LHON mutations, as well as the m.3376G > A LHON/MELAS overlap syndrome mutation. Based on the constellation of clinical symptoms and signs presented in our patient, including the muscular involvement, and the results of the investigations, the diagnosis of optic neuropathy as a stroke-like event affecting the optic disc was performed. L-arginine and ubidecarenone therapies were started with the aim to improve stroke-like episode symptoms and prevention. The visual defect remained stable with no further progression or outbreak of new symptoms.

Conclusions Atypical clinical presentations must be always considered in mitochondrial disorders, even in well-described phenotypes and when mutational load in peripheral tissue is low. Mitotic segregation of mitochondrial DNA (mtDNA) does not allow to know the exact degree of heteroplasmy existent within different tissue, such as retina

†Simone Scarcella and Laura Dell'Arti equally contributed to this work.

*Correspondence:
Megi Meneri
megi.meneri@unimi.it
Full list of author information is available at the end of the article

middle-aged woman harbouring MELAS classic mutation, possibly secondary to a stroke-like event affecting the optic nerve. The pathogenesis of this SLE could be in our opinion better explained by the angiopathy existent within MELAS, thus needed to be separated from the pathogenesis of a classic NAION. In our opinion, the simultaneous onset of diffuse muscular pain, typical for myopathic involvement within mitochondrial disorders, further supports this hypothesis. L-arginine and ubidecarenone therapies were therefore started to improve stroke-like episode symptoms and prevention of new ones, as well as avoiding the involvement of the other eye. After the initiation of therapy we did not assist to further progression of symptoms or to the outbreak of new possible ones.

This is the second case of a possible SLE described in a carrier of mutation m.3243A > G with swollen optic disc; previously, a case with bilateral transient optic disc oedema was described [10]. However, differently from our case, the recovery of the visual acuity was complete. Considering that SLE may be at least partially reversible, the authors speculated that a similar phenomenon had occurred in their patient [10].

In conclusion, atypical clinical presentations must be always considered in mitochondrial disorders, even in well-described phenotypes and even if mutational load in peripheral tissue (that are easily accessible for analysis) is low. Indeed, the stochastic mitotic segregation of mtDNA molecules does not allow to foresee the exact degree of heteroplasmy existent within different tissues, such as retina and optic nerve. As a consequence, important therapeutic implications arise from a correct diagnosis of atypical presentations of mitochondrial disorders.

In conclusion, mitochondrial gene mutations may present not only with different and less frequently atypical phenotypes, but they may also be considered as an adjunctive risk factor for ischemic events. In this case, we think that better knowledge of the genetic background could help not only in a correct diagnosis, but also for a tempestive and tailored therapy.

Abbreviations

ADC	Apparent diffusion coefficient
BBB	Blood barrier brain
CNS	Central nervous system
CRP	C reactive protein
CSF	Cerebrospinal fluid
DWI	Diffusion weighted imaging
ESR	Erythrocyte sedimentation rate
ETC	Electron transport chain
FA	Fluorescein angiography
FAF	Fundus autofluorescence
FDG-PET	Fludeoxyglucose positron emission tomography
FLAIR	Fluid-attenuated inversion recovery
GCA	Giant cell arteritis
GCL	Ganglion cell layer
LE	Left eye
LHON	Leber hereditary optic neuropathy
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
mtDNA	Mitochondrial DNA
MT-TL1	Mitochondrially encoded tRNA leucine 1
NAION	Non-arteritic ischemic optic neuropathy
NMO	Neuromyelitis optica
NMOSD	Neuromyelitis optica spectrum disorders
OCT	Ocular coherence tomography
PCR	Polymerase chain reaction
RE	Right eye
RNFL	Retinal nerve fiber layer
SLE	Stroke-like episode
STIR	Short tau inverion recovery
tRNA	Transfer RNA

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-023-03198-3.

Additional file 1. Supplementary materials. Family history and pedigree.

Acknowledgements
This work was partially supported by Italian Ministry of Health (Ministero della Salute), Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico Grant Ricerca Corrente 2022 to GPC. We would like to thank "Associazione Centro Dino Ferrari" for its support.

Authors' contributions
SS and MM: drafted the manuscript for intellectual content and collected and analyzed the data. DG, LD, DV, CM, VM, DP and MM: collected and analyzed the data and revised the manuscript for intellectual content. FM, MM, AG, GPC,DR and SC: revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

Funding
This work was partially supported by Italian Ministry of Health (Ministero della Salute), Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico Grant Ricerca Corrente 2022 to GPC.

Availability of data and materials
The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Declarations

Ethics approval and consent to participate
Not available.

Consent for publication
Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Competing interests
The authors declare no competing interests.

Author details
[1]Neuroscience Section, Dino Ferrari Centre, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Via Francesco Sforza 35, 20122 Milan, Italy. [2]Neurology Unit, Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy. [3]Ophthalmological Unit, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy. [4]Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy.

frontiers | Frontiers in Neurology

TYPE Case Report
PUBLISHED 02 June 2023
DOI 10.3389/fneur.2023.1170071

OPEN ACCESS

EDITED BY
Serdar Ceylaner,
Intergen Genetic Diagnosis and Research Center, Turkey

REVIEWED BY
Per Harald Jonson,
University of Helsinki, Finland
Afagh Alavi,
University of Social Welfare and Rehabilitation Sciences, Iran

*CORRESPONDENCE
Dario Ronchi
✉ dario.ronchi@unimi.it

RECEIVED 20 February 2023
ACCEPTED 16 May 2023
PUBLISHED 02 June 2023

CITATION
Velardo D, Antognozzi S, Rimoldi M, Pagliarani S, Cogiamanian F, Barbieri S, Corti S, Comi GP and Ronchi D (2023) Case report: Clinical and molecular characterization of two siblings affected by Brody myopathy. *Front. Neurol.* 14:1170071. doi: 10.3389/fneur.2023.1170071

Case report: Clinical and molecular characterization of two siblings affected by Brody myopathy

Daniele Velardo[1], Sara Antognozzi[2], Martina Rimoldi[3], Serena Pagliarani[2], Filippo Cogiamanian[4], Sergio Barbieri[4], Stefania Corti[2,3], Giacomo Pietro Comi[1,3] and Dario Ronchi[3]*

[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuromuscular and Rare Disease Unit, Milan, Italy, [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy, [3]Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy, [4]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurophysiology Unit, Milan, Italy

Exercise-induced muscle stiffness is the hallmark of Brody disease, an autosomal recessive myopathy due to biallelic pathogenic variants in *ATP2A1*, encoding the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase SERCA1. About 40 patients have been reported so far. Our knowledge about the natural history of this disorder, genotype–phenotype correlations and the effect of symptomatic treatment is partial. This results in incomplete recognition and underdiagnosis of the disease. Here, we report the clinical, instrumental, and molecular features of two siblings presenting childhood-onset exercise-induced muscle stiffness without pain. Both the probands display difficulty in climbing stairs and running, frequent falls, delayed muscle relaxation after exertion. Cold temperatures worsen these symptoms. No myotonic discharges were observed at electromyography. Whole Exome Sequencing analysis in the probands revealed the presence of two *ATP2A1* variants: the previously reported frameshift microdeletion c.2464delC and the likely pathogenic novel splice-site variant c.324+1G>A, whose detrimental effect was demonstrated in *ATP2A1* transcript analysis. The bi-allelic inheritance was verified by Sanger sequencing in the unaffected parents. This study expands the molecular defects associated with Brody myopathy.

KEYWORDS

Brody myopathy, SERCA1, *ATP2A1*, WES, neuromuscular disorder

1. Introduction

Brody Myopathy (BM, MIM # 601003) is a muscle disorder characterized by childhood onset exercise-induced progressive impairment of muscle relaxation, stiffness, cramps, and myalgia, predominantly in upper and lower limbs and face (eyelids). Symptoms generally improve after a few minutes of rest and may be exacerbated by cold. This disorder is recessively inherited and associated with pathogenic variants in the *ATP2A1* gene encoding for the Sarco(Endoplasmic) Reticulum Calcium ATPase protein SERCA1 (1–3).

SERCA1 catalyzes the ATP-dependent uptake of Ca^{2+} from the cytosol to sarcoplasmic reticulum taking part in the regulation of calcium levels in the sarcoplasmic reticulum and therefore muscle contraction (4, 5). In Brody myopathy patients, the activity of SERCA1 in type II muscle fibres is reduced, resulting in delayed muscle relaxation, silent cramps, muscle weakness and muscle atrophy. The reduction of SERCA1 activity has been documented in

Neuromuscular Disorders 33 (2023) 911–916

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Respiratory function in a large cohort of treatment-naïve adult spinal muscular atrophy patients: a cross-sectional study

Alex Vicino[a,b], Luca Bello[c], Silvia Bonanno[a], Alessandra Govoni[d,e], Federica Cerri[f], Manfredi Ferraro[g], Giuliana Capece[c], Giulio Gadaleta[g], Megi Meneri[d], Veria Vacchiano[h], Giulia Ricci[e], Eustachio D'Errico[i], Irene Tramacere[j], Paolo Banfi[k], Sara Bortolani[g], Riccardo Zanin[l], Maria Antonietta Maioli[m], Mauro Silvestrini[n,o], Stefano Carlo Previtali[f], Angela Berardinelli[p], Mara Turri[q], Michela Coccia[o], Renato Mantegazza[a], Rocco Liguori[h,r], Massimiliano Filosto[s,t], Gabriele Siciliano[e], Isabella Laura Simone[i,u], Tiziana Mongini[g], Giacomo Comi[d,v], Elena Pegoraro[c], Lorenzo Maggi[a,*]

[a] *Neuroimmunology and Neuromuscular Disease Unit, Foundation IRCCS Carlo Besta Neurological Institute,* Via *Celoria 11, Milano 20133, Italy*
[b] *Nerve-Muscle Unit, Neurology Service, Department of Clinical Neurosciences, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland*
[c] *Department of Neurosciences, University of Padua, Padova, Italy*
[d] *Neuromuscular and Rare Disease Unit, La Fondazione IRCCS Ca' Granda Ospedale Maggiore di Milano Policlinico, Milano, Italy*
[e] *Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy*
[f] *Division of Neuroscience, Department of Neurology & INSPE, San Raffaele Hospital, Milano, Italy*
[g] *Department of Neuroscience Rita Levi Montalcini, Università degli Studi di Torino, Torino, Italy*
[h] *IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC Clinica Neurologica, Bologna, Italy*
[i] *Neurology Unit, Azienda Ospedaliero-Universitaria, Policlinico of Bari, Bari, Italy*
[j] *Department of Research and Clinical Development, Scientific Directorate, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy*
[k] *Heart-Respiratory Rehabilitation Unit, IRCCS Fondazione Don Carlo Gnocchi, Milan, Italy*
[l] *Developmental Neurology, Foundation IRCCS Carlo Besta Neurological Institute, Milano, Italy*
[m] *Multiple Sclerosis Center, Ospedale Binaghi, Cagliari, Italy*
[n] *Department of Experimental and Clinical Medicine, Universita Politecnica delle Marche Facolta di Medicina e Chirurgia, Ancona, Italy*
[o] *Department of Neurological Sciences, AOU Ospedali Riuniti di Ancona, Ancona, Italy*
[p] *Department of Child Neuropsychiatry, Fondazione Istituto Neurologico Nazionale C Mondino Istituto di Ricovero e Cura a Carattere Scientifico, Pavia, Italy*
[q] *Department of Neurology/Stroke Unit, Bolzano Hospital, Bolzano, Italy*
[r] *Dipartimento di Scienze Biomediche e Neuromotorie, Universita degli Studi di Bologna, Bologna, Italy*
[s] *Department of Clinical and Experimental Sciences, University of Brescia, Italy*
[t] *NeMO-Brescia Clinical Center for Neuromuscular Diseases, Brescia, Italy*
[u] *School of Medicine, University of Bari "Aldo Moro" Bari, Bari, Italy*
[v] *Department of Pathophysiology and Transplantation (DEPT), Dino Ferrari Centre, University of Milan, Milano, Italy*

ARTICLE INFO

Article history:
Received 3 September 2023
Revised 3 October 2023
Accepted 9 October 2023

Keywords:
Spinal muscular atrophy
Spirometry
Respiratory function
FVC
Non-invasive ventilation
Outcome measure

ABSTRACT

Due to poor data in literature, we aimed to investigate the respiratory function in a large cohort of naïve Italian adult (≥18 years) SMA patients in a multi-centric cross-sectional study. The following respiratory parameters were considered: forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and need for non-invasive ventilation (NIV). We included 145 treatment-naïve adult patients (SMA2=18, SMA3=125; SMA4=2), 58 females (40 %), with median age at evaluation of 37 years (range 18–72). Fifty-six (37 %) and 41 (31 %) patients had abnormal (<80 %) values of FVC and FEV1, respectively. Fourteen (14 %) patients needed NIV, started at median age of 21 (range 4–68). Motor function, measured by Hammersmith Functional Motor Scale Expanded and Revised Upper Limb Module as well as SMA2, loss of walking ability, surgery for scoliosis, use of NIV, and cough assisting device (CAD) were all significantly associated to lower FVC and FEV1 values, while no association with age at baseline, disease duration, gender or 6 min walking test was observed, except for a correlation between FVC and age in SMA3 walkers ($p < 0.05$). In conclusion, respiratory function in adult SMA patients is relatively frequently impaired, substantially stable, and significantly correlated with motor function and disease severity.

* Corresponding author.
E-mail address: Lorenzo.maggi@istituto-besta.it (L. Maggi).

https://doi.org/10.1016/j.nmd.2023.10.002
0960-8966/

Cellular and Molecular Life Sciences (2023) 80:241
https://doi.org/10.1007/s00018-023-04885-7

Cellular and Molecular Life Sciences

ORIGINAL ARTICLE

Multi-omics profiling of CSF from spinal muscular atrophy type 3 patients after nusinersen treatment: a 2-year follow-up multicenter retrospective study

Irene Faravelli[1] · Delia Gagliardi[1,2] · Elena Abati[1,2] · Megi Meneri[1,2] · Jessica Ongaro[2] · Francesca Magri[2] · Valeria Parente[2] · Lucia Petrozzi[3] · Giulia Ricci[3] · Fiorenza Farè[4] · Giulia Garrone[4] · Manuela Fontana[4] · Donatella Caruso[4,5] · Gabriele Siciliano[3] · Giacomo Pietro Comi[1,2] · Alessandra Govoni[1] · Stefania Corti[1,2] · Linda Ottoboni[1]

Received: 2 April 2023 / Revised: 16 July 2023 / Accepted: 17 July 2023 / Published online: 5 August 2023

Abstract

Spinal muscular atrophy (SMA) is a neurodegenerative disorder caused by mutations in the *SMN1* gene resulting in reduced levels of the SMN protein. Nusinersen, the first antisense oligonucleotide (ASO) approved for SMA treatment, binds to the *SMN2* gene, paralogue to *SMN1*, and mediates the translation of a functional SMN protein. Here, we used longitudinal high-resolution mass spectrometry (MS) to assess both global proteome and metabolome in cerebrospinal fluid (CSF) from ten SMA type 3 patients, with the aim of identifying novel readouts of pharmacodynamic/response to treatment and predictive markers of treatment response. Patients had a median age of 33.5 [29.5; 38.25] years, and 80% of them were ambulant at time of the enrolment, with a median HFMSE score of 37.5 [25.75; 50.75]. Untargeted CSF proteome and metabolome were measured using high-resolution MS (nLC-HRMS) on CSF samples obtained before treatment (T0) and after 2 years of follow-up (T22). A total of 26 proteins were found to be differentially expressed between T0 and T22 upon VSN normalization and LIMMA differential analysis, accounting for paired replica. Notably, key markers of the insulin-growth factor signaling pathway were upregulated after treatment together with selective modulation of key transcription regulators. Using CombiROC multimarker signature analysis, we suggest that detecting a reduction of SEMA6A and an increase of COL1A2 and GRIA4 might reflect therapeutic efficacy of nusinersen. Longitudinal metabolome profiling, analyzed with paired *t*-Test, showed a significant shift for some aminoacid utilization induced by treatment, whereas other metabolites were largely unchanged. Together, these data suggest perturbation upon nusinersen treatment still sustained after 22 months of follow-up and confirm the utility of CSF multi-omic profiling as pharmacodynamic biomarker for SMA type 3. Nonetheless, validation studies are needed to confirm this evidence in a larger sample size and to further dissect combined markers of response to treatment.

Keywords Spinal muscular atrophy · Antisense oligonucleotides · Proteomic · Metabolomic

I. Faravelli, D. Gagliardi have equally contributed to this work.

✉ Irene Faravelli
irene.faravelli@unimi.it

✉ Stefania Corti
stefania.corti@unimi.it

✉ Linda Ottoboni
linda.ottoboni@unimi.it

[1] Department of Pathophysiology and Transplantation (DEPT), Dino Ferrari Centre, University of Milan, Milan, Italy

[2] Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[3] Department of Clinical and Experimental Medicine, Neurological Clinics, University of Pisa, Pisa, Italy

[4] Unitech OMICs, University of Milan, Milan, Italy

[5] Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy

Cellular and Molecular Life Sciences (2023) 80:373
https://doi.org/10.1007/s00018-023-05018-w

Cellular and Molecular Life Sciences

ORIGINAL ARTICLE

Combined RNA interference and gene replacement therapy targeting *MFN2* as proof of principle for the treatment of Charcot–Marie–Tooth type 2A

Federica Rizzo[1] · Silvia Bono[1] · Marc David Ruepp[3] · Sabrina Salani[1] · Linda Ottoboni[1] · Elena Abati[1] · Valentina Melzi[1] · Chiara Cordiglieri[4] · Serena Pagliarani[1] · Roberta De Gioia[1] · Alessia Anastasia[1] · Michela Taiana[1] · Manuela Garbellini[1] · Simona Lodato[5,6] · Paolo Kunderfranco[5,6] · Daniele Cazzato[7] · Daniele Cartelli[7] · Caterina Lonati[8] · Nereo Bresolin[1,2] · Giacomo Comi[1,2] · Monica Nizzardo[1] · Stefania Corti[2,9]

Received: 14 April 2023 / Revised: 23 October 2023 / Accepted: 26 October 2023 / Published online: 25 November 2023

Abstract

Mitofusin-2 (MFN2) is an outer mitochondrial membrane protein essential for mitochondrial networking in most cells. Autosomal dominant mutations in the *MFN2* gene cause Charcot–Marie–Tooth type 2A disease (CMT2A), a severe and disabling sensory-motor neuropathy that impacts the entire nervous system. Here, we propose a novel therapeutic strategy tailored to correcting the root genetic defect of CMT2A. Though mutant and wild-type *MFN2* mRNA are inhibited by RNA interference (RNAi), the wild-type protein is restored by overexpressing cDNA encoding functional *MFN2* modified to be resistant to RNAi. We tested this strategy in CMT2A patient-specific human induced pluripotent stem cell (iPSC)-differentiated motor neurons (MNs), demonstrating the correct silencing of endogenous *MFN2* and replacement with an exogenous copy of the functional wild-type gene. This approach significantly rescues the CMT2A MN phenotype in vitro, stabilizing the altered axonal mitochondrial distribution and correcting abnormal mitophagic processes. The *MFN2* molecular correction was also properly confirmed in vivo in the MitoCharc1 CMT2A transgenic mouse model after cerebrospinal fluid (CSF) delivery of the constructs into newborn mice using adeno-associated virus 9 (AAV9). Altogether, our data support the feasibility of a combined RNAi and gene therapy strategy for treating the broad spectrum of human diseases associated with *MFN2* mutations.

Keywords *MFN2* · RNA interfering · Gene therapy · Motor neuron · MitoCharc1 · CMT2A

Abbreviations

AD	Alzheimer's disease
PD	Parkinson's disease
SMA	spinal muscular atrophy
FDA	Food and Drug Administration or
EMA	European Medical Agency
ICV	intracerebroventricularly injection
MFN2	Mitofusin-2

Monica Nizzardo and Stefania Corti contributed equally.

✉ Stefania Corti
stefania.corti@unimi.it

[1] Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[2] Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Milan, Italy

[3] United Kingdom Dementia Research Institute Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, Maurice Wohl Clinical Neuroscience Institute, London, UK

[4] Istituto Di Genetica Molecolare "Romeo Ed Enrica Invernizzi", Milan, Italy

[5] Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, 20072 Milan, Italy

[6] IRCCS Humanitas Research Hospital, Rozzano, 20089 Milan, Italy

[7] Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

[8] Center for Preclinical Research, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Pace 9, 20100 Milan, Italy

[9] Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuromuscular and Rare Diseases Unit, Milan, Italy

Ageing Research Reviews 92 (2023) 102126

Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review Article

Genomic and transcriptomic advances in amyotrophic lateral sclerosis

Mafalda Rizzuti [a], Luca Sali [a], Valentina Melzi [a], Simone Scarcella [b], Gianluca Costamagna [b], Linda Ottoboni [b], Lorenzo Quetti [a], Lorenzo Brambilla [a], Dimitra Papadimitriou [c], Federico Verde [b,d], Antonia Ratti [d,e], Nicola Ticozzi [b,d], Giacomo Pietro Comi [a,b,f], Stefania Corti [a,b,*], Delia Gagliardi [b,*]

[a] Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[b] Department of Pathophysiology and Transplantation, Dino Ferrari Center, Università degli Studi di Milano, Milan, Italy
[c] Neurological Department, Henry Dunant Hospital Center, Athens, Greece
[d] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy
[e] Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy
[f] Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

ARTICLE INFO

Keywords:
ALS
Genomics
Epigenomics
Transcriptomics
MicroRNAs
Biomarkers

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder and the most common motor neuron disease. ALS shows substantial clinical and molecular heterogeneity. In vitro and in vivo models coupled with multiomic techniques have provided important contributions to unraveling the pathomechanisms underlying ALS. To date, despite promising results and accumulating knowledge, an effective treatment is still lacking. Here, we provide an overview of the literature on the use of genomics, epigenomics, transcriptomics and microRNAs to deeply investigate the molecular mechanisms developing and sustaining ALS. We report the most relevant genes implicated in ALS pathogenesis, discussing the use of different high-throughput sequencing techniques and the role of epigenomic modifications. Furthermore, we present transcriptomic studies discussing the most recent advances, from microarrays to bulk and single-cell RNA sequencing. Finally, we discuss the use of microRNAs as potential biomarkers and promising tools for molecular intervention. The integration of data from multiple omic approaches may provide new insights into pathogenic pathways in ALS by shedding light on diagnostic and prognostic biomarkers, helping to stratify patients into clinically relevant subgroups, revealing novel therapeutic targets and supporting the development of new effective therapies.

1. Introduction

Motor neuron diseases (MNDs) are a heterogeneous group of neurodegenerative disorders characterized by progressive loss of upper and lower motor neurons (MNs). Amyotrophic lateral sclerosis (ALS) is the most common MND, and affected patients may present with different clinical phenotypes associated with variable disease progression and prognosis. The broad molecular background and pathophysiological heterogeneity of ALS may contribute to the variety of clinical phenotypes. The pathological mechanisms underlying the disease include oxidative stress, inflammation, mitochondrial dysfunction, nucleocytoplasmic transport impairment, axonal transport defects and alterations in RNA processing (Goutman et al., 2022).

The analysis of clinical data and biological samples using a high-throughput approach may allow a more precise stratification of disease subtypes beyond improving both biomarker discovery and personalized treatment development. Multiomic approaches can quantify and integrate enormous amounts of data obtained from large samples in a forward-looking perspective toward the identification of new potential molecular pathways associated with the disease. Specifically, multiomic studies may be performed on human biological samples or on in vitro and in vivo ALS models.

In the Answer ALS (AALS) program, demographic and clinical data from ALS patients and multiomic data from patient-derived induced pluripotent stem cells (iPSCs) and MNs are being collected with the aim of building an open source of integrated clinical and biological records on ALS (Baxi et al., 2022). This program represents a robust and high-powered tool to extract and analyze data from biologically relevant

* Corresponding authors.
E-mail addresses: stefania.corti@unimi.it (S. Corti), delia.gagliardi@unimi.it (D. Gagliardi).

https://doi.org/10.1016/j.arr.2023.102126
Received 1 June 2023; Received in revised form 9 November 2023; Accepted 10 November 2023
Available online 14 November 2023

current issues in molecular biology

MDPI

Article

Distribution of Exonic Variants in Glycogen Synthesis and Catabolism Genes in Late Onset Pompe Disease (LOPD)

Paola De Filippi [1], Edoardo Errichiello [1,2], Antonio Toscano [3], Tiziana Mongini [4], Maurizio Moggio [5], Sabrina Ravaglia [1], Massimiliano Filosto [6], Serenella Servidei [7], Olimpia Musumeci [8], Fabio Giannini [9], Alberto Piperno [10], Gabriele Siciliano [11], Giulia Ricci [11], Antonio Di Muzio [12], Miriam Rigoldi [13], Paola Tonin [14], Michele Giovanni Croce [1], Elena Pegoraro [15], Luisa Politano [16], Lorenzo Maggi [17], Roberta Telese [12], Alberto Lerario [5], Cristina Sancricca [7], Liliana Vercelli [4], Claudio Semplicini [15], Barbara Pasanisi [16], Bruno Bembi [18], Andrea Dardis [18], Ilaria Palmieri [1,2], Cristina Cereda [19], Enza Maria Valente [1,2] and Cesare Danesino [2,*]

1 IRCCS Mondino Foundation, 27100 Pavia, Italy
2 Department of Molecular Medicine, University of Pavia, 27100 Pavia, Italy
3 ERN-NMD Center of Messina for Neuromuscular Disorders, Department of Clinical and Experimental Medicine, University of Messina, 98125 Messina, Italy
4 Neuromuscular Unit, Department of Neuroscience RLM, University of Torino, 10126 Torino, Italy
5 Neuromuscular and Rare Diseases Unit, BioBank of Skeletal Muscle, Peripheral Nerve, DNA and Dino Ferrari Center, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, 20100 Milan, Italy
6 Department of Clinical and Experimental Sciences, NeMO-Brescia Clinical Center for Neuromuscular Diseases, University of Brescia, 25121 Brescia, Italy
7 Department of Neuroscience, Catholic University, 00100 Rome, Italy
8 Department of Clinical and Experimental Medicine, University of Messina, 98125 Messina, Italy
9 Department of Medical, Surgical and Neurological Sciences, University of Siena, "Le Scotte" Hospital, 53100 Siena, Italy
10 Fondazione IRCCS San Gerardo, Centro Ricerca Testamenti, Monza-European Reference Network–MetabERN, 20900 Monza, Italy
11 Department of Clinical and Experimental Medicine, Neurological Clinics, University of Pisa, 56100 Pisa, Italy
12 Centre for Neuromuscular Disease, CeSI, University "G. d'Annunzio", 66100 Chieti, Italy
13 Dipartimento di Ricerca Malattie Rare, Istituto Mario Negri IRCCS, 24020 Ranica, Italy
14 Department of Neurosciences, Biomedicine and Movement Sciences, Section of Clinical Neurology, University of Verona, 37100 Verona, Italy
15 Department of Neurosciences, University of Padova, 35100 Padova, Italy
16 Cardiomiologia e Genetica Medica, Dipartimento di Medicina Sperimentale, Seconda Università di Napoli, 80100 Napoli, Italy
17 Neuroimmunology and Neuromuscular Diseases Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20100 Milano, Italy
18 Regional Coordinator Centre for Rare Diseases, University Hospital "Santa Maria della Misericordia", 33100 Udine, Italy
19 Center of Functional Genomic and Rare Diseases-Buzzi Children's Hospital, 20100 Milano, Italy
* Correspondence: cidi@unipv.it

check for updates

Citation: De Filippi, P.; Errichiello, E.; Toscano, A.; Mongini, T.; Moggio, M.; Ravaglia, S.; Filosto, M.; Servidei, S.; Musumeci, O.; Giannini, F.; et al. Distribution of Exonic Variants in Glycogen Synthesis and Catabolism Genes in Late Onset Pompe Disease (LOPD). *Curr. Issues Mol. Biol.* **2023**, *45*, 2847–2860. https://doi.org/10.3390/cimb45040186

Academic Editor: Grzegorz Wegrzyn

Received: 2 March 2023
Revised: 20 March 2023
Accepted: 22 March 2023
Published: 1 April 2023

Abstract: Pompe disease (PD) is a monogenic autosomal recessive disorder caused by biallelic pathogenic variants of the *GAA* gene encoding lysosomal alpha-glucosidase; its loss causes glycogen storage in lysosomes, mainly in the muscular tissue. The genotype–phenotype correlation has been extensively discussed, and caution is recommended when interpreting the clinical significance of any mutation in a single patient. As there is no evidence that environmental factors can modulate the phenotype, the observed clinical variability in PD suggests that genetic variants other than pathogenic GAA mutations influence the mechanisms of muscle damage/repair and the overall clinical picture. Genes encoding proteins involved in glycogen synthesis and catabolism may represent excellent candidates as phenotypic modifiers of PD. The genes analyzed for glycogen synthesis included *UGP2*, glycogenin (*GYG1*-muscle, *GYG2*, and other tissues), glycogen synthase (*GYS1*-muscle and *GYS2*-liver), *GBE1*, *EPM2A*, *NHLRC1*, *GSK3A*, and *GSK3B*. The only enzyme involved in glycogen catabolism in lysosomes is α-glucosidase, which is encoded by *GAA*, while two cytoplasmic enzymes, phosphorylase (*PYGB*-brain, *PGL*-liver, and *PYGM*-muscle) and glycogen debranching (*AGL*) are

diseases

MDPI

Case Report

Unraveling the Neurological Complexity of Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal Protein, and Skin Changes Syndrome: A Report of a Challenging Case of a Young Woman and Cutting-Edge Advancements in the Field

Gioconda Furciniti [1], Giuseppe Casalino [2], Francesco M. Lo Russo [3], Niccolò Bolli [4,5], Megi Meneri [1,6], Giacomo P. Comi [1,6], Stefania P. Corti [1,7,*,†] and Daniele Velardo [6,*,†]

1 Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, 20122 Milano, Italy; gioconda.furciniti@unimi.it (G.F.); megi.meneri@unimi.it (M.M.); giacomo.comi@unimi.it (G.P.C.)
2 Eye Clinic, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milano, Italy; giuseppe.casalino@policlinico.mi.it
3 Neuroradiology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milano, Italy; francesco.lorusso@policlinico.mi.it
4 Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milano, Italy; niccolo.bolli@unimi.it
5 Department of Oncology and Onco-Hematology, Università degli Studi di Milano, 20122 Milano, Italy
6 Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milano, Italy
7 Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milano, Italy
* Correspondence: stefania.corti@unimi.it (S.P.C.); daniele.velardo@policlinico.mi.it (D.V.)
† These authors contributed equally to this work.

Abstract: POEMS syndrome—characterized by polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes—is an uncommon and complex paraneoplastic disorder encompassing a diverse array of symptoms. Here we report the challenging case of a 34-year-old female who sought medical attention at the emergency department due to distal lower limb weakness. She was breastfeeding her first child at that time. Her condition rapidly deteriorated, making it difficult for her to perform simple tasks independently. Initially, she struggled with activities like jumping or climbing stairs. Eventually, her ability to walk was also compromised. These symptoms underscored the swift evolution of her polyneuropathy. Nerve conduction studies and electromyography confirmed a diagnosis of mixed demyelinating and axonal polyneuropathy. Subsequent investigations, including bone marrow biopsy and immunochemistry testing, revealed a plasma cell disorder characterized by lambda monoclonal gammopathy, along with elevated levels of vascular endothelial growth factor (VEGF > 8000 pg/mL). This pivotal finding led to the diagnosis of POEMS syndrome, prompting the initiation of antineoplastic therapy (daratumumab-lenalidomide-dexamethasone) to manage this condition. An autologous cell transplantation was planned. The rarity of POEMS syndrome and its diverse clinical manifestations often lead to an incorrect or delayed diagnosis. Our case underscores the importance of considering this syndrome in patients presenting with acute or subacute polyneuropathy, even if the patients are young. In conclusion, this case elucidates the diagnostic complexities of POEMS syndrome, emphasizing the integral role of comprehensive multidisciplinary evaluations and the potential influence of increased VEGF as a diagnostic key element and possible therapeutic target.

Keywords: POEMS syndrome; polyneuropathy; endocrinopathy; M-protein; VEGF

Citation: Furciniti, G.; Casalino, G.; Lo Russo, F.M.; Bolli, N.; Meneri, M.; Comi, G.P.; Corti, S.P.; Velardo, D. Unraveling the Neurological Complexity of Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal Protein, and Skin Changes Syndrome: A Report of a Challenging Case of a Young Woman and Cutting-Edge Advancements in the Field. *Diseases* **2023**, *11*, 167. https://doi.org/10.3390/diseases11040167

Academic Editor: Maurizio Battino

Received: 30 October 2023
Revised: 1 November 2023
Accepted: 2 November 2023
Published: 10 November 2023

International Journal of
Molecular Sciences

MDPI

Article

The Profiling of 179 miRNA Expression in Serum from Limb Girdle Muscular Dystrophy Patients and Healthy Controls

Francesca Magri [1], Laura Napoli [2], Michela Ripolone [2], Patrizia Ciscato [2], Maurizio Moggio [2], Stefania Corti [2,3], Giacomo Pietro Comi [1,3], Monica Sciacco [2] and Simona Zanotti [2,*]

[1] Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[2] Neuromuscular and Rare Disease Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy; maurizio.moggio@policlinico.mi.it (M.M.)
[3] Dino Ferrari Centre, Department of Pathophysiology and Transplantation (DEPT), University of Milan, 20122 Milan, Italy
* Correspondence: simona.zanotti@policlinico.mi.it; Tel.: +39-0255-036-504

Abstract: Limb girdle muscular dystrophies (LGMDs) are a group of genetically inherited neuromuscular diseases with a very variable clinical presentation and overlapping traits. Over the last few years there has been an increasing interest in the use of non-invasive circulating biomarkers to monitor disease progression and to evaluate the efficacy of therapeutic approaches. Our aim was to identify the miRNA signature with potential value for LGMD patient screening and stratification. Using miRCURY LNA miRNA qPCR Serum/Plasma Panel, we analyzed 179 miRNAs from 16 patients, divided in four pools based on their genetic diagnosis, and from healthy controls. The miRNAs analysis showed a total of 107 dysregulated miRNAs in LGMD patients when compared to the healthy controls. After filtering via skeletal tissue expression and gene/pathways target analysis, the number of dysregulated miRNAs drastically reduced. Six selected miRNAs—let-7f-5p (in LGMDR1), miR-20a-5p (in LGMDR2), miR-130b-5p, miR-378a-5p (both in LGMDR3), miR-376c-3p and miR-382-5p (both in LGMDR4)—whose expression was significantly lower compared to controls in the different LGMD pools, were further investigated. The bioinformatic analysis of the target genes in each selected miRNA revealed ECM–receptor interaction and TGF-beta signaling as the most involved pathways. The correlation analysis showed a good correlation of let-7f-5p with fibrosis and with the cross sectional area of type I and type II fibers, while miR-130b-5p showed a good correlation with the age of onset of the disease. The receiver operating characteristic curves showed how single miRNAs were able to discriminate a specific group of LGMD patients and how the combination of six miRNAs was able to discriminate LGMD patients from controls.

Keywords: limb girdle muscle dystrophy; miRNAs; fibrosis; inflammation; atrophy; biomarkers

Citation: Magri, F.; Napoli, L.; Ripolone, M.; Ciscato, P.; Moggio, M.; Corti, S.; Comi, G.P.; Sciacco, M.; Zanotti, S. The Profiling of 179 miRNA Expression in Serum from Limb Girdle Muscular Dystrophy Patients and Healthy Controls. *Int. J. Mol. Sci.* **2023**, *24*, 17402. https://doi.org/10.3390/ijms242417402

Academic Editor: Toshifumi Yokota

Received: 24 October 2023
Revised: 5 December 2023
Accepted: 7 December 2023
Published: 12 December 2023

1. Introduction

Limb girdle muscular dystrophies (LGMDs) are a group of genetically inherited neuromuscular diseases. The most recent classification encompasses 31 genetically transmitted LGMD variants including autosomal dominant, autosomal recessive and X-linked forms. The most common are calpainopathies, dysferlinopathies, sarcoglycanopathies, dystroglycanopathies and anoctaminopathies [1]. The clinical presentation is quite variable in accordance with each disorder's main features, i.e., the groups of primarily affected muscles, the degree of weakness, and the age of onset and progression rate. Though the diagnostic process is well defined for most of these pathologies, some aspects, including differences in the age of onset or in the disease progression, are still poorly defined.

Calpainopathy (LGMDR1) is caused by pathogenic variants in the *CAPN3* gene codifying for a non-lysosomal calcium-dependent cysteine protease. The primary symptom is a progressive worsening of muscle weakness of the hip and shoulder muscles, enlarged calf muscles, shortening and hardening of muscles leading to contractures, scoliosis, and

Journal of *Personalized Medicine*

MDPI

Case Report

MERRF Mutation A8344G in a Four-Generation Family without Central Nervous System Involvement: Clinical and Molecular Characterization

Michela Ripolone [1,†], Simona Zanotti [1,†], Laura Napoli [1], Dario Ronchi [2], Patrizia Ciscato [1], Giacomo Pietro Comi [1,2], Maurizio Moggio [1] and Monica Sciacco [1,*]

[1] Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[2] Dino Ferrari Center, Department of Pathophysiology and Transplantation (DEPT), University of Milan, 20122 Milan, Italy
* Correspondence: monica.sciacco@policlinico.mi.it; Tel.: +39-0255-036-504
† These authors contributed equally to this work.

Abstract: A 53-year-old man approached our Neuromuscular Unit following an incidental finding of hyperckemia. Similar to his mother who had died at the age of 77 years, he was diabetic and had a few lipomas. The patient's two sisters, aged 60 and 50 years, did not have any neurological symptoms. Proband's skeletal muscle biopsy showed several COX-negative fibers, many of which were "ragged red". Genetic analysis revealed the presence of the A8344G mtDNA mutation, which is most commonly associated with a maternally inherited multisystem mitochondrial disorder known as MERRF (myoclonus epilepsy with ragged-red fibers). The two sisters also carry the mutation. Family members on the maternal side were reported healthy. Although atypical phenotypes have been reported in association with the A8344G mutation, central nervous system (CSN) manifestations other than myoclonic epilepsy are always reported in the family tree. If present, our four-generation family manifestations are late-onset and do not affect CNS. This could be explained by the fact that the mutational load remains low and therefore prevents tissues/organs from reaching the pathologic threshold. The fact that this occurs throughout generations and that CNS, which has the highest energetic demand, is clinically spared, suggests that regulatory genes and/or pathways affect mitochondrial segregation and replication, and protect organs from progressive dysfunction.

Keywords: MERRF; lipoma; central nervous system

Citation: Ripolone, M.; Zanotti, S.; Napoli, L.; Ronchi, D.; Ciscato, P.; Comi, G.P.; Moggio, M.; Sciacco, M. MERRF Mutation A8344G in a Four-Generation Family without Central Nervous System Involvement: Clinical and Molecular Characterization. *J. Pers. Med.* **2023**, *13*, 147. https://doi.org/10.3390/jpm13010147

Academic Editors: Svetlana Viktorovna Demyanenko and Denis Silachev

Received: 9 December 2022
Revised: 10 January 2023
Accepted: 10 January 2023
Published: 11 January 2023

1. Introduction

The A-to-G transition at nucleotide 8344 (m.8344A > G) of mtDNA is the prevalent mutation found in a multisystem disorder, and is known with the acronym MERRF (myoclonus epilepsy with ragged-red fibers). It is characterized by myoclonus, generalized epilepsy, ataxia, weakness, dementia as well as signs of multisystem involvement [1–3]. The histopathological study of the skeletal muscle tissue typically shows ragged-red fibers (RRFs) with the modified Gomori trichrome (MGT) stain and hyperactive fibers with the succinate dehydrogenase (SDH) stain. Histochemical reaction for cytochrome c oxidase (COX) shows lack of activity in RRFs and some non-RRFs [4–6]. Occasionally, RRFs may not be observed [7]. The presence of lipomas has often been reported in patients affected with MERRF and/or in their maternally-related family members [8–10].

Moreover, the m.8344A > G variant has been reported in association with isolated myopathy, lipomatosis with muscle lipid storage, or Leigh syndrome [11–13]. Other unusual manifestations include sudden infant death syndrome [14], spasmodic dysphonia [15], Parkinsonism with neuropathy and myopathy [16], infantile-onset ataxia, myoclonus and bilateral putaminal necrosis on brain MRI [17], sudden respiratory failure in adulthood [18], acute central and peripheral nervous system demyelinating disease [19], and

AMERICAN JOURNAL OF PHYSIOLOGY
CELL PHYSIOLOGY

Am J Physiol Cell Physiol 324: C769–C776, 2023.
First published February 6, 2023; doi:10.1152/ajpcell.00002.2023

RAPID REPORT

Muscle Wasting: Cellular and Molecular Mechanisms

Myosin post-translational modifications and function in the presence of myopathy-linked truncating *MYH2* mutations

Alexander Sonne,[1] Lorenzo Peverelli,[2] Aurelio Hernandez-Lain,[3,4] Cristina Domínguez-González,[4,5] Jesper L. Andersen,[6,7] Margherita Milone,[8] Alan H. Beggs,[9] and Julien Ochala[1,10]

[1]*Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark;* [2]*Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Fondazione, IRCCS Ca' Granda Ospedale Maggiore, Policlinico, Milan, Italy;* [3]*Neuropathology Unit, Department of Pathology, 12 de Octubre University Hospital, Madrid, Spain;* [4]*imas12 Research Institute, Rare Diseases Network Biomedical Research Center (CIBERER), 12 de Octubre University Hospital, Madrid, Spain;* [5]*Neuromuscular Unit, Department of Neurology, 12 de Octubre University Hospital, Madrid, Spain;* [6]*Department of Orthopaedic Surgery, Institute of Sports Medicine Copenhagen, University Copenhagen Hospital-Bispebjerg and Frederiksberg, Copenhagen, Denmark;* [7]*Center for Healthy Aging, Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark;* [8]*Department of Neurology, Mayo Clinic, Rochester, Minnesota, United States;* [9]*Division of Genetics and Genomics, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, United States; and* [10]*Centre for Human and Applied Physiological Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom*

Abstract

Congenital myopathies are a vast group of genetic muscle diseases. Among the causes are mutations in the *MYH2* gene resulting in truncated type IIa myosin heavy chains (MyHCs). The precise cellular and molecular mechanisms by which these mutations induce skeletal muscle symptoms remain obscure. Hence, in the present study, we aimed to explore whether such genetic defects would alter the presence as well as the post-translational modifications of MyHCs and the functionality of myosin molecules. For this, we dissected muscle fibers from four myopathic patients with *MYH2* truncating mutations and from five human healthy controls. We then assessed *1*) MyHCs presence/post-translational modifications using LC/MS; *2*) relaxed myosin conformation and concomitant ATP consumption with a loaded Mant-ATP chase setup; *3*) myosin activation with an unloaded in vitro motility assay; and *4*) cellular force production with a myofiber mechanical setup. Interestingly, the type IIa MyHC with one additional acetylated lysine (Lys35-Ac) was present in the patients. This was accompanied by *1*) a higher ATP demand of myosin heads in the disordered-relaxed conformation; *2*) faster actomyosin kinetics; and *3*) reduced muscle fiber force. Overall, our findings indicate that *MYH2* truncating mutations impact myosin presence/functionality in human adult mature myofibers by disrupting the ATPase activity and actomyosin complex. These are likely important molecular pathological disturbances leading to the myopathic phenotype in patients.

congenital myopathy; myosin; skeletal muscle

INTRODUCTION

Congenital myopathies are a heterogeneous group of muscle disorders with varying ages of onset and clinical symptoms (1). A fraction of these genetic diseases is caused by mutations in genes encoding myosin heavy chains (mainly *MYH7* and *MYH2* with an estimated prevalence of 1:26,000) (2). The well-studied *MYH7* mutations have been associated with either hypertrophic, dilated cardiomyopathy and/or skeletal myopathies whereas the under-appreciated *MYH2* mutations have solely been related to skeletal myopathies, often characterized by late-onset and progressive proximal limb muscle weakness as well as ophthalmoplegia (2). *MYH7* mutations are typically dominant missense variants that change just one amino acid in the β/slow myosin heavy chain (MyHC) protein whereas *MYH2* mutations are more diverse with notably the presence of truncated type IIa MyHC molecules in muscles from the patients due to biallelic recessive variants often predicted to cause loss of function (2). The mechanisms by which these *MYH2* truncating mutations lead to congenital myopathies remain totally unexplored. Truncating mutations in *MYH2* usually induce

Correspondence: J. Ochala (julien.ochala@sund.ku.dk).
Submitted 3 January 2023 / Revised 27 January 2023 / Accepted 28 January 2023

we used the patients' leftover tissue and a technique named unloaded in vitro motility assay. We observed a relatively high level of nonmobile actin filaments in both patients and controls (Fig. 3*A*) that may relate to the flash freezing procedure known to increase the number of dead myosin motors. Nevertheless, we also noticed that the motility speed from patients with *MYH2* truncating mutations was significantly faster than controls (Fig. 3*B*). To get insights into the potential consequences of such increased actin sliding velocity, we attempted to measure the force production of isolated membrane-permeabilized muscle fibers. This procedure turned to be very difficult as patients' myofibers were unusually fragile and broke at *p*Ca 4.5. We only succeeded to do so for one of the four patients. A total of 55 muscle fibers were included in the analysis (46 muscle fibers from three controls and 9 from one patient). The force produced by myofibers expressing the type II MyHC was significantly lower in the patient than in the controls (Fig. 3*C*).

Opposite observations have been made for one dominant *MYH2* missense mutation (with a presumed gain of function) where there is one amino acid replacement (position 706) from a negatively charged glutamic acid to a positively charged lysine in the myosin converter domain (close to the head region) (30). In this study, even though they report a high number of nonmoving filaments and dead myosin heads, the motility speed was significantly reduced in the patients (30). Our opposite increase in the motility speed of actin filaments (where the mutations are presumably loss of function) is unlikely to be caused by the fiber-type disproportion observed in patients or by the higher variability in the amount of moving filaments, which would theoretically induce a slower motility speed. Other underlying mechanisms are likely to play a role.

There is a close relationship between the speed at which actin filaments move and the enzymatic properties of myosin molecules (31, 32). Hence, it is plausible that the actual mutation or/and Lys35-Ac promotes the dissociation of myosin heads from actin filaments; thereby increasing the cross-bridge detachment rate. This would then suggest a shortened cross-bridge duty cycle, i.e., a shorter time spent by individual myosin heads in a strong-binding state, resulting in a decreased myofiber force. This would explain, at the molecular level, why patients suffer from limb muscle weakness (2).

Conclusions

Importantly, here, we found the type IIa MyHC isoform to be present and one acetylated lysine (Lys35-Ac) to be significantly increased in the patients with *MYH2* truncating mutations. In parallel, we also observed that the ATP consumption of myosin molecules in the disordered-relaxed conformation was significantly increased as was the speed at which myosin molecules moved actin filaments. Altogether, these results indicate a myosin dysfunctional remodeling likely contributing to the cellular force depression and the myopathic phenotype of the patients at the molecular level.

DATA AVAILABILITY

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (25) with the data set identifier PXD039178. Data analyzed are also presented here in Figs. 1, 2, and 3.

ACKNOWLEDGMENTS

Mass spectrometry analyses were performed by the Proteomics Research Infrastructure (PRI) at the University of Copenhagen (UCPH), supported by the Novo Nordisk Foundation (Grant agreement number NNF19SA0059305). Some of the patients' samples came from the Centro Dino Ferrari and Biobank of skeletal muscle, peripheral nerve, DNA, and cell cultures, member of the Telethon Network of Genetic Biobanks and the Eurobiobank Network.

GRANTS

This work was generously funded by the Novo Nordisk Foundation Grant NNF0070539 and Carlsberg Foundation Grant CF20-0113 (to J. Ochala).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.S., L.P., A.H.-L., C.D., J.L.A., M.M., A.H.B., and J.O. conceived and designed research; A.S., L.P., A.H.-L., C.D., J.L.A., M.M., A.H.B., and J.O. performed experiments; A.S., J.L.A., and J.O. analyzed data; A.S., J.L.A., and J.O. interpreted results of experiments; A.S. and J.O. prepared figures; A.S. and J.O. drafted manuscript; A.S., L.P., A.H.-L., C.D., J.L.A., M.M., A.H.B., and J.O. edited and revised manuscript; A.S., L.P., A.H.-L., C.D., J.L.A., M.M., A.H.B., and J.O. approved final version of manuscript.

REFERENCES

1. **Jungbluth H**, **Treves S**, **Zorzato F**, **Sarkozy A**, **Ochala J**, **Sewry C**, **Phadke R**, **Gautel M**, **Muntoni F.** Congenital myopathies: disorders of excitation-contraction coupling and muscle contraction. *Nat Rev Neurol* 14: 151–167, 2018. doi:10.1038/nrneurol.2017.191.
2. **Tajsharghi H**, **Oldfors A.** Myosinopathies: pathology and mechanisms. *Acta Neuropathol* 125: 3–18, 2013. doi:10.1007/s00401-012-1024-2.
3. **Lossos A**, **Oldfors A**, **Fellig Y**, **Meiner V**, **Argov Z**, **Tajsharghi H.** MYH2 mutation in recessive myopathy with external ophthalmoplegia linked to chromosome 17p13.1-p12. *Brain* 136: e238, 2013. doi:10.1093/brain/aws365.
4. **Tajsharghi H**, **Hammans S**, **Lindberg C**, **Lossos A**, **Clarke NF**, **Mazanti I**, **Waddell LB**, **Fellig Y**, **Foulds N**, **Katifi H**, **Webster R**, **Raheem O**, **Udd B**, **Argov Z**, **Oldfors A.** Recessive myosin myopathy with external ophthalmoplegia associated with MYH2 mutations. *Eur J Hum Genet* 22: 801–808, 2014. doi:10.1038/ejhg.2013.250.
5. **Willis T**, **Hedberg-Oldfors C**, **Alhaswani Z**, **Kulshrestha R**, **Sewry C**, **Oldfors A.** A novel MYH2 mutation in family members presenting with congenital myopathy, ophthalmoplegia and facial weakness. *J Neurol* 263: 1427–1433, 2016. doi:10.1007/s00415-016-8154-8.
6. **Vanhooren V**, **Navarrete Santos A**, **Voutetakis K**, **Petropoulos I**, **Libert C**, **Simm A**, **Gonos ES**, **Friguet B.** Protein modification and maintenance systems as biomarkers of ageing. *Mech Ageing Dev* 151: 71–84, 2015. doi:10.1016/j.mad.2015.03.009.
7. **Smith K**, **Rennie MJ.** The measurement of tissue protein turnover. *Baillieres Clin Endocrinol Metab* 10: 469–495, 1996. doi:10.1016/s0950-351x(96)80651-3.
8. **Gordon AM**, **Homsher E**, **Regnier M.** Regulation of contraction in striated muscle. *Physiol Rev* 80: 853–924, 2000. doi:10.1152/physrev.2000.80.2.853.
9. **Hooijman P**, **Stewart MA**, **Cooke R.** A new state of cardiac myosin with very slow ATP turnover: a potential cardioprotective mechanism

cells

MDPI

Article

Characterization of Skeletal Muscle Biopsy and Derived Myoblasts in a Patient Carrying Arg14del Mutation in *Phospholamban* Gene

Simona Zanotti [1], Michela Ripolone [1], Laura Napoli [1], Daniele Velardo [1], Sabrina Salani [2], Patrizia Ciscato [1], Silvia Priori [3,4,5], Deni Kukavica [3,4,5], Andrea Mazzanti [3,4,5], Luca Diamanti [6], Elisa Vegezzi [7,8], Maurizio Moggio [1], Stefania Corti [2,9], Giacomo Comi [1,9] and Monica Sciacco [1,*]

1 Neuromuscular and Rare Disease Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy
2 Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
3 Department of Molecular Medicine, University of Pavia, 27100 Pavia, Italy
4 Department of Molecular Cardiology, IRCCS ICS Maugeri, 27100 Pavia, Italy
5 Laboratory of Molecular Cardiology, Centro Nacional de Investigaciones Cardiovasculares Carlos III, 28029 Madrid, Spain
6 Neuroncology Unit, IRCCS Mondino Foundation, 27100 Pavia, Italy
7 Department of Brain and Behavioral Sciences, University of Pavia, 27100 Pavia, Italy
8 IRCCS Mondino Foundation, 27100 Pavia, Italy
9 Dino Ferrari Centre, Department of Pathophysiology and Transplantation (DEPT), University of Milan, 20122 Milan, Italy
* Correspondence: monica.sciacco@policlinico.mi.it; Tel.: +39-0255-036-504

Abstract: Phospholamban is involved in the regulation of the activity and storage of calcium in cardiac muscle. Several mutations have been identified in the *PLN* gene causing cardiac disease associated with arrhythmogenic and dilated cardiomyopathy. The patho-mechanism underlying *PLN* mutations is not fully understood and a specific therapy is not yet available. *PLN* mutated patients have been deeply investigated in cardiac muscle, but very little is known about the effect of *PLN* mutations in skeletal muscle. In this study, we investigated both histological and functional features in skeletal muscle tissue and muscle-derived myoblasts from an Italian patient carrying the *Arg14del* mutation in *PLN*. The patient has a cardiac phenotype, but he also reported lower limb fatigability, cramps and fasciculations. The evaluation of a skeletal muscle biopsy showed histological, immunohistochemical and ultrastructural alterations. In particular, we detected an increase in the number of centronucleated fibers and a reduction in the fiber cross sectional area, an alteration in p62, LC3 and VCP proteins and the formation of perinuclear aggresomes. Furthermore, the patient's myoblasts showed a greater propensity to form aggresomes, even more marked after proteasome inhibition compared with control cells. Further genetic and functional studies are necessary to understand whether a definition of PLN myopathy, or cardiomyopathy *plus*, can be introduced for selected cases with clinical evidence of skeletal muscle involvement. Including skeletal muscle examination in the diagnostic process of *PLN*-mutated patients can help clarify this issue.

Keywords: phospholamban; Arg14del; skeletal muscle; aggresomes

check for updates

Citation: Zanotti, S.; Ripolone, M.; Napoli, L.; Velardo, D.; Salani, S.; Ciscato, P.; Priori, S.; Kukavica, D.; Mazzanti, A.; Diamanti, L.; et al. Characterization of Skeletal Muscle Biopsy and Derived Myoblasts in a Patient Carrying Arg14del Mutation in *Phospholamban* Gene. *Cells* **2023**, *12*, 1405. https://doi.org/10.3390/cells12101405

Academic Editors: Prabhakara Nagareddy, Hua Zhu and Mona M. El-Refaey

Received: 16 March 2023
Revised: 10 May 2023
Accepted: 15 May 2023
Published: 17 May 2023

1. Introduction

Phospholamban (PLN) and sarcolipin (SLN) are small proteins localized in the membrane of the sarcoplasmic and endoplasmic reticula. They belong to the "regulin family", along with myoregulin (MLN), dwarf open reading frame (DWORF), endoregulin (ELN) and another-regulin (ALN). Regulins are involved in the regulation of calcium signaling and in the activity of the sarcoplasmic reticulum Ca^{+2}-ATPase pumps, SERCA1a and SERCA2a [1]. PLN is mainly expressed in cardiac, slow-twitch skeletal muscle and smooth

International Journal of
Molecular Sciences

MDPI

Article

Extracellular Matrix Disorganization and Sarcolemmal Alterations in COL6-Related Myopathy Patients with New Variants of COL6 Genes

Simona Zanotti [1], Francesca Magri [2], Sabrina Salani [2], Laura Napoli [1], Michela Ripolone [1], Dario Ronchi [3], Francesco Fortunato [3], Patrizia Ciscato [1], Daniele Velardo [1], Maria Grazia D'Angelo [4], Francesca Gualandi [5], Vincenzo Nigro [6], Monica Sciacco [1,2], Stefania Corti [2,3], Giacomo Pietro Comi [1,3] and Daniela Piga [2,*]

[1] Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[2] Neurology Unit, Department of Neuroscience Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[3] Dino Ferrari Centre, Department of Pathophysiology and Transplantation (DEPT), University of Milan, 20122 Milan, Italy
[4] IRCCS Eugenio Medea, Bosisio Parini, 23842 Lecco, Italy
[5] Medical Genetics Unit, Department of Medical Science, University of Ferrara, 44121 Ferrara, Italy
[6] Dipartimento di Medicina di Precisione, "Luigi Vanvitelli" University of Campania and Telethon Institute of Genetics and Medicine (TIGEM), 81100 Naples, Italy
* Correspondence: daniela.piga@policlinico.mi.it; Tel.: +39-02-5503-3843

Abstract: Collagen VI is a heterotrimeric protein expressed in several tissues and involved in the maintenance of cell integrity. It localizes at the cell surface, creating a microfilamentous network that links the cytoskeleton to the extracellular matrix. The heterotrimer consists of three chains encoded by *COL6A1*, *COL6A2* and *COL6A3* genes. Recessive and dominant molecular defects cause two main disorders, the severe Ullrich congenital muscular dystrophy and the relatively mild and slowly progressive Bethlem myopathy. We analyzed the clinical aspects, pathological features and mutational spectrum of 15 COL6-mutated patients belonging to our cohort of muscular dystrophy probands. Patients presented a heterogeneous phenotype ranging from severe forms to mild adult-onset presentations. Molecular analysis by NGS detected 14 different pathogenic variants, three of them so far unreported. Two changes, localized in the triple-helical domain of COL6A1, were associated with a more severe phenotype. Histological, immunological and ultrastructural techniques were employed for the validation of the genetic variants; they documented the high variability in COL6 distribution and the extracellular matrix disorganization, highlighting the clinical heterogeneity of our cohort. The combined use of these different technologies is pivotal in the diagnosis of COL6 patients.

Keywords: collagen type VI; extracellular matrix; electron microscopy; COL6-RM

Citation: Zanotti, S.; Magri, F.; Salani, S.; Napoli, L.; Ripolone, M.; Ronchi, D.; Fortunato, F.; Ciscato, P.; Velardo, D.; D'Angelo, M.G.; et al. Extracellular Matrix Disorganization and Sarcolemmal Alterations in COL6-Related Myopathy Patients with New Variants of COL6 Genes. *Int. J. Mol. Sci.* **2023**, *24*, 5551. https://doi.org/10.3390/ijms24065551

Academic Editors: Paolo Bonaldo, Shireen Lamandé and Luciano Merlini

Received: 30 November 2022
Revised: 8 March 2023
Accepted: 9 March 2023
Published: 14 March 2023

1. Introduction

Collagen VI is a non-fibrillar heterotrimeric protein expressed in the extracellular matrix (ECM) of connective tissue of several organs including skeletal muscle, skin, cornea, lung, blood vessels, intervertebral disks and joints. This complex localizes at the cell surface, links the cytoskeleton to the ECM and it is involved in cell anchoring and adhesion, maintenance of cell integrity and signal transduction. The heterotrimer consists of three main chains, alfa1, alfa2 and alfa3, which associate via their C-terminal domains and fold into triple helical monomers. These monomers align in an antiparallel manner to form dimers and tetramers representing the secreted form of collagen VI. Finally, in the extracellular space, tetramers associate end-to-end creating collagen VI microfibrils organized in a microfilamentous network [1]. These collagen chains are encoded by *COL6A1*, *COL6A2* and

brain sciences

MDPI

Article

Speech, Gait, and Vestibular Function in Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome

Giulia Di Rauso [1,2,3], Andrea Castellucci [4], Francesco Cavallieri [3,*], Andrea Tozzi [5], Valentina Fioravanti [3], Edoardo Monfrini [6,7], Annalisa Gessani [2], Jessica Rossi [3,8], Isabella Campanini [9], Andrea Merlo [9], Dario Ronchi [6,7], Manuela Napoli [10], Rosario Pascarella [10], Sara Grisanti [8], Giuseppe Ferrulli [5], Rossella Sabadini [3], Alessio Di Fonzo [6], Angelo Ghidini [4] and Franco Valzania [3]

1 Department of Biomedical, Metabolic and Neural Science, University of Modena and Reggio Emilia, 41125 Modena, Italy; giuliadirauso3@gmail.com
2 Neurology, Neuroscience Head Neck Department, Azienda Ospedaliero-Universitaria di Modena, 41126 Modena, Italy; gessani.annalisa@aou.mo.it
3 Neurology Unit, Neuromotor & Rehabilitation Department, Azienda USL-IRCCS di Reggio Emilia, 42123 Reggio Emilia, Italy; valentina.fioravanti@ausl.re.it (V.F.); jessica.rossi@ausl.re.it (J.R.); rossella.sabadini@ausl.re.it (R.S.); franco.valzania@ausl.re.it (F.V.)
4 Otolaryngology Unit, Azienda USL-IRCCS di Reggio Emilia, 42123 Reggio Emilia, Italy; andrea.castellucci@ausl.re.it (A.C.); angelo.ghidini@ausl.re.it (A.G.)
5 Otorhinolaryngology-Head and Neck Surgery Department, University Hospital of Modena, 41125 Modena, Italy; andreatozzi29@gmail.com (A.T.); dottorgiuseppeferrulli@gmail.com (G.F.)
6 Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy; edoardo.monfrini@unimi.it (E.M.); dario.ronchi@unimi.it (D.R.); alessio.difonzo@policlinico.mi.it (A.D.F.)
7 Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy
8 Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, 41125 Reggio Emilia, Italy; grisanti.sara@gmail.com
9 LAM-Motion Analysis Laboratory, Neuromotor and Rehabilitation Department, Azienda USL-IRCCS di Reggio Emilia, 42123 Reggio Emilia, Italy; isabella.campanini@ausl.re.it (I.C.); andrea.merlo@ausl.re.it (A.M.)
10 Neuroradiology Unit, Azienda USL-IRCCS di Reggio Emilia, 42123 Reggio Emilia, Italy; manuela.napoli@ausl.re.it (M.N.); rosario.pascarella@ausl.re.it (R.P.)
* Correspondence: francesco.cavallieri@ausl.re.it; Tel.: +39-0522-295565

Abstract: (1) Background: Cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is characterized by late-onset cerebellar ataxia, bilateral vestibulopathy, and sensory neuronopathy mostly due to biallelic RFC1 expansion. (2) Objectives: The aim of this case series is to describe vestibular, gait, and speech alterations in CANVAS via a systematic approach. (3) Methods: All patients (n = 5) underwent a standardized clinical–instrumental examination, including the perceptual and acoustic analysis of speech, instrumental gait, and balance analysis (posturographic data were acquired using a force plate [Kistler, Winterthur, Switzerland] while 3D gait analysis, inclusive of surface electromyography, was acquired using a motion capture system [SMART DX, BTS Bioengineering, Milan, Italy], a wireless electromyograph [FreeEMG, BTS Bioengineering, Milan, Italy]), and vestibular assessment with video-oculography. (4) Results: Five patients were included in the analysis: three females (patients A, B, C) and two males (patients D and E) with a mean age at evaluation of 62 years (SD ± 15.16, range 36–74). The mean age of symptoms' onset was 55.6 years (SD ± 15.04, range 30–68), and patients were clinically and instrumentally evaluated with a mean disease duration of 6.4 years (SD ± 0.54, range 6–7). Video-Frenzel examination documented spontaneous downbeat nystagmus enhanced on bilateral gaze in all patients, except for one presenting with slight downbeat nystagmus in the supine position. All patients exhibited different degrees of symmetrically reduced VOR gain for allsix semicircular canals on the video-head impulse test and an unexpectedly normal ("false negative") VOR suppression, consistent with combined cerebellar dysfunction and bilateral vestibular loss. Posturographic indices were outside their age-matched normative ranges in all patients, while 3D gait analysis highlighted a reduction in ankle dorsiflexion (limited forward rotation of the tibia over the stance foot during the stance phase of gait and fatigue of the dorsiflexor muscles)

check for updates

Citation: Di Rauso, G.; Castellucci, A.; Cavallieri, F.; Tozzi, A.; Fioravanti, V.; Monfrini, E.; Gessani, A.; Rossi, J.; Campanini, I.; Merlo, A.; et al. Speech, Gait, and Vestibular Function in Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome. *Brain Sci.* **2023**, *13*, 1467. https://doi.org/10.3390/brainsci13101467

Academic Editor: Dominic Thyagarajan

Received: 24 September 2023
Revised: 10 October 2023
Accepted: 12 October 2023
Published: 17 October 2023

RESEARCH ARTICLE

Harmonizing Genetic Testing for Parkinson's Disease: Results of the PARKNET Multicentric Study

Alessio Di Fonzo, MD, PhD,[1,2*] Marco Percetti, MD,[1,3,4] Edoardo Monfrini, MD, PhD,[1,2] Ilaria Palmieri, PhD,[5] Alberto Albanese, MD,[6] Micol Avenali, MD, PhD,[5,7] Anna Bartoletti-Stella, BSc, PhD,[8,9] Fabio Blandini, MD, PhD,[10] Gloria Brescia, PhD,[10] Giovanna Calandra-Buonaura, MD, PhD,[9,11] Rosa Campopiano, BSc,[12] Sabina Capellari, MD, PhD,[9,11] Isabel Colangelo, MSc,[13] Giacomo Pietro Comi, MD,[1,2] Giada Cuconato, BSc,[14] Rosangela Ferese, BSc,[12] Caterina Galandra, BSc,[5,14] Stefano Gambardella, BSc,[15] Barbara Garavaglia, PhD,[13] Andrea Gaudio, BSc,[16] Emiliano Giardina, PhD,[17,18] Federica Invernizzi, MSc,[13] Paola Mandich, MD, PhD,[16,19] Rossana Mineri, PhD,[6] Celeste Panteghini, MSc,[13] Chiara Reale, MSc,[13] Lucia Trevisan, MD,[16] Stefania Zampatti, MD,[17] Pietro Cortelli, MD, PhD,[9,11] Enza Maria Valente, MD, PhD,[5,14] and on behalf of the PARKNET study group

[1]*Neuroscience Section, Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, Milan, Italy*
[2]*Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy*
[3]*School of Medicine and Surgery, Milan Center for Neuroscience, University of Milan-Bicocca, Milan, Italy*
[4]*Foundation IRCCS San Gerardo dei Tintori, Monza, Italy*
[5]*IRCCS Mondino Foundation, Pavia, Italy*
[6]*IRCCS Humanitas Research Hospital, Milan, Italy*
[7]*Department of Brain and Behavior Sciences, University of Pavia, Pavia, Italy*
[8]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy*
[9]*DIMEC, University of Bologna, Bologna, Italy*
[10]*Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
[11]*DIBINEM, University of Bologna, Bologna, Italy*
[12]*IRCCS Neuromed, Pozzilli, Italy*
[13]*Medical Genetics and Neurogenetics Unit, Fondazione IRCCS Istituto Neurologico C. Besta, Milan, Italy*
[14]*Department of Molecular Medicine, University of Pavia, Pavia, Italy*
[15]*Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy*
[16]*IRCCS Ospedale Policlinico San Martino, Genoa, Italy*
[17]*Genomic Medicine Laboratory-UILDM, Santa Lucia Foundation IRCCS, Rome, Italy*
[18]*Department of Biomedicine and Prevention, Tor Vergata University, Rome, Italy*
[19]*DINOGMI, University of Genoa, Genoa, Italy*

ABSTRACT: Background and Objective: Early-onset Parkinson's disease (EOPD) commonly recognizes a genetic basis; thus, patients with EOPD are often addressed to diagnostic testing based on next-generation sequencing (NGS) of PD-associated multigene panels. However, NGS interpretation can be challenging in a diagnostic setting, and few studies have addressed this issue so far.
Methods: We retrospectively collected data from 648 patients with PD with age at onset younger than 55 years who underwent NGS of a minimal shared panel of 15 PD-

*__Correspondence to:__ Dr. Alessio Di Fonzo, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan 20122, Italy; E-mail: alessio.difonzo@policlinico.mi.it

Alessio Di Fonzo and Marco Percetti share first co-authorship.

[Correction added on 12 October 2023, after first online publication: The ninth author's degree is updated in this version.].

Relevant conflicts of interest/financial disclosures: A.D.F. reports advisory board fees from Sanofi and speaking honoraria from Sanofi and Zambon. A.A. is Specialty Chief Editor of *Frontiers in Neurology* and President-Elect of the International Association for Parkinsonism and Related Disorders. A.A. received speaker's honoraria from Merz Pharma and Ipsen Pharma. G.C.B. received speakers's honoraria from Bial Italia. G.P.C. reports participation to advisory boards of Roche and Biogen. S. Z. received financial support from National Research council CNR. P.C. received speakers's honoraria from Abbvie. E.M.V. is Associate Editor of *Journal of Medical Genetics*; is Genetics Section Editor of *Pediatric Research*, of *The Cerebellum*, and of *Neurological Sciences*; is member of the Editorial Board of *Movement Disorders clinical Practice*; is member of the Steering Committee of ASAP GP2 (Global Parkinson genetic Program). E.M.V. received research support from the Italian Ministry of Health, CARIPLO Foundation, Telethon Foundation Italy, Pierfranco and Luisa Mariani Foundation, and European Community (Eranet Neuron). None of the other authors report any conflicts of interest.

Funding agencies: This work was supported by grants from the Italian Ministry of Health (Ricerca Corrente Reti 2021–2022-PARKNET project).

Received: 9 March 2023; **Revised:** 30 August 2023; **Accepted:** 11 September 2023

Published online 26 September 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29617

https://doi.org/10.14802/jmd.23125 / J Mov Disord 2024;17(1):102-105
pISSN 2005-940X / eISSN 2093-4939

LETTER TO THE EDITOR

A Novel Pathogenic *PSEN1* Variant in a Patient With Dystonia-Parkinsonism Without Dementia

Maria Chiara Malaguti,[1] Alessio Di Fonzo,[2] Chiara Longo,[1,3] Raffaella Di Giacopo,[4] Costanza Papagno,[5] Davide Donner,[6,7] Umberto Rozzanigo,[8] Edoardo Monfrini[2,9]

[1]Department of Neurology, Santa Chiara Hospital, APSS, Trento, Italy
[2]Department of Neurology, Foundation Istituti di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[3]Department of Psychology, University of Milano-Bicocca, Milan, Italy
[4]Department of Neurology, Rovereto Hospital, APSS, Rovereto, Italy
[5]Center for Mind/Brain Sciences (CIMeC), University of Trento, Rovereto, Italy
[6]Department of Nuclear Medicine, Santa Chiara Hospital, APSS, Trento, Italy
[7]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy
[8]Department of Diagnostic Imaging, Santa Chiara Hospital, APSS, Trento, Italy
[9]Department of Pathophysiology and Transplantation, Neuroscience Section, Dino Ferrari Center, University of Milan, Milan, Italy

Dear Editor,

The *PSEN1* gene is located on chromosome 14 and encodes the presenilin 1 protein, which is a key component of the γ-secretase complex that is involved in the cleavage of amyloid precursor protein (APP) in amyloid beta (Aβ) peptides.[1] Recent works have suggested that Aβ peptides may also play a role in the pathophysiology of motor symptoms in Alzheimer's disease (AD) patients carrying *PSEN1* mutations (*PSEN1*-AD) through accumulation in the striatum.[2] Aβ peptides may disrupt the function of the basal ganglia, possibly leading to the development of extrapyramidal symptoms.[3] In addition, *PSEN1* mutations can be associated with the accumulation of other proteins, such as alpha-synuclein and tau, which have also been linked with motor signs.[4] Parkinsonism, ataxia, and spasticity are the most frequently described motor symptoms in *PSEN1*-AD patients.[5] Motor impairment has been reported in the early stages of the disease and may even precede cognitive decline in a small subset of patients.[5] Few cases have been reported of *PSEN1* mutation carriers with parkinsonism as an isolated presenting feature without dementia or significant cognitive decline over time.[6-8] Here, we report a male patient with adult-onset dystonia-parkinsonism with positive AD biomarkers carrying a novel *PSEN1* frameshift variant.

A 52-year-old man came to our observation for painful sustained muscular contractions, cramps, and subjective mild attention deficit. Neurological examination revealed lower limb dystonia and symmetric rigidity causing gait impairment, rest and postural right-hand tremor, mild spontaneous and sensory-induced myoclonus, global and symmetric bradykinesia, mild cerebellar dysarthria and fluctuating diplopia (Supplementary Video 1 in the online-only Data Supplement). He had a positive family history of neurological diseases on the maternal side of the family with a hereditary pattern compatible with autosomal dominant inheritance (i.e., head tremor in his mother and Parkinson's disease evolving to dementia in a second-degree cousin and dementia in a great aunt, both from the maternal side) (Figure 1A).

Blood test results were normal, including systemic autoimmunity, neurological paraneoplastic antibodies, and ceruloplasmin. Brain magnetic resonance imaging (MRI) was unremarkable (Figure 1B, C), while single photon emission computed tomography (SPECT) with ioflupane (123I) showed a moderate reduction in radiotracer uptake in the striatum with a slight right-side prevalence (Figure 1D). In-depth cognitive assessment revealed substantially preserved cognition with selective difficulty in visuospatial short-term memory and learning verbal material

Received: June 30, 2023 **Revised:** August 10, 2023 **Accepted:** September 30, 2023
✉ **Corresponding author:** Maria Chiara Malaguti, MD
Department of Neurology, Santa Chiara Hospital, APSS, Largo Medaglie d'Oro, 9, Trento 38122, Italy / **Tel:** +39-0461-903281 / **Fax:** +39-0461-902732 / **E-mail:** mariachiara.malaguti@apss.tn.it

Movement Disorders
CLINICAL PRACTICE

CASE REPORT

Chorea-Acanthocytosis Presenting with Parkinsonism-Dystonia without Chorea

Edoardo Monfrini, MD, PhD,[1,2] Alessio Di Fonzo, MD, PhD,[2,*] and Francesca Morgante, MD, PhD[3,4]

Chorea-acanthocytosis (ChAc) is a rare genetic neurodegenerative disorder caused by biallelic VPS13A pathogenic variants. The phenotype is characterized by progressive chorea, neuropsychiatric features, seizures, and hyperCKemia due to myopathy, that may often be subclinical, and neuropathy. The phenotype is characterized by progressive chorea, neuropsychiatric features, seizures, and subclinical myopathy associated with hyperCKemia.[1] The term "acanthocytosis" is due to the observation of abnormal erythrocytes with spiked cell membrane (acanthocytes) in the blood smear of affected individuals.[1,2] ChAc is part of the "Neuroacanthocytosis syndromes," which is a group of rare progressive disorders displaying acanthocytes and neurological abnormalities also including McLeod syndrome.[1] Here, we present a case of ChAc without chorea, but with prominent parkinsonism combined with dystonia.

Case Report

The proband was an Italian male with normal psychomotor development. His parents were first cousins. One maternal cousin had epilepsy. At the age of 34 he developed generalized epileptic seizures which were controlled with oxcarbazepine, levetiracetam, and clonazepam. Over the following years, cognitive disturbances, behavioral disinhibition, prominent gait disturbances, and generalized slowness were also noted. These symptoms gradually progressed and within 10 years from onset, he needed assistance in all activities of daily living.

Neurological examination performed at age 48 showed action-induced dystonia in the lower limbs combined with signs of symmetrical parkinsonism (moderate bradykinesia and rigidity). He had knee bending when walking forward, which resolved when walking backwards. He also had freezing of gait and festination. Other findings included: slow horizontal saccades, nystagmus on lateral gaze; facial hypomimia, hypophonia, and stuttering dysarthria; stimulus-sensitive myoclonus was evident in the upper and lower limbs; motor perseverations, hyperreflexia except for absent ankle reflexes, extensor plantar responses. No motor impersistence or chorea were observed (Video 1).

Laboratory investigations including full blood count, ceruloplasmin, copper studies, liver enzymes, protein electrophoresis, and alpha-fetoprotein were all normal, but for CK which was mildly elevated (258 U/L). Blood films were not performed. Skeletal muscle biopsy showed a moderate reduction in size of some fibers. A liver ultrasound showed hepatomegaly and steatosis. Echocardiography, nerve conduction studies, and electromyography were normal. Brain Magnetic Resonance Imaging showed T_2-weighted symmetrical hyperintensity surrounding the putamen, caudate atrophy, moderate atrophy of the cerebellar vermis, and mild generalized atrophy (Fig. 1A–C). Whole-exome sequencing (WES) was performed upon obtaining written informed consent from the patient. Variant prioritization looking for rare (AF ≤ 0.001) nonsynonymous variants in genes associated with movement disorders revealed a novel homozygous frameshift truncating variant affecting the *VPS13A* gene (NM_033305.3): c.4351delT, p.(Phe1451Serfs*3), which was confirmed by Sanger sequencing (Fig. 1D, E). Vps13a loss-of-function is the recognized disease mechanism of ChAc. The variant reported here is predicted to lead to nonsense-mediated decay, and consequently to a complete loss of the Vps13a protein.

On long-term follow-up, the patient's condition deteriorated with worsening of parkinsonian symptoms and dementia. Higher doses of levodopa determined excessive daytime sleepiness and behavioral abnormalities. He died of pneumonia at age 51.

[1]Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy; [2]Foundation IRCCS Ca'Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy; [3]Neurosciences Research Centre, Molecular and Clinical Sciences Research Institute, St George's, University of London, London, UK; [4]Department of Experimental and Clinical Medicine, University of Messina, Messina, Italy

***Correspondence to:** Dr. Alessio Di Fonzo, Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy; E-mail: alessio.difonzo@policlinico.mi.it
Keywords: *VPS13A*, chorea-acanthocytosis, parkinsonism, dystonia, chorea.

Received 24 October 2022; revised 22 December 2022; accepted 18 January 2023.
Published online 00 Month 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mdc3.13771

Received: 16 March 2023 | Accepted: 12 July 2023

DOI: 10.1002/epd2.20132

Epileptic Disorders

CLINICAL COMMENTARY

GABRB1-related early onset developmental and epileptic encephalopathy: Clinical trajectory and novel de novo mutation

Edoardo Monfrini[1,2] | **Linda Borellini**[3] | **Eleonora Zirone**[3] | **Vidal Yahya**[1,2] | **Eleonora Mauri**[3] | **Maria Takeko Molisso**[3] | **Francesca Mameli**[3] | **Fabiana Ruggiero**[3] | **Giacomo Pietro Comi**[1,2] | **Sergio Barbieri**[3] | **Alessio Di Fonzo**[2] | **Robertino Dilena**[3]

[1]Neuroscience Section, Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, Milan, Italy

[2]Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[3]Neurophysiopathology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Correspondence
Robertino Dilena, Via Francesco Sforza 35, 20122 Milan, Italy.
Email: robertino.dilena@policlinico.mi.it

Funding information
Italian Ministry of Health

Abstract

Developmental and epileptic encephalopathy 45 (DEE45) is a neurogenetic disorder caused by heterozygous pathogenic variants of *GABRB1*, encoding the beta1 subunit of the GABA type A receptor. Only three infants with DEE45 have been reported so far, and a detailed description of the disease history of these patients is still lacking. We describe the clinical and genetic findings of a 21-year-old woman with DEE45 carrying a novel de novo *GABRB1* mutation (c.841A>G, p.T281A). The patient presented at birth with hypotonia and focal apneic seizures evolving in a phenotype of epilepsy of infancy with migrating focal seizures that were refractory to antiseizure medications. Epileptic spasms partially responsive to steroid therapy appeared in the second year of life. Acquired microcephaly, profound mental retardation, and tetraparesis became evident with development. During childhood and adolescence, the epileptic phenotype evolved toward a Lennox–Gastaut Syndrome. Atypical absence status and clusters of tonic seizures occurred, often triggered by respiratory infections. The main strengths of this work are the identification of a novel pathogenic *GABRB1* variant localized in the same transmembrane domain of a previously described mutation and the detailed description of the clinical trajectory of GABRB1-related encephalopathy along 21 years of disease history.

KEYWORDS

electroencephalography, epilepsy of infancy with migrating focal seizures, epileptic spasms, GABRB1, Lennox–Gastaut syndrome

1 | INTRODUCTION

Developmental and epileptic encephalopathy 45 (DEE45) is a recently described neurogenetic disease associated with *GABRB1* pathogenic variants (OMIM #617153). Three patients with *GABRB1*-related DEE have been reported so far.[1–3] The gene *GABRB1* plays a fundamental role in central neurotransmission since it encodes the

REPORT

A form of inherited hyperferritinemia associated with bi-allelic pathogenic variants of *STAB1*

Edoardo Monfrini,[1,2,9] Sara Pelucchi,[3,9] Maija Hollmén,[4] Miro Viitala,[4] Raffaella Mariani,[5] Francesca Bertola,[6] Silvia Majore,[7] Alessio Di Fonzo,[2] and Alberto Piperno[5,8,*]

Summary

Hyperferritinemia is a frequent finding in several conditions, both genetic and acquired. We previously studied eleven healthy subjects from eight different families presenting with unexplained hyperferritinemia. Their findings suggested the existence of an autosomal-recessive disorder. We carried out whole-exome sequencing to detect the genetic cause of hyperferritinemia. Immunohistochemistry and flow cytometry assays were performed on liver biopsies and monocyte-macrophages to confirm the pathogenic role of the identified candidate variants. Through a combined approach of whole-exome sequencing and homozygosity mapping, we found bi-allelic *STAB1* variants in ten subjects from seven families. *STAB1* encodes the multifunctional scavenger receptor stabilin-1. Immunohistochemistry and flow cytometry analyses showed absent or markedly reduced stabilin-1 in liver samples, monocytes, and monocyte-derived macrophages. Our findings show a strong association between otherwise unexplained hyperferritinemia and bi-allelic *STAB1* mutations suggesting the existence of another genetic cause of hyperferritinemia without iron overload and an unexpected function of stabilin-1 in ferritin metabolism.

Introduction

Hyperferritinemia is a frequent finding in clinical practice and often requires an extensive diagnostic workup. A large spectrum of conditions, both genetic and acquired, associated or not with iron overload, displays high serum ferritin.[1–3] The diagnostic strategy to reveal the cause of hyperferritinemia includes family and personal medical history, biochemical and genetic tests, and evaluation of liver iron by direct (biopsy) or indirect (quantitative magnetic resonance) methods.[1] Despite this complex and time-consuming approach, often the precise etiology remains elusive.

Ferritin expression in mammals is regulated by iron through a well-characterized mechanism of coordinated cytosolic post-transcriptional regulation.[4] In addition to iron, ferritin synthesis is regulated by cytokines during development, cellular differentiation, proliferation, and inflammation.[5] In mammals, a small amount of ferritin (normally 0.025% of the total body ferritin)[6] is present in a secreted form in serum. It mostly consists of variably glycosylated L-ferritin and trace amounts of H-ferritin.[7,8] Different from cytosolic ferritin, extracellular ferritin is relatively poor in iron.[7,8] Serum ferritin measurement has become a routine laboratory test to indirectly evaluate iron stores, although it is known that many additional factors, including inflammation, infection, liver diseases, and dietary and metabolic abnormalities—all of which may elevate serum ferritin—complicate its interpretation.[1,2,9] Despite this long history of clinical use, fundamental aspects of the biology of serum ferritin are still unclear. For example, tissue of origin, secretory pathway, receptor interactions, clearance, and functions remain topics of active debate.[10–12]

Stabilin receptors belong to class H scavenger receptors that consists of two members, stabilin-1 (also known as Clever-1 and FEEL-1) and stabilin-2.[13,14] The stabilins are enigmatic proteins whose physiological functions are still not entirely understood.[15] They comprise a large extracellular N terminus of multiple epidermal growth factor (EGF)/EGF-like domains, seven fasciclin-1 domains, an X-link domain, and a short intracellular C-terminal domain, linked by a transmembrane region.[14] Their extracellular domains share 55% similar homology, but their short intracellular domains are highly diverse, which results in differential abundance and function in different tissues and cells.[15,16] More specifically, stabilin-1 is primarily expressed on human monocytes, immunosuppressive macrophage populations, lymphatic endothelial cells, and sinusoidal endothelial cells of the liver, spleen, adrenal cortex, and bone marrow, and is involved in scavenging, angiogenesis, and cell adhesion.[14,16,17] As a scavenger receptor, stabilin-1 is known to bind and endocytose a wide range of ligands and, therefore, plays an important role in tissue homoeostasis and remodeling, and is involved in receptor-mediated endocytosis, intracellular sorting, and recycling.

[1]Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milano, Milano, Italy; [2]Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, Neurology Unit, Milano, Italy; [3]School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy; [4]MediCity Research Laboratory and InFLAMES flagship, University of Turku, Turku, Finland; [5]Centre for Rare Disease – Disorders of Iron Metabolism, Fondazione IRCCS, San Gerardo dei Tintori, European Reference Network – EuroBloodNet, Monza, Italy; [6]Cytogenetics and Medical Genetics, Fondazione IRCCS, San Gerardo dei Tintori, Monza, Italy; [7]Medical Genetics, Department of Molecular Medicine, Sapienza University, San Camillo-Forlanini Hospital, Roma, Italy; [8]Centro Ricerca Tettamanti, Monza, Italy
[9]These authors contributed equally
*Correspondence: alberto.piperno@unimib.it
https://doi.org/10.1016/j.ajhg.2023.07.004.

Journal of the Neurological Sciences 451 (2023) 120737

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Levodopa responsive asymmetric parkinsonism as clinical presentation of progranulin gene mutation.

ARTICLE INFO

Keywords
Progranuline
GNR
Parkinsonism
Fronto-temporal dementia spectrum disorders
Precision medicine

Dear Editor

Early diagnosis of neurodegenerative diseases is a challenge that we must face given the increasing emergence of promising treatments tackling the molecular mechanisms underlying each specific neurodegenerative form. Progranulin (*GRN*) gene mutations were discovered in families with frontotemporal dementia (FTD) in 2006 [1]. Since then, about 130 mutations linked with FTD have been reported. Frameshift and nonsense mutations are the most common pathogenic variants, inserting premature stop codons that lead to mRNA degradation and haploinsufficiency [6]. Heterozygous *GRN* mutations are responsible for about one third of autosomal dominant FTD cases worldwide [14]. The phenotypic spectrum is quite heterogeneous [7,15]. Extrapyramidal symptoms can be observed over the course of the disease, but very rarely they may precede cognitive disturbances profiling into an atypical parkinsonism or a phenotype similar to Parkinson's disease (PD) [12].

Here we describe the case of a 53 years-old man with a family history of PD (both parents and one paternal uncle) and dementia (one paternal uncle) who came to our attention for rest tremor, clumsiness, bradykinesia, and rigidity in the right hand. His past medical history included only diabetes mellitus.

Neurological examination confirmed a right-sided hemiparkinsonism. Brain MRI was normal while the DaTscan (ioflupane [123I] SPECT) revealed a severe reduction of tracer uptake in the left putamen followed by caudate nucleus with moderate reduced uptake of the tracer in the right putamen (Fig. 1A). A diagnosis of PD was made and a treatment with ropinirole was initially started. After two years, Carbidopa/Levodopa was also introduced with good control of motor symptoms. An acute levodopa challenge test was performed three years after the diagnosis showing a significant reduction of the UPDRS part III motor score after levodopa intake (carbidopa/levodopa 200 mg; med-off score: 35; med-on score: 20; % of reduction: 42.86%).

About four years after symptoms' onset, the patient developed motor complications in the form of wearing-off phenomena and peak-dose dyskinesias. DaTscan was repeated showing a slight worsening particularly in the left nigrostriatal pathway (Fig. 1B). During the following years, a progressive cognitive decline emerged, associated with sleep disturbances, delusions, hallucinations and axial symptoms in the form of instability and gait disturbances. Clinical evaluation showed mutacism; bilateral akinetic-rigid syndrome without resting tremor; and frontal release signs (bilateral grasping and glabellar reflex). Brain-MRI was repeated, showing moderate diffuse atrophy while 18F-FDG PET study showed bilateral hypometabolism involving frontal, parietal, and occipital cortices, precuneus, posterior cingulate cortex and basal ganglia (Fig. 1C-D). The neuropsychological assessment showed severe cognitive impairment with predominant dysexecutive, attentional, and visuospatial alterations. A diagnosis of dementia associated with PD was made and treatment with quetiapine and rivastigmine was started with some benefits. Given the strong familiarity for PD and dementia and the presence of a significant cognitive/behavioral dysfunction, a genetic analysis was performed (Whole-Exome Sequencing) revealing a null variant in the *GRN* gene (c.328C > T, p.Arg110*), which was classified as pathogenic according to ACMG criteria (PSV1, PP5, PM2). No additional candidate variants in genes associated with PD or dementia were identified. Unfortunately, the patient died at the age of 61 due to complications of a severe bilateral pneumonia.

Levodopa-responsive parkinsonism is a quite rare clinical presentation in *GRN* mutation carriers and, according to some case series, it is estimated to involve about 1.4% of patients [9]. Recently, Carneiro et al., described three patients with idiopathic PD phenotype carrying a *GRN* mutation (GRN-PD); in particular, the second case described had overlapping clinical and neurological features with our patient [3]. In addition, Carneiro et al. described some red flags in their GRN-PD patients that could help with diagnosis, including a family history of early-onset dementia/ALS, the presence of cognitive/behavioral dysfunction and subtle motor characteristics (i.e., postural myoclonic/jerky tremor; lower-limb onset) [3]). In our case the positive family history of dementia and the presence of relevant cognitive/behavioral dysfunction were the red flags that prompted us to proceed with the genetic testing.

Neuronal ubiquitinated inclusions of TDP-43 are the neuropathological hallmark of GRN-FTD patients [8]. Neuropathological studies reported different TDP-43 conformations that could present different clinical aspects. In particular it has been assumed that different banding patterns of abnormal TDP-43 fragments in ALS and FTLD might

https://doi.org/10.1016/j.jns.2023.120737
Received 21 June 2023; Received in revised form 12 July 2023; Accepted 16 July 2023
Available online 17 July 2023

Niccolò Biagioli[a], Francesco Cavallieri[b,*], Alessandro Marti[b], Giulia Di Rauso[a], Valentina Fioravanti[b], Edoardo Monfrini[c,d], Federico Gasparini[b], Daniela Beltrami[b], Sara Grisanti[e], Jessica Rossi[b,e], Giulia Toschi[b], Alessandro Fraternali[f], Annibale Versari[f], Manuela Napoli[g], Rosario Pascarella[g], Alessio Di Fonzo[c,d], Franco Valzania[b]

[a] *Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy*

[b] *Neurology Unit, Neuromotor & Rehabilitation Department, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy*

[c] *Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy*

[d] *Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy*

[e] *Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Italy*

[f] *Nuclear Medicine Unit, Azienda Unità Sanitaria Locale-IRCCS of Reggio Emilia, Reggio Emilia, Italy*

[g] *Neuroradiology Unit, Radiology Department, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy*

[*] Corresponding author.
E-mail address: Francesco.Cavallieri@ausl.re.it (F. Cavallieri).

CHAPTER TEN

Early-onset inherited dystonias versus late-onset idiopathic dystonias: Same or different biological mechanisms?

Roberto Erro[a,*], **Edoardo Monfrini**[b,c], **and Alessio Di Fonzo**[b]
[a]Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi, SA, Italy
[b]Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy
[c]Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy
*Corresponding author. e-mail address: rerro@unisa.it

Contents

Abstract

Dystonia syndromes encompass a heterogeneous group of movement disorders which might be differentiated by several clinical-historical features. Among the latter, age-at-onset is probably the most important in predicting the likelihood both for the symptoms to spread from focal to generalized and for a genetic cause to be found. Accordingly, dystonia syndromes are generally stratified into early-onset and late-onset forms, the former having a greater likelihood of being monogenic disorders and the latter to be possibly multifactorial diseases, despite being currently labeled as idiopathic. Nonetheless, there are several similarities between these two groups of dystonia, including shared pathophysiological and biological mechanisms. Moreover, there is also initial evidence of age-related modifiers of early-onset dystonia syndromes and of critical periods of vulnerability of the sensorimotor network, during which a combination of genetic and non-genetic insults is more likely to produce symptoms. Based on these lines of evidence, we reappraise the double-hit hypothesis

International Review of Neurobiology, Volume 169
ISSN 0074-7742, https://doi.org/10.1016/bs.irn.2023.05.002

Neurological Sciences (2023) 44:3703–3705
https://doi.org/10.1007/s10072-023-06961-z

BRIEF COMMUNICATION

Don't forget Allgrove syndrome in adult patients as a bulbar-ALS mimicker

Martina Vigano'[1] · Vittorio Mantero[1] · Paola Basilico[1] · Fiammetta Pirro[2] · Dario Ronchi[3] · Alessio Di Fonzo[4] · Andrea Salmaggi[1]

Received: 17 February 2023 / Accepted: 11 July 2023 / Published online: 17 July 2023

Abstract

Introduction Allgrove syndrome is a genetic disorder characterized by a multisystem involvement manifesting mainly in childhood with esophageal achalasia, adrenal insufficiency, and alacrima. Associated neurological manifestations are frequent in patients with late-onset forms and include peripheral, central, and autonomic dysfunction. The definitive diagnosis remains genetic, but neurological symptoms/signs could be a relevant clue for the diagnosis.

Discussion This syndrome is rare, but it is not impossible for it to occur in adults, so all neurologists must be alert. Moreover, in this regard, neurological symptoms can sometimes be very similar to those of motor neuron disease patients, so that, although rare, Allgrove syndrome may also enter into the differential diagnosis with the bulbar variant of amyotrophic lateral sclerosis. Nevertheless, attention to extra-neurological symptoms must remain high as these play an equally important role in reaching the diagnosis.

Case Report Here we present the case of a patient with some peculiarities that are onset at an advanced age, genetic confirmation of the diagnosis, and prominent neurological involvement, which also opens the differential diagnosis to amyotrophic lateral sclerosis.

Keywords Allgrove syndrome · ALS mimicker · Peripheral neuropathy · Achalasia

Introduction

Allgrove syndrome, also known as the triple-A syndrome (TAS, Achalasia – Addisonianism – Alacrima), is a rare autosomal recessive disorder, mostly caused by mutations in the *AAAS gene* (chromosome 12q13) [1]. This multisystem syndrome first manifests in childhood with alacrima and achalasia, whereas adrenal insufficiency develops gradually over the first 2 decades [2]. Neurological dysfunction is reported in up to 70% of patients with progressive course [3], with autonomic dysfunction and amyotrophy possibly representing the "fourth" and "fifth-A" of the syndrome [4].

Even if rare, late-onset forms exist, and neurologists should maintain a high index of suspicion, especially in adults, where the diagnosis could be very challenging partly mimicking bulbar motor neuron disease, as it happened in the case report we describe.

Case report

A 67-year-old woman presented to our Emergency Department complaining of progressive worsening of dysphagia in the last few weeks with significant weight loss (20 kg). She developed acute dyspnea and tirage, which were treated with bronchodilators, adrenaline, and non-invasive ventilation. She had a medical history of osteoporosis, hysterectomy, and surgical myotomy for esophageal achalasia at the age of 52; her family history was not

✉ Martina Vigano'
mart.vigano@asst-lecco.it

[1] Department of Neurology and Stroke Unit, ASST Lecco Ospedale Alessandro Manzoni, Via Eremo 9/11, LC 23900 Lecco, Italy

[2] Department of Neurology and Stroke Unit, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

[3] Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

[4] Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Journal of Neuropathology & Experimental Neurology, 2023, **82**, 818–820
https://doi.org/10.1093/jnen/nlad055
Advance access publication 10 July 2023
Letter to the Editor

Letter to the Editor

The unexpected finding of CNS autoantibodies in GBA1 mutation carriers with atypical parkinsonism

Francesca Di Biasio, MD, PhD[1]*, Giulia Lazzeri, MD[2,3], Edoardo Monfrini, MD, PhD[2,3], Paola Mandich, MD, PhD[1,4,5], Lucia Trevisan, MD, PhD[4,5], Silvia Morbelli, MD, PhD[1,4], Tiziana Benzi Markushi, MD[4], Laura Avanzino, MD, PhD[1,6], Alessio Di Fonzo, MD, PhD[2]

[1]Neurology Unit, IRCCS Policlinico San Martino, Genoa, Italy
[2]Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy
[3]Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy
[4]Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genoa, Genoa, Italy
[5]Medical Genetics Unit, IRCCS San Martino Hospital, Genoa, Italy
[6]Section of Human Physiology, Department of Experimental Medicine, University of Genoa, Genoa, Italy

*Send correspondence to: Francesca Di Biasio, MD, PhD, IRCCS San Martino, Largo Rosanna Benzi, 10, Genova, Italy; E-mail: fradibias@gmail.com

To the Editor:

Although mutations in the beta-glucocerebrosidase gene (GBA1) are the most important risk factor for developing Parkinson disease (PD) and Lewy body dementia (1), very few reports have linked atypical parkinsonism to GBA1 pathogenic variants (2, 3); moreover, little is known about their pathogenic role in these cases. We report the cases of 2 unrelated subjects with atypical parkinsonism carrying the GBA1 L444P mutation (c.1448 T>C; p.Leu483Pro) who also tested positive for anti-basal ganglia antibodies (ABGAs) and anti-AMPA-glutamate receptor3 (anti-GluR3) antibodies. Genetic analyses were performed by next-generation sequencing (NGS) on genomic DNA extracted from venous blood. The gene panel included the following genes: *APP, ATP13A2, ATP7B, CHMP2B, DCTN1, DNAJC6, FBXO7, FUS, GBA1, GCH1, GRN, LRRK2, MAPT, PARK7, PINK1, PLA2G6, POLG, PRKN, PRNP, PSEN1, PSEN2, RAB39B, SYNJ1, SNCA, TARDBP, TREM2, TWNK, UBQLN2, VCP,* and *VPS35*.

The first case was a 51-year-old woman with a 1-month history of falls, bradykinesia, and rigidity with negative family history. She presented with severe akinetic-rigid parkinsonism associated with mild involuntary movements of the right limbs, limitation of upward conjugate gaze, and postural instability, without dysautonomia or psychiatric disorder. Her cognitive assessment showed mild deficiency on attentive functions. Levodopa administration (1000 mg qd) showed a poor response (UPDRS-III OFF state 56, ON state 51). The brain MRI revealed atrophy of the posterior putamen (L>R) in T2-weighted sequences. Dopamine transporter (DAT)-SPECT demonstrated reduced specific binding ratio values in bilateral putamen (L>R) compared to healthy subjects; 18F-Fluorodeoxyglucose PET (FDG–PET) showed markedly reduced uptake in the right putamen and moderately reduced uptake in the left putamen. Due to the rapidly worsening course, a cerebrospinal fluid (CSF) analysis was performed with normal results. Suspecting a possible autoimmune encephalitis, a steroid bolus therapy was attempted without benefit. Autoimmune screening carried out by Western immunoblotting (Fig. 1), revealed the presence in both serum and CSF of ABGAs and anti-GluR3 antibodies. Finally, genetic analysis (whole-exome sequencing) identified the GBA1 L444P mutation in the heterozygous state. No other candidate pathogenic variants associated with PD, Alzheimer disease, or frontotemporal dementia genes were identified. Immunotherapy with intravenous immunoglobulin (400 mg/kg daily for 5 days) produced mild improvement of the symptoms.

The second patient was a 66-year-old woman with asymmetric extrapyramidal syndrome started at age 63 years with rigidity and bradykinesia of the left arm, and progressive worsening because of falls and postural instability. Her medical history revealed a previous diagnosis of breast cancer with negative oncologic follow-up, and hypertension. Parkinsonism was found in her grandmother's records. The neurological examination disclosed severe rigidity and bradykinesia of the left limbs, associated with dystonic postures, limitation of upward conjugate gaze, and a marche à petit pas. No significant improvement with Levodopa (800 mg daily) was observed (UPDRS-III OFF state 53, ON state 48). Her brain MRI revealed putaminal atrophy and T2 and SWI hypointensity of the posterolateral part of both putamina, together with mild

Bitetto *et al.*
Orphanet Journal of Rare Diseases (2023) 18:152
https://doi.org/10.1186/s13023-023-02763-w

Orphanet Journal of Rare Diseases

RESEARCH

Open Access

SCARB1 downregulation in adrenal insufficiency with Allgrove syndrome

Giacomo Bitetto[1], Gianluca Lopez[2], Dario Ronchi[1], Alessandra Pittaro[2], Valentina Melzi[1], Erika Peverelli[3], Fulvia Milena Cribiù[2], Giacomo P. Comi[1], Giovanna Mantovani[3,4] and Alessio Di Fonzo[1*]

Abstract

Background Allgrove disease is a rare genetic syndrome characterized by adrenal insufficiency, alacrimia, achalasia and complex neurological involvement. Allgrove disease is due to recessive mutations in the *AAAS* gene, which encodes for the nucleoporin Aladin, implicated in the nucleocytoplasmic transport. The adrenal insufficiency has been suggested to rely on adrenal gland-ACTH resistance. However, the link between the molecular pathology affecting the nucleoporin Aladin and the glucocorticoid deficiency is still unknown.

Results By analyzing postmortem patient's adrenal gland, we identified a downregulation of Aladin transcript and protein. We found a downregulation of Scavenger receptor class B-1 (SCARB1), a key component of the steroidogenic pathway, and SCARB1 regulatory miRNAs (mir125a, mir455) in patient's tissues. With the hypothesis of an impairment in the nucleocytoplasmic transport of the SCARB1 transcription enhancer cyclic AMP-dependent protein kinase (PKA), we detected a reduction of nuclear Phospho-PKA and a cytoplasmic mislocalization in patient's samples.

Conclusions These results shed a light on the possible mechanisms linking ACTH resistance, SCARB1 impairment, and defective nucleocytoplasmic transport.

Keywords Allgrove syndrome, Adrenal cortex, Adrenal insufficiency, PKA, SCARB1

Introduction

The adrenal gland is a complex endocrine gland composed of two developmentally unrelated tissues, an outer layer of adrenal cortex and an inner layer of adrenal medulla [1].

The adrenal cortex is an important site of synthesis for three different classes of steroid hormones. Mineralocorticoids (aldosterone) are produced by the cells of the adrenal zone glomerulosa, which is the outermost layer, glucocorticoids (cortisol and corticosterone) are synthesized in the adrenal cortical zone fasciculata, and androgens (androstenedione and dehydroepiandrosterone) in the inner zone reticularis [2, 3].

Aldosterone synthesis mainly responds to the renin-angiotensin regulatory pathway, whereas adrenal cortical zona fasciculata and reticularis produce hormones in response to adrenocorticotropic hormone (ACTH) stimulation [4–6].

ACTH exerts its role in promoting steroidogenic cell growth, leading to adrenal cellular hypertrophy and hyperplasia, and stimulates acute and chronic adrenal response [7]. The ACTH stimulation is provided by cyclic-AMP which increases in the adrenal cells and the consequent cyclic AMP-dependent protein kinase (PKA) cleavage and phosphorylation. Phosphorylated

*Correspondence:
Alessio Di Fonzo
Alessio.difonzo@policlinico.mi.it
[1] Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy
[2] Division of Pathology, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy
[3] Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy
[4] Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

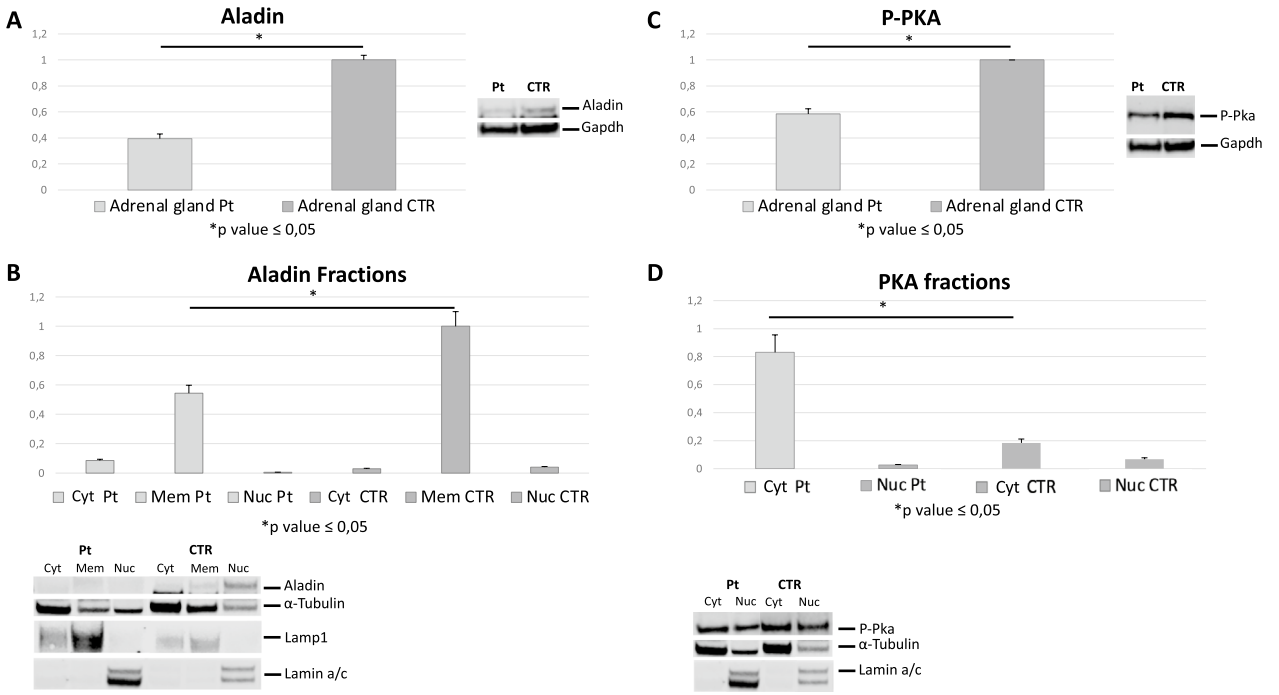


Fig. 4 **A** Reduction of Aladin protein amount in total lysate of patient's cortical adrenal gland (Pt). **B** Reduction of Aladin protein in membrane fraction from patient's cortical adrenal gland with a slight increasing in patient's cytoplasmic fraction. **C** Reduction in P-PKA concentration in cortical adrenal gland from patient compared to two controls (CTR). **D** Increase of P-PKA protein in cytosolic fraction from patient cortical adrenal gland compared to controls and not significative reduction in P-PKA protein amount in patient's nuclear fraction

enhance *SCARB1* transcription. We found P-PKA protein slightly reduced into the nucleus of patient's cortical adrenal gland and significantly increased in the cytosolic fraction. This result suggests that PKA or other PKA-shuttling proteins may be possible cargoes needed to be imported into cell nucleus and that this specific mechanism is impaired by damages of the nucleoporin Aladin.

Interestingly, we observed a strongly reduced expression of the ACTH receptor MC2R in patient's cortical adrenal gland. This result could open different scenarios: either the compensatory response to an ACTH overstimulation because of adrenal gland insufficiency, or the impairment of MC2R expression pathway on cellular membrane. The latter opens an intriguing pathogenic mechanism implying Aladin dysfunction associated to an aberrant nucleocytoplasmic transport of specific transcription factors or transcripts.

The comprehension of the mechanism underlying the adrenal insufficiency in Allgrove syndrome and the elucidation of the possible link with the nucleocytoplasmic transport may represent the initial step for future research and therapeutic approaches in ACTH-resistant hypocortisolism.

Conclusions

This study provides a unique pathological description of adrenal glands affected by Allgrove's syndrome. The findings could help to shed a light on the mechanism underlying adrenal insufficiency, providing new insight that could link ACTH resistance, SCARB1 impairment, and defective nucleocytoplasmic transport. We aim to confirm the molecular anomalies found in a future biobank in a cohort of several cases of this rare disease. These observations may represent the initial step for future research and new therapeutic strategies for this rare disease.

Acknowledgements
The authors would like to acknowledge the Dino Ferrari Center Association for support.

Author contributions
GB, ADF: design of the study, writing manuscript. GB, ADF, DR, VM: molecular analysis. GL, AP, FMC: neuropathological analysis. EP, GPC, GM: clinical evaluation and collection of samples. All authors approved the final manuscript.

Funding
None.

Availability of data and materials
Please contact author for data requests.

RESEARCH ARTICLE OPEN ACCESS

Whole-Exome Sequencing Study of Fibroblasts Derived From Patients With Cerebellar Ataxia Referred to Investigate CoQ10 Deficiency

Edoardo Monfrini, MD, Alba Pesini, PhD, Fabio Biella, PhD, Claudia F.R. Sobreira, MD, Valentina Emmanuele, MD, PhD, Gloria Brescia, PhD, Luis Carlos Lopez, MD, PhD, Saba Tadesse, MS, Michio Hirano, MD, Giacomo P. Comi, MD, Catarina Maria Quinzii, MD,* and Alessio Di Fonzo, MD, PhD*

Correspondence
Dr. Di Fonzo
alessio.difonzo@policlinico.mi.it

Neurol Genet 2023;9:e200058. doi:10.1212/NXG.0000000000200058

Abstract

Background and Objectives

Coenzyme Q_{10} (CoQ_{10})–deficient cerebellar ataxia can be due to pathogenic variants in genes encoding for CoQ_{10} biosynthetic proteins or associated with defects in protein unrelated to its biosynthesis. Diagnosis is crucial because patients may respond favorably to CoQ_{10} supplementation. The aim of this study was to identify through whole-exome sequencing (WES) the pathogenic variants, and assess CoQ_{10} levels, in fibroblasts from patients with undiagnosed cerebellar ataxia referred to investigate CoQ_{10} deficiency.

Methods

WES was performed on genomic DNA extracted from 16 patients. Sequencing data were filtered using a virtual panel of genes associated with CoQ_{10} deficiency and/or cerebellar ataxia. CoQ_{10} levels were measured by high-performance liquid chromatography in 14 patient-derived fibroblasts.

Results

A definite genetic etiology was identified in 8 samples of 16 (diagnostic yield = 50%). The identified genetic causes were pathogenic variants of the genes *COQ8A* (*ADCK3*) (n = 3 samples), *ATP1A3* (n = 2), *PLA2G6* (n = 1), *SPG7* (n = 1), and *MFSD8* (n = 1). Five novel mutations were found (*COQ8A* n = 3, *PLA2G6* n = 1, and *MFSD8* n = 1). CoQ_{10} levels were significantly decreased in 3/14 fibroblast samples (21.4%), 1 carrying compound heterozygous *COQ8A* pathogenic variants, 1 harboring a homozygous pathogenic *SPG7* variant, and 1 with an unknown molecular defect.

Discussion

This work confirms the importance of *COQ8A* gene mutations as a frequent genetic cause of cerebellar ataxia and CoQ_{10} deficiency and suggests *SPG7* mutations as a novel cause of secondary CoQ_{10} deficiency.

*These authors contributed equally to this work.

From the Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico (E.M., G.B., A.D.F.), Neurology Unit, Milan, Italy; Dino Ferrari Center (E.M., F.B., G.P.C.), Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Italy; Department of Neurology (A.P., V.E., S.T., M.H., C.M.Q.), Columbia University Medical Center, New York; Universidade de São Paulo (C.F.R.S.), Ribeirão Preto Medical School, Department of Neurosciences, Brazil; Departamento de Fisiología (L.C.L.), Facultad de Medicina, Universidad de Granada, Spain; and Centro de Investigación Biomédica (L.C.L.), Instituto de Biotecnología, Universidad de Granada, Spain.

Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

The Article Processing Charge was funded by the authors.

Movement Disorders
CLINICAL PRACTICE

RESEARCH ARTICLE

Levodopa Equivalent Dose of Safinamide: A Multicenter, Longitudinal, Case–Control Study

Roberto Cilia, MD,[1,*] Emanuele Cereda, MD, PhD,[2] Marco Piatti, MD,[3,4] Andrea Pilotto, MD,[5] Luca Magistrelli, MD, PhD,[6] Nico Golfrè Andreasi, MD,[1] Salvatore Bonvegna, MD,[4] Elena Contaldi, MD,[6] Francesca Mancini, MD,[7] Gabriele Imbalzano, MD,[8,9] Rosa De Micco, MD,[10] Fabiana Colucci, MD,[11,12] Arianna Braccia, MD,[11,12] Gabriele Bellini, MD,[13] Francesco Brovelli, MD,[3] Roberta Zangaglia, MD,[14] Giulia Lazzeri, MD,[15] Maria Claudia Russillo, MD,[16] Enrica Olivola, MD,[17] Chiara Sorbera, MD,[18] Viviana Cereda, MSc,[19] Patrizia Pinto, MD,[20] Patrizia Sucapane, MD,[21] Giorgio Gelosa, MD,[22] Mario Meloni, MD, PhD,[23] Francesca Pistoia, MD, PhD,[21,24] Maria Sessa, MD,[20] Margherita Canesi, MD,[19] Nicola Modugno, MD,[17] Claudio Pacchetti, MD,[14] Laura Brighina, MD, PhD,[3] Maria Teresa Pellecchia, MD, PhD,[16] Roberto Ceravolo, MD, PhD,[13] Mariachiara Sensi, MD, PhD,[11,12] Maurizio Zibetti, MD, PhD,[8,9] Cristoforo Comi, MD, PhD,[6] Alessandro Padovani, MD, PhD,[5] Anna L. Zecchinelli, MD,[4] Alessio Di Fonzo, MD, PhD,[15] Alessandro Tessitore, MD, PhD,[10] Francesca Morgante, MD, PhD,[25,26] and Roberto Eleopra, MD[1]

Abstract: Background: Effects of dopaminergic medications used to treat Parkinson's disease (PD) may be compared with each other by using conversion factors, calculated as Levodopa equivalent dose (LED). However, current LED proposals on MAO-B inhibitors (iMAO-B) safinamide and rasagiline are still based on empirical approaches.

Objectives: To estimate LED of safinamide 50 and 100 mg.

Methods: In this multicenter, longitudinal, case–control study, we retrospectively reviewed clinical charts of 500 consecutive PD patients with motor complications and treated with (i) safinamide 100 mg ($N = 130$), safinamide 50 mg ($N = 144$), or rasagiline 1 mg ($N = 97$) for 9 ± 3 months and a control group of patients never treated with any iMAO-B ($N = 129$).

Results: Major baseline features (age, sex, disease duration and stage, severity of motor signs and motor complications) were similar among the groups. Patients on rasagiline had lower UPDRS-II scores and Levodopa dose than control subjects. After a mean follow-up of 8.8-to-10.1 months, patients on Safinamide 50 mg and 100 mg had lower UPDRS-III and OFF-related UPDRS-IV scores than control subjects, who in turn had larger increase in total LED than the three iMAO-B groups. After adjusting for age, disease duration, duration of follow-up, baseline values and taking change in UPDRS-III scores into account (sensitivity analysis), safinamide 100 mg

[1]Department of Clinical Neurosciences, Parkinson and Movement Disorders Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; [2]Clinical Nutrition and Dietetics Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [3]Neurology Unit, Department of Neurology, Milan Center for Neuroscience, San Gerardo Hospital, Monza, Italy; [4]Centro Parkinson e Parkinsonismi, ASST Gaetano Pini-CTO, Milan, Italy; [5]Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy; [6]Department of Translational Medicine, Movement Disorders Centre, Neurology Unit, University of Piemonte Orientale, Novara, Italy; [7]IRCCS, Department of Neurology-Stroke Unit and Laboratory of Neuroscience – Milan, Istituto Auxologico Italiano, Milan, Italy; [8]Department of Neuroscience "Rita Levi Montalcini", University of Torino, Turin, Italy; [9]SC Neurologia 2U, AOU Città della Salute e della Scienza, Turin, Italy; [10]Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy; [11]Azienda Ospedaliera Univerisitaria S. Anna, U.O. Neurologia, Ferrara, Italy; [12]University of Ferrara, Ferrara, Italy; [13]Unit of Neurology, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; [14]Parkinson's Disease and Movement Disorders Unit, IRCCS Mondino Foundation, Pavia, Italy; [15]Neurology Unit, Department of Neuroscience, Dino Ferrari Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [16]Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, Neuroscience Section, University of Salerno, Italy; [17]Parkinson and Movement Disorders Unit, IRCCS Neuromed, Pozzilli, Italy; [18]IRCCS Centro Neurolesi "Bonino-Pulejo", Messina, Italy; [19]Department of Neurological Rehabilitation, Parkinson's Disease and Movement Disorders Center, Moriggia-Pelascini Hospital, Gravedona ed Uniti, Gravedona, Italy; [20]Neurology Unit, ASST Papa Giovanni XXIII, Bergamo, Italy; [21]Neurology Unit, San Salvatore Hospital, L'Aquila, Italy; [22]Neurology Unit, ASST "Grande Ospedale Metropolitano" Niguarda, Milan, Italy; [23]IRCCS Fondazione Don Carlo Gnocchi ONLUS, Milan, Italy; [24]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy; [25]Neuroscience Research Centre, Molecular and Clinical Sciences Institute, St. George's, University of London, London, UK; [26]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

***Correspondence to:** Roberto Cilia, Fondazione IRCCS Istituto Neurologico Carlo Besta, Parkinson and Movement Disorders Unit, via Celoria 11, 20133, Milan, Italy; E-mail: roberto.cilia@istituto-besta.it

Keywords: Parkinson's disease, levodopa equivalent dose, LED, safinamide, Rasagiline.

Received 9 December 2022; accepted 21 January 2023.
Published online 15 February 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mdc3.13681

Chunyu Li, MD, Junyu Lin, MD, Qirui Jiang, MD, Tianmi Yang, MD, Yi Xiao, MD, Jingxuan Huang, MD, Yanbing Hou, MD, Qianqian Wei, MD, Shichan Wang, MD, Xiaoting Zheng, MD, Ruwei Ou, MD, Kuncheng Liu, MD, Xueping Chen, MD, Wei Song, MD, Bi Zhao, MD and Huifang Shang, MD*

Department of Neurology, Laboratory of Neurodegenerative Disorders, Rare Diseases Center, West China Hospital, Sichuan University, Chengdu, China

References

1. Percetti M, Franco G, Monfrini E, et al. TWNK in Parkinson's disease: a movement disorder and mitochondrial disease center perspective study. Mov Disord 2022;37(9):1938–1943.
2. Senkevich K, Gan-Or Z. No association between rare TWNK variants and Parkinson's disease in European cohorts. Mov Disord 2022; 37(11):2318–2319.
3. Li C, Lin J, Gu X, et al. Mutation screening of TFG in α-Synucleinopathy and amyotrophic lateral sclerosis. Mov Disord 2022;37(8):1756–1761.
4. Bandres-Ciga S, Diez-Fairen M, Kim JJ, Singleton AB. Genetics of Parkinson's disease: an introspection of its journey towards precision medicine. Neurobiol Dis 2020;137:104782.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Reply to: "Lack of Association between *TWNK* Rare Variants and Parkinson's Disease in a Chinese Cohort"

We thank Dr. Chunyu Li and colleagues for their interest in our work[1] and for further exploring the link between rare variants in *TWNK* and Parkinson's disease (PD). To this aim, they performed a burden analysis using data obtained from two cohorts of patients with PD and control subjects of Chinese ancestry who underwent whole-exome sequencing. They conclude against an association between rare variants in *TWNK* and PD.[2] Recently, Drs. Senkevich and Gan-Or[3] performed similar analyses in European cohorts, leading to the same conclusions. As mentioned in our previous correspondence,[4] although we acknowledge the importance of replication studies, we raise some perplexities regarding the possible contribution of burden analyses for rare variants in *TWNK* and PD. Monoallelic *TWNK* variants are an established cause of several mitochondrial disorders, such as autosomal dominant progressive external ophthalmoplegia. To this regard, the screening of soft mitochondrial signs should be considered in selection criteria of both cases and controls in genetic association studies, keeping in mind that such syndromes could remain underdiagnosed until the advanced age. Moreover, none of the *TWNK* variants reported in our work in patients with PD was identified, strengthening their possible role of private variants.

In conclusion, we agree that caution is needed when assessing the possible contribution of *TWNK* to the etiology of PD, which requires additional genetic and functional studies. However, we point out that association studies even when complemented by rare variant burden analyses may not be adequate to detect the pathogenic impact of rare variants with incomplete penetrance. Functional studies based on patient-derived cell models could help in the future to elucidate the effect of specific *TWNK* variants in the pathogenesis of PD.

Acknowledgments: V.C. acknowledges the support of the Italian region Emilia-Romagna funding (ER-MITO project—Programma di ricerca Regione-Università 2010-2012, PRUa1RI-2012-008). A.D.F and V.C. acknowledge the Italian Ministry of Health (Ricerca Corrente funding). We are grateful to the patients and families for taking part in this study.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Marco Percetti, MD,[1,2,3] Edoardo Monfrini, MD,[3,4] Leonardo Caporali, PhD,[5] Raffaella Minardi, PhD,[5] Valerio Carelli, MD, PhD,[5,6] Enza Maria Valente, MD, PhD,[7,8] and Alessio Di Fonzo, MD, PhD[3,4*]

[1]*School of Medicine and Surgery and Milan Center for Neuroscience, University of Milan-Bicocca, Milan, Italy,* [2]*Foundation IRCCS San Gerardo dei tintori, Neurology Unit, Monza, Italy,* [3]*Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy,* [4]*Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy,* [5]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy,* [6]*Unit of Neurology, Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy,* [7]*Neurogenetics Research Center, IRCCS Mondino Foundation, Pavia, Italy, and* [8]*Department of Molecular Medicine, University of Pavia, Pavia, Italy*

***Correspondence to:** Dr. Alessio Di Fonzo, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Via Francesco Sforza 35, Milan 20122, Italy; E-mail: alessio.difonzo@policlinico.mi.it

Funding agencies: This work was supported by the Italian Ministry of Health Ricerca Corrente 2020–2021 (PARKNET project to A.D.F., E.M.V., and V.C.) and Italian region Emilia-Romagna funding (ER-MITO project—Programma di ricerca Regione-Universita 2010–2012, PRUa1RI-2012-008 to V.C.).

Relevant conflicts of interest/financial disclosures: A.D.F. reports advisory board fees from Sanofi and speaking honoraria from Sanofi and Zambon. V.C. reports consultant and advisory board fees from GenSight Biologics, Pretzel Therapeutics, Stealth Biotherapeutics, and Chiesi Farmaceutici and speaker honoraria from Chiesi Farmaceutici, First Class, and Medscape. None of the other authors reports any conflict of interest.
Full financial disclosures and author roles may be found in the online version of this article.

Received: 9 February 2023; **Accepted:** 16 February 2023

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29373

International Journal of
Molecular Sciences

MDPI

Review

Genetic Evidence for Endolysosomal Dysfunction in Parkinson's Disease: A Critical Overview

Vidal Yahya [1,2], Alessio Di Fonzo [2] and Edoardo Monfrini [1,2,*]

[1] Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy; vidal.yahya@unimi.it
[2] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, 20122 Milan, Italy; alessio.difonzo@policlinico.mi.it
* Correspondence: edoardo.monfrini@unimi.it

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder in the aging population, and no disease-modifying therapy has been approved to date. The pathogenesis of PD has been related to many dysfunctional cellular mechanisms, however, most of its monogenic forms are caused by pathogenic variants in genes involved in endolysosomal function (*LRRK2*, *VPS35*, *VPS13C*, and *ATP13A2*) and synaptic vesicle trafficking (*SNCA*, *RAB39B*, *SYNJ1*, and *DNAJC6*). Moreover, an extensive search for PD risk variants revealed strong risk variants in several lysosomal genes (e.g., *GBA1*, *SMPD1*, *TMEM175*, and *SCARB2*) highlighting the key role of lysosomal dysfunction in PD pathogenesis. Furthermore, large genetic studies revealed that PD status is associated with the overall "lysosomal genetic burden", namely the cumulative effect of strong and weak risk variants affecting lysosomal genes. In this context, understanding the complex mechanisms of impaired vesicular trafficking and dysfunctional endolysosomes in dopaminergic neurons of PD patients is a fundamental step to identifying precise therapeutic targets and developing effective drugs to modify the neurodegenerative process in PD.

Keywords: Parkinson's disease; genetics; lysosomes; endolysosomes; synaptic vesicles

Citation: Yahya, V.; Di Fonzo, A.; Monfrini, E. Genetic Evidence for Endolysosomal Dysfunction in Parkinson's Disease: A Critical Overview. *Int. J. Mol. Sci.* **2023**, *24*, 6338. https://doi.org/10.3390/ijms24076338

Academic Editors: José G. Castaño and Beatriz Álvarez-Castelao

Received: 10 January 2023
Revised: 23 March 2023
Accepted: 26 March 2023
Published: 28 March 2023

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the aging population [1–4]. It is clinically defined by the presence of bradykinesia in combination with either rest tremor and/or rigidity, and a clear beneficial response to dopaminergic therapy [5]. Neuropathologically, it is characterized by the loss of dopaminergic neurons in the substantia nigra (SN) and the presence of α-synuclein positive inclusions (Lewy bodies, LB) in surviving neurons [6–8]. At present, there are no approved treatments capable of slowing neurodegeneration in PD. Therefore, it is of paramount importance to shed light on the molecular mechanism causing PD neurodegeneration, because this knowledge is the indispensable prerequisite to identifying therapeutic compounds that can address the dysfunctional cellular machinery specific to this neurodegenerative disorder [9,10]. In the past two decades, PD etiopathogenesis has been linked with several deranged cellular mechanisms, ranging from mitochondrial impairment (*PRKN*, *PINK1*, *PARK7*) and ubiquitination defects (*FBXO7*) to dysfunction of the endolysosomal pathway (*LRRK2*, *VPS35*, *VPS13C*, *ATP13A2*) and synaptic vesicle trafficking (*SNCA*, *RAB39B*, *SYNJ1*, *DNAJC6*). In addition, significant parts of the risk genes associated with PD encode for endolysosomal and synaptic vesicle proteins, confirming a particular susceptibility of PD-related brain structures to the impairment of these pathways (Figure 1) [11–18].

Glycobiology, 2023, **33**, 2, 88–94
https://doi.org/10.1093/glycob/cwac081
Advance access publication date 12 December 2022
GlycoForum - Technical Note

A sensitive method for determining UDP-glucose: ceramide glucosyltransferase (UGCG) activity in biological samples using deuterated glucosylceramide as acceptor substrate

Michele Dei Cas[1], Sara Casati[2], Gabriella Roda[3], Sergio Pablo Sardi[4], Rita Paroni[1], Alessio di Fonzo[5,6], Marco Trinchera[7,*]

[1]Department of Health Sciences, San Paolo Hospital, Università degli Studi di Milano, 20142 Milano, Italy, [2]Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, 20133 Milan, Italy, [3]Department of Pharmaceutical Sciences, Università degli Studi di Milano, 20133 Milan, Italy, [4]Rare and Neurologic Diseases Research, Sanofi, 350 Water St., Cambridge MA 02141, USA, [5]Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, 20122 Milan, Italy, [6]Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, 20122 Milan, Italy, [7]Department of Medicine and Surgery (DMC), University of Insubria, 21100 Varese, Italy

*Corresponding author: Dipartimento di Medicina e Chirurgia, Università dell'Insubria, via JH Dunant 5, 21100 Varese, Italy. Email: marco.trinchera@uninsubria.it

Glucosylceramide synthase (UGCG) is a key enzyme in the biosynthesis of glycosphingolipids and its activity is related to the resistance to anticancer drugs and is involved in the derangement of metabolism in various diseases. Moreover, UGCG acts as a major controller of the balanced levels of individual brain sphingolipids that may trigger neurodegeneration in Gaucher disease and in Parkinson disease associated to pathogenic variants in the glucocerebrosidase-encoding gene *GBA*. We have developed an effective method for determining UGCG activity in vitro using deuterated ceramide as an acceptor, and quantitation of the formed deuterated glucosylceramide by liquid chromatography coupled with tandem mass spectrometry. The method enabled us to determine the kinetic parameters of UGGC and the effect of the inhibitor GZ667161 on the enzyme activity expressed in model cells, as well as to measure UGCG specific activity in human fibroblasts using a simple crude cell homogenate. This novel approach may be useful in determining the actual UGCG activity levels in patient cells and tissues of animal models of diseases, and to study novel drugs targeting glycosphingolipid metabolism.

Key words: ganglioside; glycosphingolipid; gaucher disease; mass spectrometry.

Introduction

UGCG, UDP-Glucose: Ceramide β1–1′ glucosyltransferase, EC:2.4.1.80, also known as glucosylceramide (GlcCer) synthase (see chemical reaction in Supplementary Fig. S1) is the enzyme responsible for the biosynthesis of the bulk of glycosphingolipids in mammals; since the reaction product, GlcCer, is the immediate precursor of lactosylceramide, the core structure common to almost all classes of complex glycosphingolipids, such as globosides, gangliosides and lacto- or neolacto neutral or sulfated glycosphingolipids (Belarbi et al. 2020). In addition to such a relevant physiologic role, UGCG merited special attention due to the involvement in drug resistance in various cancers (Wegner, Gruber, et al. 2018; Wegner, Schömel, et al. 2018; Madigan et al. 2020; Salustiano et al. 2020; Bataller et al. 2021; Chueakwon et al. 2022), in the metabolic derangement occurring in malignant (Schömel et al. 2020; Jennemann et al. 2021; Zhang and Zhang 2021) and nonmalignant diseases (Andersson et al. 2021; Baccam et al. 2022), and more recently, in the development of Parkinson disease (PD) in patients carrying mutations in the *GBA* gene (Sidransky et al. 2009). According to the hypothesis that reduced glucocerebrosidase activity gives rise to an imbalance between GlcCer and Cer that triggers α-synuclein accumulation and Parkinson disease (Riboldi and Di Fonzo 2019; Belarbi et al. 2020), reducing GlcCer biosynthesis appeared a logical therapeutic approach that could be addressed inhibiting UGCG ubiquitously, mainly in the central nervous system. To this aim, potential inhibitors, including those able to pass the blood brain barrier, have been designed and tested in vivo and in vitro (Cabrera-Salazar et al. 2012; Marshall et al. 2016; Sardi et al. 2017; Fujii et al. 2021; Dodge et al. 2022; Sabnis 2022; Tanaka et al. 2022). One of the main tools for addressing such issues is an assay for measuring UGCG activity in vitro. Between the assay methods reported so far (Roy et al. 2019), none concurrently overcomes the three main obstacles encountered since several years: a reliable enzyme source, a suitable acceptor substrate not far from the natural one, and an accurate but convenient method for reaction product quantification. Taking advantage from our recent work with other glycosyltransferases (Indellicato et al. 2019, 2020), we have thought to bypass the first obstacle by obtaining relevant amounts of stable UGCG through transient over-expression in mammalian cells upon transfection of cDNA placed in an effective vector. Regarding the acceptor, we decided to incubate the enzyme with a commercially available isotope of Cer resembling the naturally occurring one: the deuterated C15-acyl-sphingosine. In the end, we detected and quantitate the formed deuterated GlcCer by LC–MS/MS of the reaction mixture. To test the efficacy of the procedure, we determined the GlcCer synthase specific activity in human skin fibroblasts and evaluated the inhibitory effect of GZ667161 (Venglustat), a brain-penetrant clinical candidate GCS inhibitor already tested in a phase 2 clinical trial for *GBA*-PD, (Viel et al. 2021; Peterschmitt et al. 2022), on the enzyme activity.

Results and discussion

HEK-293 T cells, which are efficiently transfected with plasmid DNAs and able to replicate plasmids carrying the

Received: October 11, 2022. **Revised:** December 1, 2022. **Accepted:** December 1, 2022

Neurobiology of Disease 176 (2023) 105947

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Oligomeric α-synuclein and tau aggregates in NDEVs differentiate Parkinson's disease from atypical parkinsonisms

Mario Meloni [a,1], Cristina Agliardi [a,*,1], Franca Rosa Guerini [a], Milena Zanzottera [a], Elisabetta Bolognesi [a], Silvia Picciolini [a], Massimo Marano [b], Alessandro Magliozzi [b], Alessio Di Fonzo [c], Andrea Arighi [c], Chiara Fenoglio [d], Giulia Franco [c], Federica Arienti [c], Francesca Lea Saibene [a], Jorge Navarro [a], Mario Clerici [a,d]

[a] IRCCS Fondazione Don Carlo Gnocchi ONLUS, Via Capecelatro, 66, 20148, Milan, Italy
[b] Unit of Neurology, Neurophysiology and Neurobiology, Department of Medicine, Fondazione Policlinico Campus Bio-Medico, Rome, Italy
[c] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy
[d] Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy

ARTICLE INFO

Keywords:
Parkinson's disease
Atypical parkinsonian syndromes
Corticobasal degeneration (CBD)
Supranuclear palsy (PSP)
Neural-derived extracellular vesicles (NDEVs),
α-Synuclein, tau
Biomarker
Exosomes

ABSTRACT

The early differential diagnosis of Parkinson's disease (PD) and atypical Parkinsonian syndromes (APS), including corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), is challenging because of an overlap of clinical features and the lack of reliable biomarkers. Neural-derived extracellular vesicles (NDEVs) isolated from blood provide a window into the brain's biochemistry and may assist in distinguishing between PD and APS. We verified in a case-control study whether oligomeric α-Synuclein and Tau aggregates isolated from NDEVs could allow the differential diagnosis of these conditions.

Blood sampling and clinical data, including disease duration, motor severity, global cognition, and levodopa equivalent daily dose (LEDD), were collected from patients with a diagnosis of either PD ($n = 70$), PSP ($n = 21$), or CBD ($n = 19$). NDEVs were isolated from serum by immunocapture using an antibody against the neuronal surface marker L1CAM; oligomeric α-Synuclein and aggregated Tau were measured by ELISA.

NDEVs analyses showed that oligomeric α-Synuclein is significantly augmented in PD compared to APS, whereas Tau aggregates are significantly increased in APS compared to PD ($p < 0.0001$). ROC analyses showed that these two biomarkers have a "good" power of classification ($p < 0.0001$ for both proteins), with high sensitivity and specificity, with NDEVs concentration of Tau aggregates and oligomeric α-Synuclein being respectively the best biomarker for PD/PSP and PD/CBD diagnostic differentiation.

Logistic and multiple regression analysis confirmed that NDEVs-derived oligomeric α-Synuclein and Tau aggregates differentiate PD from CBD and PSP ($p < 0.001$). Notably, a positive correlation between NDEVs oligomeric α-Synuclein and disease severity (disease duration, $p = 0.023$; Modified H&Y, $p = 0.015$; UPDRS motor scores, $p = 0.004$) was found in PD patients and, in these same patients, NDEVs Tau aggregates concentration inversely correlated with global cognitive scores ($p = 0.043$).

A minimally invasive blood test measuring the concentration of α-synuclein and Tau aggregates in NDEVs can represent a promising tool to distinguish with high sensitivity and specificity PD from CBD or PSP patients. Optimization and validation of these data will be needed to confirm the diagnostic value of these biomarkers in distinguishing synucleinopathies from taupathies.

Abbreviations: APS, Atypical Parkinsonian Syndromes; CD81, Cluster of Differentiation 81; CI, confidence interval; L1CAM, L1 Cell Adhesion Molecule; LEDD, Levodopa Equivalent Daily Dose; MISEV, Minimal Information for Studies of Extracellular Vesicles; NDEVs, Neural Derived Extracellular Vesicles.

* Corresponding author.
E-mail address: cagliardi@dongnocchi.it (C. Agliardi).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.nbd.2022.105947
Received 26 September 2022; Received in revised form 22 November 2022; Accepted 4 December 2022
Available online 5 December 2022

2021) L1CAM -based immunoprecipitation extracellular vesicles is nevertheless expected to enrich NDEVs rather than to yield a pure population. Moreover, in-depth characterization of L1CAM-isolated NDEV's clearly showed that these particles carry specific exosomal and neural markers (Agliardi et al., 2021; Dutta et al., 2021). Reinforcing the idea that NDEVs isolated in this way are indeed an extremely useful tool to allow a glimpse into the CNS.

It has become evident that the clinicopathological heterogeneity of PSP and CBD impedes the development of specific clinical diagnostic criteria. Many studies have attempted to identify clinical features from clinicopathologic series in order to predict the underlying pathology. The overlapping clinical spectrum of PD and APS can make the differential diagnosis of these conditions very challenging. The difficulty in discriminate between these forms is particularly evident in the early stages, when neurological sings and neuroimaging features can be indistinguishable. In this scenario, the need for precise, reliable and easily measurable biomarkers is warranted.

The results presented here will need to be validated in larger independent cohorts and will need to be confirmed using next generation ELISA methods, that reach sub-picogram concentration sensitivity. It also has to be noted that the final diagnosis of patients, which was used to determine diagnostic accuracy, was based on clinical evaluation alone and has not yet been confirmed by neuropathologic examination. Although a team of movement disorders specialists has identified clinical diagnoses according to international diagnostic criteria, we cannot rule out that some patients may have received an erroneous diagnosis. These limitations notwithstanding, these results strongly suggest that NDEVs-associated oligomeric α-Synuclein and Tau aggregates concentration may serve as minimally invasive biomarkers for the early differential diagnosis of PD and APS, and could have a prognostic value in PD patients.

5. Conclusions

Data herein not only confirm very recent studies showing that increased α-synuclein in NDEVs can predict and differentiates PD from APS (Jiang et al., 2020), but also expand the knowledge by showing that the evaluation of α-synuclein and aggregated Tau in NDEVs allows to distinguish between PD and APS. This new observation suggests that these proteins have a promising potential to become disease-specific biomarkers in the clinical settings.

Funding

This work was supported by Italian Ministry of health [Ricerca Corrente 2021] and partially supported by Fondazione Alessandro e Vincenzo Negroni Prati Morosini and Fondazione Romeo ed Enrica Invernizzi

Relevant conflicts of interest

Nothing to declare.

Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

CRediT authorship contribution statement

Mario Meloni: Conceptualization, Project administration, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. **Cristina Agliardi:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Franca Rosa Guerini:** Formal analysis, Visualization, Writing – review & editing. **Milena Zanzottera:** Investigation, Methodology. **Elisabetta Bolognesi:** Investigation, Formal analysis, Writing – review & editing. **Silvia Picciolini:** Investigation, Writing – review & editing. **Massimo Marano:** Resources, Writing – review & editing. **Alessandro Magliozzi:** Resources, Writing – review & editing. **Alessio Di Fonzo:** Resources, Writing – review & editing. **Andrea Arighi:** Resources, Writing – review & editing. **Chiara Fenoglio:** Resources, Writing – review & editing. **Giulia Franco:** Resources, Writing – review & editing. **Federica Arienti:** Resources, Writing – review & editing. **Francesca Lea Saibene:** Resources, Writing – review & editing. **Jorge Navarro:** Resources, Writing – review & editing. **Mario Clerici:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

We thank Dr. Melissa Saibene for TEM imaging. ADF, GD and FA acknowledge the Associazione Centro Dino Ferrari and the Fresco Institute for their continuing support.

References

Adler, C.H., Beach, T.G., Hentz, J.G., Shill, H.A., Caviness, J.N., Driver-Dunckley, E., Sabbagh, M.N., Sue, L.I., Jacobson, S.A., Belden, C.M., Dugger, B.N., 2014. Low clinical diagnostic accuracy of early vs advanced Parkinson disease: clinicopathologic study. Neurology. 83 (5), 406–412. https://doi.org/10.1212/WNL.0000000000000641.

Agliardi, C., Clerici, M., 2020. Blood extracellular vesicles (EVs) of central nervous system origin: A window into the brain. Neural Regen. Res. 15 (1), 55–56. https://doi.org/10.4103/1673-5374.264454.

Agliardi, C., Meloni, M., Guerini, F.R., Zanzottera, M., Bolognesi, E., Baglio, F., Clerici, M., 2021. Oligomeric α-Syn and SNARE complex proteins in peripheral extracellular vesicles of neural origin are biomarkers for Parkinson's disease. Neurobiol. Dis. 148, 105185 https://doi.org/10.1016/j.nbd.2020.105185.

Armstrong, M.J., Litvan, I., Lang, A.E., Bak, T.H., Bhatia, K.P., Borroni, B., Boxer, A.L., Dickson, D.W., Grossman, M., Hallett, M., Josephs, K.A., Kertesz, A., Lee, S.E., Miller, B.L., Reich, S.G., Riley, D.E., Tolosa, E., Tröster, A.I., Vidailhet, M., Weiner, W.J., 2013. Criteria for the diagnosis of corticobasal degeneration. Neurology. 80 (5), 496–503. https://doi.org/10.1212/WNL.0b013e31827f0fd1.

Beach, T.G., Adler, C.H., 2018. Importance of low diagnostic accuracy for early Parkinson's disease. Mov. Disord. 33 (10), 1551–1554. https://doi.org/10.1002/mds.27485.

Buée, L., Delacourte, A., 1999. Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease. Brain Pathol. 9 (4), 681–693. https://doi.org/10.1111/j.1750-3639.1999.tb00550.x.

Dickson, D.W., Bergeron, C., Chin, S.S., Duyckaerts, C., Horoupian, D., Ikeda, K., Jellinger, K., Lantos, P.L., Lippa, C.F., Mirra, S.S., Tabaton, M., Vonsattel, J.P., Wakabayashi, K., Litvan, I., 2002. Office of Rare Diseases of the National Institutes of Health. Office of Rare Diseases neuropathologic criteria for corticobasal degeneration. J. Neuropathol. Exp. Neurol. 61 (11), 935–946. https://doi.org/10.1093/jnen/61.11.935.

Dickson, D.W., Ahmed, Z., Algom, A.A., Tsuboi, Y., Josephs, K.A., 2010. Neuropathology of variants of progressive supranuclear palsy. Curr. Opin. Neurol. 23 (4), 394–400. https://doi.org/10.1097/WCO.0b013e32833be924.

Duran, R., Barrero, F.J., Morales, B., Luna, J.D., Ramirez, M., Vives, F., 2010. Plasma alpha-synuclein in patients with Parkinson's disease with and without treatment. Mov. Disord. 25 (4), 489–493. https://doi.org/10.1002/mds.22928.

Dutta, S., Hornung, S., Kruayatidee, A., Maina, K.N., Del Rosario, I., Paul, K.C., Wong, D. Y., Duarte Folle, A., Markovic, D., Palma, J.A., Serrano, G.E., Adler, C.H., Perlman, S. L., Poon, W.W., Kang, U.J., Alcalay, R.N., Sklerov, M., Gylys, K.H., Kaufmann, H., Fogel, B.L., Bronstein, J.M., Ritz, B., Bitan, G., 2021. α-synuclein in blood exosomes immunoprecipitated using neuronal and oligodendroglial markers distinguishes Parkinson's disease from multiple system atrophy. Acta Neuropathol. 142 (3), 495–511. https://doi.org/10.1007/s00401-021-02324-0.

Eusebi, P., Giannandrea, D., Biscetti, L., Abraha, I., Chiasserini, D., Orso, M., Calabresi, P., Parnetti, L., 2017. Diagnostic utility of cerebrospinal fluid α-synuclein

Case report

EJO European Journal of Ophthalmology

European Journal of Ophthalmology
2023, Vol. 33(4) NP1–NP4

DOI: 10.1177/11206721221107798
journals.sagepub.com/home/ejo
Sage

Genetic evaluation in phenotypically discordant monozygotic twins with Coats Disease

Rosario Alfio Umberto Lizzio[1], Edoardo Monfrini[2,3], Simona Romano[1], Gloria Brescia[3,4], Stela Vujosevic[1,5], Matteo Sacchi[1], Alessio Di Fonzo[2,3] and Paolo Nucci[5]

Abstract

Purpose: To report the unique case of a pair of phenotypically discordant monozygotic twins, with one of them affected by unilateral Coats disease.

Case report: Both patients underwent a complete ophthalmologic evaluation and were genetically tested with whole-exome sequencing (WES). Any known or unknown potential genetic determinant of Coats disease wasn't found.

Conclusion: It may suggest a non-genetic etiology for this disorder. This represents, to the best of our knowledge, the first case of genetic analysis of monozygotic twins, one of whom is affected by Coats disease. Further studies are warranted, including performing genetic analysis directly on retinal biopsy tissue.

Keywords

Coats disease, Coats, genetic, monozygotic twins, genetic analysis, genetic evaluation, phenotypically discordant monozygotic twins, retinal telangiectasia, idiopatic retinal vasculopathy

Date received: 10 March 2022; accepted: 17 May 2022

Introduction

Coats disease is an idiopathic retinal vasculopathy characterized by retinal telangiectasia, intraretinal or subretinal exudation, micro and macro-aneurysm, and exudative retinal detachment.[1] Vascular abnormalities are more common in the peripheral retina, and exudation occurs mostly in the macular area.[2] Coats disease can manifest at any age, but the majority of patients are children with a diagnosis in their first or second decades of life.[3] It's a rare disease, with an incidence estimated at 0.09 per 100,000 population in the UK.[4] It occurs predominantly in males without any ethnic differences. This disease is usually unilateral, with a bilateral manifestation in less than 10% of cases.[2] In the last decades more sophisticated diagnostic techniques[2,5] and treatments of Coats disease have been proposed. Vitreoretinal or subretinal/external drainage surgery, laser photocoagulation,[6] and periocular and/or intravitreal medications have led to a reduction in the need for enucleation, especially in advanced-stage Coats disease.[1] Coats disease is usually not associated with systemic disease and its genetic etiology is still debated. Several candidate gene mutations have been described, including the Norrie Disease Protein (*NDP*),[7] *CRB1*,[8] *PANK2*,[9] *TERC*,[10] *ABCD4*.[11] In addition, the hypothesis of a somatic mutation has been proposed in the years given the congenital, nonfamilial, and unilateral features of the disease[7]

[1]University Eye Clinic, IRCCS Multimedica, Milan, Italy
[2]Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy
[3]Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[4]Laboratory of Medical Genetics, Foundation IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy
[5]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

Corresponding author:
Rosario Alfio Umberto Lizzio, Univeristy Eye Clinic, IRCCS MultiMedica, Via San Vittore 12, 20123 Milano, Italy.
Email: umberto.lizzio@live.it

International Journal of
Molecular Sciences

MDPI

Review

Brain Calcifications: Genetic, Molecular, and Clinical Aspects

Edoardo Monfrini [1,2], Federica Arienti [2], Paola Rinchetti [3], Francesco Lotti [3] and Giulietta M. Riboldi [4,*]

[1] Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy
[2] Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, 20122 Milan, Italy
[3] Columbia University Irving Medical Center, Center for Motor Neuron Biology and Diseases, Departments of Pathology & Cell Biology and Neurology, New York, NY 10032, USA
[4] The Marlene and Paolo Fresco Institute for Parkinson's and Movement Disorders, Department of Neurology, NYU Langone Health, New York, NY 10017, USA
* Correspondence: giulietta.riboldi@nyulangone.org

Abstract: Many conditions can present with accumulation of calcium in the brain and manifest with a variety of neurological symptoms. Brain calcifications can be primary (idiopathic or genetic) or secondary to various pathological conditions (e.g., calcium–phosphate metabolism derangement, autoimmune disorders and infections, among others). A set of causative genes associated with primary familial brain calcification (PFBC) has now been identified, and include genes such as *SLC20A2*, *PDGFB*, *PDGFRB*, *XPR1*, *MYORG*, and *JAM2*. However, many more genes are known to be linked with complex syndromes characterized by brain calcifications and additional neurologic and systemic manifestations. Of note, many of these genes encode for proteins involved in cerebrovascular and blood–brain barrier functions, which both represent key anatomical structures related to these pathological phenomena. As a growing number of genes associated with brain calcifications is identified, pathways involved in these conditions are beginning to be understood. Our comprehensive review of the genetic, molecular, and clinical aspects of brain calcifications offers a framework for clinicians and researchers in the field.

Keywords: primary familial brain calcification (PFBC); *SLC20A2*; *PDGFB*; *PDGFRB*; *XPR1*; *JAM2*; *MYORG*

Citation: Monfrini, E.; Arienti, F.; Rinchetti, P.; Lotti, F.; Riboldi, G.M. Brain Calcifications: Genetic, Molecular, and Clinical Aspects. *Int. J. Mol. Sci.* **2023**, *24*, 8995. https://doi.org/10.3390/ijms24108995

Academic Editor: Antonio Orlacchio

Received: 1 March 2023
Revised: 21 April 2023
Accepted: 9 May 2023
Published: 19 May 2023

1. Introduction

Brain calcifications (BC) are intracranial calcium deposits localized in the brain parenchyma and its microvasculature [1,2]. Their prevalence ranges from 1% in young individuals up to 38% in elderly subjects [2–4]. Calcified areas are easily identified by clinicians as hyperdense alterations on brain CT. A certain degree of intracranial calcifications, particularly of the basal ganglia, pineal gland, choroid plexus, and habenula, can be considered a normal phenomenon associated with aging [2]. Indeed, BC are often incidental findings on neuroimaging of asymptomatic individuals; however, they can also be associated with many genetic and acquired disorders [5,6].

BC can be primary, as observed in several early- and late-onset genetic syndromes, or can be secondary to systemic alterations of phosphate–calcium metabolism (genetic and also acquired forms), intrauterine (e.g., TORCH) and post-natal infections (e.g., neurocysticercosis), hypoxic-ischemic injuries, toxic exposures (e.g., lead), brain tumors (e.g., oligodendrogliomas), and autoimmune disorders (e.g., systemic lupus erythematosus) [2,5].

Although large-scale epidemiological studies are lacking, the most common neurological disorder associated with late-onset BC is traditionally known as Fahr disease [5]. It is clinically defined by the variable presence of movement disorders, recurrent headaches, and psychiatric manifestations, in association with the presence of bilateral BC, most commonly in the basal ganglia, but also in the subcortical white matter, thalamus, and cerebellum [1]. Historically, different names have been used to refer to this neurological condition, including: idiopathic basal ganglia calcification (IBCG), bilateral striopallidodentate calcinosis

Received: 5 July 2022 | Revised: 13 September 2022 | Accepted: 19 September 2022

DOI: 10.1002/alz.12824

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

RESEARCH ARTICLE

Temporal dynamics predict symptom onset and cognitive decline in familial frontotemporal dementia

David J. Whiteside[1,2] | Maura Malpetti[1] | P. Simon Jones[1] | Boyd C. P. Ghosh[3] | Ian Coyle-Gilchrist[4] | John C. van Swieten[5] | Harro Seelaar[5] | Lize Jiskoot[5] | Barbara Borroni[6] | Raquel Sanchez-Valle[7] | Fermin Moreno[8,9] | Robert Laforce[10] | Caroline Graff[11,12] | Matthis Synofzik[13,14] | Daniela Galimberti[15,16] | Mario Masellis[17] | Maria Carmela Tartaglia[18] | Elizabeth Finger[19] | Rik Vandenberghe[20,21,22] | Alexandre de Mendonça[23] | Fabrizio Tagliavini[24] | Chris R. Butler[25,26] | Isabel Santana[27,28] | Isabelle Le Ber[29,30,31] | Alexander Gerhard[32,33] | Simon Ducharme[34,35] | Johannes Levin[36,37,38] | Adrian Danek[36] | Markus Otto[39] | Sandro Sorbi[40,41] | Florence Pasquier[42,43,44] | Arabella Bouzigues[45] | Lucy L. Russell[45] | Jonathan D. Rohrer[45] | James B. Rowe[1,2,46] | Timothy Rittman[1,2] | The GENFI consortium[#]

[1]Department of Clinical Neurosciences, University of Cambridge, Cambridge, Cambridgeshire, UK

[2]Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

[3]Wessex Neurological Centre, University Hospital Southampton, Southampton, UK

[4]Norfolk and Norwich University Hospital, Norwich, UK

[5]Department of Neurology, Erasmus Medical Centre, Rotterdam, Netherlands

[6]Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

[7]Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Institut d'Investigacións Biomèdiques August Pi I Sunyer, University of Barcelona, Barcelona, Spain

[8]Cognitive Disorders Unit, Department of Neurology, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain

[9]Neuroscience Area, Biodonostia Health Research Institute, San Sebastian, Gipuzkoa, Spain

[10]CHU de Québec, and Faculté de Médecine, Département des Sciences Neurologiques, Clinique Interdisciplinaire de Mémoire, Université Laval, QC, Canada

[11]Center for Alzheimer Research, Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Solna, Sweden

[12]Unit for Hereditary Dementias, Theme Aging, Karolinska University Hospital, Solna, Sweden

[13]Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research, Tübingen, Germany

[14]Center of Neurology, University of Tübingen, Tübingen, Germany

[15]Fondazione IRCCS Ospedale Policlinico, Milan, Italy

[16]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

[17]Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada

[18]Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada

[19]Department of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada

[20]Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium

APPENDIX

GENFI consortium authors

Aitana Sogorb Esteve (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK; UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK)

Annabel Nelson (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Arabella Bouzigues (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Carolin Heller (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Caroline V Greaves (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

David Cash (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

David L Thomas (Neuroimaging Analysis Centre, Department of Brain Repair and Rehabilitation, UCL Institute of Neurology, Queen Square, London, UK)

Emily Todd (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Hanya Benotmane (UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK)

Henrik Zetterberg (UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK Department of Psychiatry and Neurochemistry,; the Sahlgrenska Academy at the University of Gothenburg, MölIndal, Sweden)

Imogen J Swift (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK; UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK)

Jennifer Nicholas (Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK)

Kiran Samra (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Lucy L Russell (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Martina Bocchetta (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Rachelle Shafei (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Rhian S Convery (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK)

Carolyn Timberlake (Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK)

Thomas Cope (Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK)

Timothy Rittman (Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK)

Alberto Benussi (Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy)

Enrico Premi (Stroke Unit, ASST Brescia Hospital, Brescia, Italy)

Roberto Gasparotti (Neuroradiology Unit, University of Brescia, Brescia, Italy)

Silvana Archetti (Biotechnology Laboratory, Department of Diagnostics, ASST Brescia Hospital, Brescia, Italy)

Stefano Gazzina (Neurology, ASST Brescia Hospital, Brescia, Italy)

Valentina Cantoni (Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy)

Andrea Arighi (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy)

Chiara Fenoglio (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy)

Elio Scarpini (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy)

Giorgio Fumagalli (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy)

Vittoria Borracci

Giacomina Rossi (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy)

Giorgio Giaccone (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy)

Giuseppe Di Fede (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy)

Journal of Neurology (2023) 270:1573–1586
https://doi.org/10.1007/s00415-022-11435-x

ORIGINAL COMMUNICATION

Loss of brainstem white matter predicts onset and motor neuron symptoms in *C9orf72* expansion carriers: a GENFI study

Agnès Pérez-Millan[1,2] · Sergi Borrego-Écija[1] · John C. van Swieten[3] · Lize Jiskoot[3,4] · Fermin Moreno[5,6] · Robert Laforce[7] · Caroline Graff[8,9] · Mario Masellis[10] · Maria Carmela Tartaglia[11] · James B. Rowe[12] · Barbara Borroni[13] · Elizabeth Finger[14] · Matthis Synofzik[15,16] · Daniela Galimberti[17,18] · Rik Vandenberghe[19,20] · Alexandre de Mendonça[21] · Chris R. Butler[22,23] · Alexander Gerhard[24,25] · Simon Ducharme[26,27] · Isabelle Le Ber[28,29] · Isabel Santana[30,31] · Florence Pasquier[32,33] · Johannes Levin[34,35] · Markus Otto[36] · Sandro Sorbi[37] · Pietro Tiraboschi[38] · Harro Seelaar[3] · Tobias Langheinrich[39] · Jonathan D. Rohrer[4] · Roser Sala-Llonch[2,40] · Raquel Sánchez-Valle[1] · The Genetic FTD Initiative, GENFI

Received: 5 August 2022 / Revised: 17 October 2022 / Accepted: 18 October 2022 / Published online: 29 November 2022

Abstract

Background and objectives The *C9orf72* expansion is the most common genetic cause of frontotemporal dementia (FTD) and/or motor neuron disease (MND). Corticospinal degeneration has been described in post-mortem neuropathological studies in these patients, especially in those with MND. We used MRI to analyze white matter (WM) volumes in presymptomatic and symptomatic *C9orf72* expansion carriers and investigated whether its measure may be helpful in predicting the onset of symptoms.

Methods We studied 102 presymptomatic *C9orf72* mutation carriers, 52 symptomatic carriers: 42 suffering from FTD and 11 from MND, and 75 non-carriers from the Genetic Frontotemporal dementia Initiative (GENFI). All subjects underwent T1-MRI acquisition. We used FreeSurfer to estimate the volume proportion of WM in the brainstem regions (midbrain, pons, and medulla oblongata). We calculated group differences with ANOVA tests and performed linear and non-linear regressions to assess group-by-age interactions.

Results A reduced WM ratio was found in all brainstem subregions in symptomatic carriers compared to both noncarriers and pre-symptomatic carriers. Within symptomatic carriers, MND patients presented a lower ratio in pons and medulla oblongata compared with FTD patients. No differences were found between presymptomatic carriers and non-carriers. Clinical severity was negatively associated with the WM ratio. *C9orf72* carriers presented greater age-related WM loss than non-carriers, with MND patients showing significantly more atrophy in pons and medulla oblongata.

Discussion We find consistent brainstem WM loss in *C9orf72* symptomatic carriers with differences related to the clinical phenotype supporting the use of brainstem measures as neuroimaging biomarkers for disease tracking.

Keywords Frontotemporal dementia · *C9orf72* · GENFI · Brainstem

Introduction

Frontotemporal dementia (FTD) refers to a heterogeneous group of neurodegenerative disorders that mainly affects the frontal and temporal lobes of the brain producing behavioral and language impairment [1]. Amyotrophic lateral sclerosis (ALS) is the most frequent motor neuron disease. It is caused by the neurodegeneration of motor neurons and the corticospinal and corticobulbar tracts leading to progressive weakness and muscular atrophy [2]. Due to the scientific advances in the last decades, it is now recognized that

Agnès Pérez-Millan and Sergi Borrego-Écija have contributed equally.

Roser Sala-Llonch and Raquel Sánchez-Valle have contributed equally.

List of GENFI consortium authors in the Appendix.

✉ Raquel Sánchez-Valle
rsanchez@clinic.cat

Extended author information available on the last page of the article

- Anne Bertrand, Sorbonne Université, Paris Brain Institute – Institut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
- Annerose Engel, Clinic for Cognitive Neurology, University Hospital Leipzig, Leipzig, Germany
- Annick Vogels, Department of Human Genetics, KU Leuven, Leuven, Belgium
- Arabella Bouzigues, Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
- Aurélie Funkiewiez, Centre de référence des démences rares ou précoces, IM2A, Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
- Benedetta Nacmias, Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
- Benjamin Bender, Department of Diagnostic and Interventional Neuroradiology, University of Tübingen, Tübingen, Germany
- Camilla Ferrari, Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
- Carlo Wilke, Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany; Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
- Carolin Heller, Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
- Carolina Maruta, Laboratory of Language Research, Centro de Estudos Egas Moniz, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
- Caroline V. Greaves, Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
- Carolyn Timberlake, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
- Catarina B. Ferreira, Laboratory of Neurosciences, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
- Catharina Prix, Neurologische Klinik, Ludwig-Maximilians-Universität München, Munich, Germany
- Chiara Fenoglio, University of Milan, Centro Dino Ferrari, Milan, Italy
- Christen Shoesmith, Department of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada
- Cristina Polito, Department of Biomedical, Experimental and Clinical Sciences “Mario Serio”, Nuclear Medicine Unit, University of Florence, Florence, Italy
- Daisy Rinaldi, Centre de référence des démences rares ou précoces, IM2A, Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière (DMU Neurosciences Paris 6), Paris, France
- Dario Saracino, Sorbonne Université, Paris Brain Institute – Institut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière (DMU Neurosciences Paris 6), Paris, France
- David Cash, Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
- David L. Thomas, Neuroimaging Analysis Centre, Department of Brain Repair and Rehabilitation, UCL Institute of Neurology, Queen Square, London, UK
- David Tang-Wai, The University Health Network, Krembil Research Institute, Toronto, Canada
- Diana Duro, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
- Ekaterina Rogaeva, Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada
- Elio Scarpini, University of Milan, Centro Dino Ferrari, Milan, Italy
- Elisabeth Wlasich, Neurologische Klinik, Ludwig-Maximilians-Universität München, Munich, Germany
- Emanuele Buratti, Molecular Pathology Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), 34149 Trieste, Italy
- Emily Todd, Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
- Enrico Premi, Stroke Unit, ASST Brescia Hospital, Brescia, Italy
- Frederico Simões do Couto, Faculdade de Medicina, Universidade Católica Portuguesa
- Gabriel Miltenberger, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
- Gemma Lombardi, IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy
- Giacomina Rossi, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
- Giorgio Fumagalli, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy
- Giorgio Giaccone, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
- Giuseppe Di Fede, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
- Gregory Kuchcinski, Univ Lille, France
- Hanya Benotmane, UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK
- Henrik Zetterberg, UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK

Journal of Neurology (2023) 270:1466–1477
https://doi.org/10.1007/s00415-022-11442-y

ORIGINAL COMMUNICATION

Motor symptoms in genetic frontotemporal dementia: developing a new module for clinical rating scales

Kiran Samra[1] · Amy M. MacDougall[2] · Georgia Peakman[1] · Arabella Bouzigues[1] · Martina Bocchetta[1] · David M. Cash[1] · Caroline V. Greaves[1] · Rhian S. Convery[1] · John C. van Swieten[3] · Lize Jiskoot[3] · Harro Seelaar[3] · Fermin Moreno[4,5] · Raquel Sanchez-Valle[6] · Robert Laforce[7] · Caroline Graff[8,9] · Mario Masellis[10] · Carmela Tartaglia[11] · James B. Rowe[12] · Barbara Borroni[13] · Elizabeth Finger[14] · Matthis Synofzik[15,16] · Daniela Galimberti[17,18] · Rik Vandenberghe[19,20,21] · Alexandre de Mendonça[22] · Chris R. Butler[23,24] · Alexander Gerhard[25,26] · Simon Ducharme[27,28] · Isabelle Le Ber[29,30,31,32] · Pietro Tiraboschi[33] · Isabel Santana[34,35] · Florence Pasquier[36,37,38] · Johannes Levin[39,40,41] · Markus Otto[42] · Sandro Sorbi[43,44] · Jonathan D. Rohrer[1] · Lucy L. Russell[1] on behalf of the Genetic FTD Initiative (GENFI)

Received: 7 September 2022 / Revised: 17 October 2022 / Accepted: 19 October 2022 / Published online: 17 November 2022

Abstract

Objective To investigate the optimal method of adding motor features to a clinical rating scale for frontotemporal dementia (FTD).

Methods Eight hundred and thirty-two participants from the international multicentre Genetic FTD Initiative (GENFI) study were recruited: 522 mutation carriers (with *C9orf72*, *GRN* and *MAPT* mutations) and 310 mutation-negative controls. A standardised clinical questionnaire was used to assess eight motor symptoms (dysarthria, dysphagia, tremor, slowness, weakness, gait disorder, falls and functional difficulties using hands). Frequency and severity of each motor symptom was assessed, and a principal component analysis (PCA) was performed to identify how the different motor symptoms loaded together. Finally, addition of a motor component to the CDR® plus NACC FTLD was investigated (CDR® plus NACC FTLD-M).

Results 24.3% of mutation carriers had motor symptoms (31.7% *C9orf72*, 18.8% *GRN*, 19.3% *MAPT*) compared to 6.8% of controls. Slowness and gait disorder were the commonest in all genetic groups while tremor and falls were the least frequent. Symptom severity scores were similar to equivalent physical motor examination scores. PCA revealed that all motor symptoms loaded together so a single additional motor component was added to the CDR® plus NACC FTLD to form the CDR® plus NACC FTLD-M. Individual global scores were more severe with the CDR® plus NACC FTLD-M, and no patients with a clinically diagnosed motor disorder (ALS/FTD-ALS or parkinsonism) were classified anymore as asymptomatic (unlike the CDR® plus NACC FTLD alone).

Conclusions Motor features are present in mutation carriers at all disease stages across all three genetic groups. Inclusion of motor symptoms in a rating scale that can be used in future clinical trials will not only ensure a more accurate severity measure is recorded but that a wider spectrum of FTD phenotypes can be included in the same trial.

Keywords Frontotemporal dementia · Genetics · Motor · Tau · Progranulin · *C9orf72*

Introduction

Frontotemporal dementia (FTD) is a neurodegenerative disorder that can present with a wide spectrum of phenotypes including behavioural, language and motor symptoms. It is often a sporadic condition but in around a third of individuals it is inherited, with the main autosomal dominant genetic mutations being found in progranulin (*GRN*), microtubule-associated protein tau (*MAPT*) and chromosome 9 open

Joint senior authors: Jonathan D. Rohrer and Lucy L. Russell.

The Genetic FTD Initiative (GENFI) consortium authors are listed in Acknowledgements.

✉ Kiran Samra
k.samra@ucl.ac.uk

Extended author information available on the last page of the article

in use of the scales, it will be important in future studies to formally assess both intra- and inter-rater variability.

In summary, motor symptoms are a key feature of genetic FTD, with differences in the type and extent of motor impairment noted between the main genetic mutation groups. Importantly, motor symptoms occur commonly in people without a primary motor diagnosis. Hence, incorporating a motor domain into a clinical rating scale for genetic FTD is essential for future trials. This will improve disease staging which in turn should optimise not only the stratification of individuals into trials but also the accuracy of clinical outcome measures.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-022-11442-y.

Acknowledgements We thank the research participants and their families for their contribution to the study. The Dementia Research Centre is supported by Alzheimer's Research UK, Alzheimer's Society, Brain Research UK, and The Wolfson Foundation. This work was supported by the National Institute for Health Research (NIHR) Queen Square Dementia Biomedical Research Unit and the University College London Hospitals Biomedical Research Centre, the Leonard Wolfson Experimental Neurology Centre (LWENC) Clinical Research Facility, and the UK Dementia Research Institute, which receives its funding from UK DRI Ltd, funded by the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK. This work was also supported by the MRC UK GENFI grant (MR/M023664/1), the Italian Ministry of Health (CoEN015 and Ricerca Corrente), the Canadian Institutes of Health Research as part of a Centres of Excellence in Neurodegeneration grant, a Canadian Institutes of Health Research operating grant, the Alzheimer's Society grant (AS-PG-16-007), the Bluefield Project and the JPND GENFI-PROX grant (2019-02248). MB is supported by a Fellowship award from the Alzheimer's Society, UK (AS-JF-19a-004-517). MB's work was also supported by the UK Dementia Research Institute which receives its funding from DRI Ltd, funded by the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK. JDR is supported by the Miriam Marks Brain Research UK Senior Fellowship and has received funding from an MRC Clinician Scientist Fellowship (MR/M008525/1) and the NIHR Rare Disease Translational Research Collaboration (BRC149/NS/MH). JBR is funded by the Wellcome Trust (103838) and the National Institute for Health Research Cambridge Biomedical Research Centre. This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy—ID 390857198). RV's work is supported by the Mady Browaeys Fonds voor Onderzoek naar Frontotemporale Degeneratie. Several authors of this publication (JCvS, MS, RSV, AD, MO, RV, JDR) are members of the European Reference Network for Rare Neurological Diseases (ERN-RND)—Project ID No 739510.

List of GENFI consortium authors: Annabel Nelson (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK), Martina Bocchetta (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK), David Cash (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK), David L. Thomas (Neuroimaging Analysis Centre, Department of Brain Repair and Rehabilitation, UCL Institute of Neurology, Queen Square, London, UK), Emily Todd (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK), Hanya Benotmane (UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK), Jennifer Nicholas (Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK), Kiran Samra (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK), Rachelle Shafei (Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK), Carolyn Timberlake (Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK), Thomas Cope (Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK), Timothy Rittman (Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK), Alberto Benussi (Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy), Enrico Premi (Stroke Unit, ASST Brescia Hospital, Brescia, Italy), Roberto Gasparotti (Neuroradiology Unit, University of Brescia, Brescia, Italy), Silvana Archetti (Biotechnology Laboratory, Department of Diagnostics, ASST Brescia Hospital, Brescia, Italy), Stefano Gazzina (Neurology, ASST Brescia Hospital, Brescia, Italy), Valentina Cantoni (Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy), Andrea Arighi (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy), Chiara Fenoglio (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy), Elio Scarpini (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy), Giorgio Fumagalli (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy), Vittoria Borracci (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy), Giacomina Rossi (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy), Giorgio Giaccone (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy), Giuseppe Di Fede (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy), Paola Caroppo (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy), Pietro Tiraboschi (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy), Sara Prioni (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy), Veronica Redaelli (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy), David Tang-Wai (The University Health Network, Krembil Research Institute, Toronto, Canada), Ekaterina Rogaeva (Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada, Miguel Castelo-Branco (Faculty of Medicine, University of Coimbra, Coimbra, Portugal), Morris Freedman (Baycrest Health Sciences, Rotman Research Institute, University of Toronto, Toronto, Canada), Ron Keren (The University Health Network, Toronto Rehabilitation Institute, Toronto, Canada), Sandra Black (Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada) (Sara Mitchell (Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada) (Christen Shoesmith (Department of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada) (Robart Bartha (Department of Medical Biophysics, The University of Western Ontario, London, Ontario, Canada; Centre for Functional and Metabolic Mapping, Robarts Research Institute, The University of Western Ontario, London, Ontario, Canada) (Rosa Rademakers (Center for Molecular Neurology, University of Antwerp) (Jackie Poos (Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands (Janne M. Papma (Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands) (Lucia Giannini (Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands) (Rick van Minkelen (Department of Clinical Genetics, Erasmus Medical Center, Rotterdam,

Journal of Neurology (2023) 270:1976–1988
https://doi.org/10.1007/s00415-022-11512-1

ORIGINAL COMMUNICATION

Language impairment in the genetic forms of behavioural variant frontotemporal dementia

Kiran Samra[1] · Amy M. MacDougall[2] · Arabella Bouzigues[1] · Martina Bocchetta[1] · David M. Cash[1] · Caroline V. Greaves[1] · Rhian S. Convery[1] · John C. van Swieten[3] · Harro Seelaar[3] · Lize Jiskoot[3] · Fermin Moreno[4,5] · Raquel Sanchez-Valle[6] · Robert Laforce[7] · Caroline Graff[8,9] · Mario Masellis[10] · Maria Carmela Tartaglia[11] · James B. Rowe[12] · Barbara Borroni[13] · Elizabeth Finger[14] · Matthis Synofzik[15,16] · Daniela Galimberti[17,18] · Rik Vandenberghe[19,20,21] · Alexandre de Mendonça[22] · Christopher R. Butler[23,24] · Alexander Gerhard[25,26] · Simon Ducharme[27,28] · Isabelle Le Ber[29,30,31,32] · Pietro Tiraboschi[33] · Isabel Santana[34,35] · Florence Pasquier[36,37,38] · Johannes Levin[39,40,41] · Markus Otto[42] · Sandro Sorbi[43,44] · Jonathan D. Rohrer[1] · Lucy L. Russell[1] · On Behalf of the Genetic FTD Initiative (GENFI)

Received: 21 October 2022 / Revised: 26 November 2022 / Accepted: 29 November 2022 / Published online: 20 December 2022

Abstract

Background Behavioural variant fronto-temporal dementia (bvFTD) is characterised by a progressive change in personality in association with atrophy of the frontal and temporal lobes. Whilst language impairment has been described in people with bvFTD, little is currently known about the extent or type of linguistic difficulties that occur, particularly in the genetic forms.

Methods Participants with genetic bvFTD along with healthy controls were recruited from the international multicentre Genetic FTD Initiative (GENFI). Linguistic symptoms were assessed using items from the Progressive Aphasia Severity Scale (PASS). Additionally, participants undertook the Boston Naming Test (BNT), modified Camel and Cactus Test (mCCT) and a category fluency test. Participants underwent a 3T volumetric T1-weighted MRI, with language network regional brain volumes measured and compared between the genetic groups and controls.

Results 76% of the genetic bvFTD cohort had impairment in at least one language symptom: 83% *C9orf72*, 80% *MAPT* and 56% *GRN* mutation carriers. All three genetic groups had significantly impaired functional communication, decreased fluency, and impaired sentence comprehension. *C9orf72* mutation carriers also had significantly impaired articulation and word retrieval as well as dysgraphia whilst the *MAPT* mutation group also had impaired word retrieval and single word comprehension. All three groups had difficulties with naming, semantic knowledge and verbal fluency. Atrophy in key left perisylvian language regions differed between the groups, with generalised involvement in the *C9orf72* group and more focal temporal and insula involvement in the other groups. Correlates of language symptoms and test scores also differed between the groups.

Conclusions Language deficits exist in a substantial proportion of people with familial bvFTD across all three genetic groups. Significant atrophy is seen in the dominant perisylvian language areas and correlates with language impairments within each of the genetic groups. Improved understanding of the language phenotype in the main genetic bvFTD subtypes will be helpful in future studies, particularly in clinical trials where accurate stratification and monitoring of disease progression is required.

Keywords Frontotemporal dementia · Genetics · Language · Tau · Progranulin · *C9orf72*

Abbreviations

ALS	Amyotrophic lateral sclerosis
bvFTD	Behavioural variant fronto-temporal dementia
BNT	Boston naming test
C9orf72	Chromosome 9 open reading frame 72
CWIT	D-KEFS colour-word interference test
FCSRT	Free and cued selective reminding test
FTD	Frontotemporal dementia

Jonathan D. Rohrer and Lucy L. Russell are joint senior authors.

The list of consortium authors are mentioned in Acknowledgements.

✉ Lucy L. Russell
l.russell@ucl.ac.uk

Extended author information available on the last page of the article

atrophy. Improved understanding of the relationship between bvFTD and its language phenotype will aid more focussed assessments and interpretations of data within FTD studies. This in turn will guide the future stratification of individuals within clinical trials as well as the monitoring of disease progression and treatment response.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-022-11512-1.

Acknowledgements We thank the research participants and their families for their contribution to the study. Several authors of this publication are members of the European Reference Network for Rare Neurological Diseases—Project ID No 739510. The Dementia Research Centre is supported by Alzheimer's Research UK, Alzheimer's Society, Brain Research UK, and The Wolfson Foundation. This work was supported by the NIHR UCL/H Biomedical Research Centre, the Leonard Wolfson Experimental Neurology Centre (LWENC) Clinical Research Facility, and the UK Dementia Research Institute, which receives its funding from UK DRI Ltd, funded by the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK. This work was also supported by the JPND GENFI-PROX grant (2019-02248; to JDR, MO, BB, CG, JvS and MS. [latter via DLR/DFG 01ED2008B]). JDR is supported by the Miriam Marks Brain Research UK Senior Fellowship and has received funding from an MRC Clinician Scientist Fellowship (MR/M008525/1) and the NIHR Rare Disease Translational Research Collaboration (BRC149/NS/MH). This work was also supported by the MRC UK GENFI grant (MR/M023664/1), the Bluefield Project and the JPND GENFI-PROX grant (2019-02248). Several authors of this publication are members of the European Reference Network for Rare Neurological Diseases—Project ID No 739510. RC/CG are supported by a Frontotemporal Dementia Research Studentships in Memory of David Blechner funded through The National Brain Appeal (RCN 290173). MB is supported by a Fellowship award from the Alzheimer's Society, UK (AS-JF-19a-004-517). MB's work is also supported by the UK Dementia Research Institute which receives its funding from DRI Ltd, funded by the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK. JCVS was supported by the Dioraphte Foundation grant 09-02-03-00, the Association for Frontotemporal Dementias Research Grant 2009, The Netherlands Organization for Scientific Research (NWO) grant HCMI 056-13-018, ZonMw Memorabel (Deltaplan Dementie, project number 733 051 042), Alzheimer Nederland and the Bluefield project. FM received funding from the Tau Consortium and the Center for Networked Biomedical Research on Neurodegenerative Disease (CIBERNED). RS-V is supported by an Alzheimer's Research UK Clinical Research Training Fellowship (ARUK-CRF2017B-2), and has received funding from Fundació Marató de TV3, Spain (grant no. 20143810). CG received funding from JPND-Prefrontals VR Dnr 529-2014-7504, VR 2015-02926 and 2018-02754, the Swedish FTD Inititative-Schörling Foundation, Alzheimer Foundation, Brain Foundation and Stockholm County Council ALF. MM has received funding from a Canadian Institute of Health Research operating grant and the Weston Brain Institute and Ontario Brain Institute. JBR has received funding from the Welcome Trust (103838) and is supported by the Cambridge University Centre for Frontotemporal Dementia, the Medical Research Council (SUAG/051 G101400) and the National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre (BRC-1215-20014). EF has received funding from a CIHR grant #327387. DG received support from the EU Joint Programme—Neurodegenerative Disease Research (JPND) and the Italian Ministry of Health (PreFrontALS) grant 733051042. RV has received funding from the Mady Browaeys Fund for Research into Frontotemporal Dementia. MO has received funding from BMBF (FTLDc). JL received funding for this work by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy—ID 390857198). Group authorship for the Genetic FTD Initiative (GENFI): Annabel Nelson: Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK. David L Thomas: Neuroimaging Analysis Centre, Department of Brain Repair and Rehabilitation, UCL Institute of Neurology, Queen Square, London, UK. Emily Todd: Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK. Hanya Benotmane: UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK. Jennifer Nicholas: Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK. Rachelle Shafei: Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK. Carolyn Timberlake: Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK. Thomas Cope: Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK. Timothy Rittman: Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK. Alberto Benussi: Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy. Enrico Premi: Stroke Unit, ASST Brescia Hospital, Brescia, Italy. Roberto Gasparotti: Neuroradiology Unit, University of Brescia, Brescia, Italy. Silvana Archetti: Biotechnology Laboratory, Department of Diagnostics, ASST Brescia Hospital, Brescia, Italy. Stefano Gazzina: Neurology, ASST Brescia Hospital, Brescia, Italy. Valentina Cantoni: Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy. Andrea Arighi: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy. Chiara Fenoglio: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy. Elio Scarpini: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy. Giorgio Fumagalli: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy. Vittoria Borracci: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy. Giacomina Rossi: Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy. Giorgio Giaccone: Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy. Giuseppe Di Fede: Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy. Paola Caroppo: Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy. Pietro Tiraboschi: Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy. Sara Prioni: Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy. Veronica Redaelli: Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy. David Tang-Wai: The University Health Network, Krembil Research Institute, Toronto, Canada. Ekaterina Rogaeva: Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada. Miguel Castelo-Branco: Faculty of Medicine, University of Coimbra, Coimbra, Portugal. Morris Freedman: Baycrest Health Sciences, Rotman Research Institute, University of Toronto, Toronto, Canada. Ron Keren: The University Health Network, Toronto Rehabilitation Institute, Toronto, Canada. Sandra Black: Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada. Sara Mitchell: Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada. Christen Shoesmith: Department of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada. Robart Bartha: Department of Medical Biophysics, The University of Western Ontario, London,

European Radiology (2023) 33:5368–5377
https://doi.org/10.1007/s00330-022-09338-6

NEURO

Quantitative susceptibility mapping of the normal-appearing white matter as a potential new marker of disability progression in multiple sclerosis

Anna M. Pietroboni[1] · Annalisa Colombi[1] · Valeria E. Contarino[1] · Francesco Maria Lo Russo[1] · Giorgio Conte[1,2] · Aurelia Morabito[3] · Silvia Siggillino[1] · Tiziana Carandini[1] · Chiara Fenoglio[2] · Andrea Arighi[1] · Milena A. De Riz[1] · Marina Arcaro[1] · Luca Sacchi[2] · Giorgio G. Fumagalli[1] · Anna Maria Bianchi[4] · Fabio Triulzi[1,2] · Elio Scarpini[1] · Daniela Galimberti[1,2]

Received: 13 April 2022 / Revised: 3 October 2022 / Accepted: 29 November 2022 / Published online: 23 December 2022

Abstract

Objectives To investigate the normal-appearing white matter (NAWM) susceptibility in a cohort of newly diagnosed multiple sclerosis (MS) patients and to evaluate possible correlations between NAWM susceptibility and disability progression.

Methods Fifty-nine patients with a diagnosis of MS (n = 53) or clinically isolated syndrome (CIS) (n = 6) were recruited and followed up. All participants underwent neurological examination, blood sampling for serum neurofilament light chain (sNfL) level assessment, lumbar puncture for the quantification of cerebrospinal fluid (CSF) β-amyloid$_{1\text{-}42}$ (Aβ) levels, and brain MRI. T2-weighted scans were used to quantify white matter (WM) lesion loads. For each scan, we derived the NAWM volume fraction and the WM lesion volume fraction. Quantitative susceptibility mapping (QSM) of the NAWM was calculated using the susceptibility tensor imaging (STI) suite. Susceptibility maps were computed with the STAR algorithm.

Results Primary progressive patients (n = 9) showed a higher mean susceptibility value in the NAWM than relapsing-remitting (n = 44) and CIS (n = 6) (p = 0.01 and p = 0.02). Patients with a higher susceptibility in the NAWM showed increased sNfL concentration (ρ = 0.38, p = 0.004) and lower CSF Aβ levels (ρ = −0.34, p = 0.009). Mean NAWM susceptibility turned out to be a predictor of the expanded disability status scale (EDSS) worsening at follow-up (β = 0.41, t = 2.66, p = 0.01) and of the MS severity scale (MSSS) (β = 0.38, t = 2.43, p = 0.019).

Conclusions QSM in the NAWM seems to predict the EDSS increment over time. This finding might provide evidence on the role of QSM in identifying patients with an increased *risk* of early disability *progression*.

Key Points

- *NAWM-QSM is higher in PPMS patients than in RRMS.*
- *NAWM-QSM seems to be a predictor of EDSS worsening over time.*
- *Patients with higher NAWM-QSM show increased sNfL concentration and lower CSF Aβ levels.*

Keywords Multiple sclerosis · Brain · White matter · Magnetic resonance imaging · Follow-up studies

✉ Anna M. Pietroboni
anna.pietroboni@policlinico.mi.it

[1] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122 Milan, Italy

[2] University of Milan, Milan, Italy

[3] Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy

[4] Politecnico di Milano, Milan, Italy

Abbreviations

Aβ	β-Amyloid$_{1\text{-}42}$
CIS	Clinically isolated syndrome
CSF	Cerebrospinal fluid
EDSS	Expanded disability status scale
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSSS	Multiple sclerosis severity score
NAWM	Normal-appearing white matter
NAWM-QSM	mean QSM value in the NAWM mask
NAWM-VF	NAWM volume fraction

treatment before the first MRI. Even though this time frame was very short (< 2 months), we cannot completely exclude that DTMs could have partly influenced the radiological parameters. Fifth, no healthy controls were included in the study. Accordingly, it is not possible to speculate whether QSM measures in the NAWM are pathological or not. The data interpretation of the increase in NAWM susceptibility is based on assumptions that need confirmation through combined advanced imaging techniques or neuropathology.

In conclusion, this study provides evidence of the potential role of QSM in the assessment of subtle early-onset WM changes, suggesting its possible use as a prognostic biomarker of disease progression in MS patients. A replication in a larger cohort of patients is required to confirm these preliminary data.

Funding This study was supported by the Italian Ministry of Health ("Ricerca Corrente" to ES and FT) and Dino Ferrari Center.

Declarations

Guarantor The scientific guarantor of this publication is Anna Pietroboni.

Conflict of interest The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Statistics and biometry No complex statistical methods were necessary for this paper.

Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Methodology
- prospective
- observational
- cross-sectional study
- performed at one institution

References

1. Reich DS, Lucchinetti CF, Calabresi PA (2018) Multiple sclerosis. N Engl J Med 378(2):169–180
2. Frischer JM, Bramow S, Dal-Bianco A et al (2009) The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 132(Pt 5):1175–1189
3. Lassmann H (2014) Multiple sclerosis: lessons from molecular neuropathology. Exp Neurol 262(Pt A):2–7
4. LeVine SM (1997) Iron deposits in multiple sclerosis and Alzheimer's disease brains. Brain Res 760(1-2):298–303
5. Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9(11):839–855
6. Thompson AJ, Banwell BL, Barkhof F et al (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 7(2):162–173
7. Bodini B, Louapre C, Stankoff B (2015) Advanced imaging tools to investigate multiple sclerosis pathology. Presse Med 44(4 Pt 2): e159–e167
8. Stankoff B, Freeman L, Aigrot MS et al (2011) Imaging central nervous system myelin by positron emission tomography in multiple sclerosis using [methyl-^{11}C]-2-(4'-methylaminophenyl)- 6-hydroxybenzothiazole. Ann Neurol 69(4):673–680
9. Filippi M, Paty DW, Kappos L et al (1995) Correlations between changes in disability and T2-weighted brain MRI activity in multiple sclerosis: a follow-up study. Neurology 45(2):255–260
10. Cree BAC, Hollenbach JA, Bove R et al (2019) Silent progression in disease activity-free relapsing multiple sclerosis. Ann Neurol 85(5):653–666
11. Langkammer C, Schweser F, Krebs N et al (2012) Quantitative susceptibility mapping (QSM) as a means to measure brain iron? A post mortem validation study. Neuroimage 62(3):1593–1599
12. Stüber C, Morawski M, Schäfer A et al (2014) Myelin and iron concentration in the human brain: a quantitative study of MRI contrast. Neuroimage 93(Pt 1):95–106
13. Gaeta M, Cavallaro M, Vinci SL et al (2021) Magnetism of materials: theory and practice in magnetic resonance imaging. Insights Imaging 12(1):179
14. Liu C, Li W, Tong KA et al (2015) Susceptibility-weighted imaging and quantitative susceptibility mapping in the brain. J Magn Reson Imaging 42(1):23–41
15. Wisnieff C, Ramanan S, Olesik J et al (2015) Quantitative susceptibility mapping (QSM) of white matter multiple sclerosis lesions: interpreting positive susceptibility and the presence of iron. Magn Reson Med 74(2):564–570
16. Bagnato F, Hametner S, Yao B et al (2011) Tracking iron in multiple sclerosis: a combined imaging and histopathological study at 7 Tesla. Brain 134(Pt 12):3602–3615
17. Lassmann H (2008) The pathologic substrate of magnetic resonance alterations in multiple sclerosis. Neuroimaging Clin N Am 18(4):563–576
18. Hametner S, Wimmer I, Haider L et al (2013) Iron and neurodegeneration in the multiple sclerosis brain. Ann Neurol 74(6):848–861
19. Zhang S, Nguyen TD, Hurtado Rúa SM et al (2019) Quantitative susceptibility mapping of time-dependent susceptibility changes in multiple sclerosis lesions. AJNR Am J Neuroradiol 40(6):987–993
20. Mehta V, Pei W, Yang G et al (2013) Iron is a sensitive biomarker for inflammation in multiple sclerosis lesions. PLoS One 8(3): e57573
21. Yu FF, Chiang FL, Stephens N et al (2019) Characterization of normal-appearing white matter in multiple sclerosis using quantitative susceptibility mapping in conjunction with diffusion tensor imaging. Neuroradiology 61(1):71–79
22. Chen W, Zhang Y, Mu K et al (2017) Quantifying the susceptibility variation of normal-appearing white matter in multiple sclerosis by quantitative susceptibility mapping. AJR Am J Roentgenol 209(4): 889–894
23. Rudko DA, Solovey I, Gati JS et al (2014) Multiple sclerosis: improved identification of disease-relevant changes in gray and white matter by using susceptibility-based MR imaging. Radiology 272(3):851–864
24. Wiggermann V, Hametner S, Hernández-Torres E et al (2017) Susceptibility-sensitive MRI of multiple sclerosis lesions and the

frontiers | Frontiers in Aging Neuroscience

TYPE Original Research
PUBLISHED 09 January 2023
DOI 10.3389/fnagi.2022.1058665

OPEN ACCESS

EDITED BY
Jeffrey Liddell,
The University of Melbourne, Australia

REVIEWED BY
Meagan McManus,
Children's Hospital of Philadelphia, United States
Heather M. Wilkins,
University of Kansas Medical Center Research Institute, United States

*CORRESPONDENCE
Stefano Salvioli
✉ stefano.salvioli@unibo.it

SPECIALTY SECTION
This article was submitted to Cellular and Molecular Mechanisms of Brain-aging, a section of the journal Frontiers in Aging Neuroscience

RECEIVED 30 September 2022
ACCEPTED 08 December 2022
PUBLISHED 09 January 2023

CITATION
Chiariello A, Valente S, Pasquinelli G, Baracca A, Sgarbi G, Solaini G, Medici V, Fantini V, Poloni TE, Tognocchi M, Arcaro M, Galimberti D, Franceschi C, Capri M, Salvioli S and Conte M (2023) The expression pattern of GDF15 in human brain changes during aging and in Alzheimer's disease.
Front. Aging Neurosci. 14:1058665.
doi: 10.3389/fnagi.2022.1058665

The expression pattern of GDF15 in human brain changes during aging and in Alzheimer's disease

Antonio Chiariello[1], Sabrina Valente[1], Gianandrea Pasquinelli[1], Alessandra Baracca[2], Gianluca Sgarbi[2], Giancarlo Solaini[2], Valentina Medici[3], Valentina Fantini[3], Tino Emanuele Poloni[3], Monica Tognocchi[4], Marina Arcaro[5], Daniela Galimberti[5], Claudio Franceschi[6], Miriam Capri[1,7], Stefano Salvioli[1,7]* and Maria Conte[1,7]

[1]Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy, [2]Department of Biomedical and Neuromotor Sciences (DIBINEM), Laboratory of Biochemistry and Mitochondrial Pathophysiology, University of Bologna, Bologna, Italy, [3]Department of Neurology and Neuropathology, Golgi-Cenci Foundation, Milan, Italy, [4]Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy, [5]Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico, Milan, Italy, [6]Department of Applied Mathematics of the Institute of ITMM, National Research Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia, [7]Interdepartmental Centre "Alma Mater Research Institute on Global Challenges and Climate Change (Alma Climate)", University of Bologna, Bologna, Italy

Introduction: Growth Differentiation Factor 15 (GDF15) is a mitochondrial-stress-responsive molecule whose expression strongly increases with aging and age-related diseases. However, its role in neurodegenerative diseases, including Alzheimer's disease (AD), is still debated.

Methods: We have characterized the expression of GDF15 in brain samples from AD patients and non-demented subjects (controls) of different ages.

Results: Although no difference in CSF levels of GDF15 was found between AD patients and controls, GDF15 was expressed in different brain areas and seems to be predominantly localized in neurons. The ratio between its mature and precursor form was higher in the frontal cortex of AD patients compared to age-matched controls ($p<0.05$). Moreover, this ratio was even higher for centenarians ($p<0.01$), indicating that aging also affects GDF15 expression and maturation. A lower expression of OXPHOS complexes I, III, and V in AD patients compared to controls was also noticed, and a positive correlation between *GDF15* and *IL-6* mRNA levels was observed. Finally, when GDF15 was silenced *in vitro* in dermal fibroblasts, a decrease in OXPHOS complexes transcript levels and an increase in *IL-6* levels were observed.

Discussion: Although GDF15 seems not to be a reliable CSF marker for AD, it is highly expressed in aging and AD brains, likely as a part of stress response aimed at counteracting mitochondrial dysfunction and neuroinflammation.

KEYWORDS

GDF15, Alzheimer's disease, aging, inflammation, mitochondrial dysfunction

exclude the possibility, though unlikely, that the chronic expression of GDF15 may play a role in the pathogenesis of AD and thus GDF15 could be considered as a potential target to treat AD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by study n. 5,802 approved on 14-09-2021 by Comitato Etico Milano Area 2 the Ethical Committee of Pavia University (Committee report 3/2009). The patients/participants provided their written informed consent to participate in this study.

Author contributions

AC: data generation and collection, statistical analysis, writing of the manuscript. SV, GP: fluorescence microscopy, transmission electron microscopy analysis, manuscript revision. AB, GSg, GSo: analysis of mitochondrial complexes, manuscript revision. VM, VF, TP, DG, MA: samples and sample data provision, manuscript revision. MT: TBARS analysis. CF, MCa: critical discussion of the manuscript. SS, MCo: study design, analysis of the data, writing of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The study was partially supported by the Roberto and Cornelia Pallotti Legacy for Cancer Research to SS and by Centro Dino Ferrari and Fondazione Gigi & Pupa Ferrari to DG.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.1058665/full#supplementary-material

SUPPLEMENTARY FIGURE S1
(A) Representative immunoblotting image of pro-GDF15, m-GDF15, and GAPDH in the frontal cortex (Fr), hippocampus (Hi), temporal cortex (Te), parietal cortex (Pa) and cerebellum (Ce). **(B–G)** Relative protein expression levels of pro-GDF15, m-GDF15, and m-GDF15/pro-GDF15 ratio from **(B–D)** 2 non-demented old subjects (NDO) and **(E–G)** 4 AD patients (AD). The bars represent mean±SE. Student's *t* and one-way ANOVA tests with Bonferroni correction were applied. Western blotting quantification was performed using ImageJ software and normalized to GAPDH expression. *$p<0.05$.

SUPPLEMENTARY FIGURE S2
(A) Representative immunoblotting image of pro-GDF15, m-GDF15, and GAPDH in the parietal cortex. **(B)** pro-GDF15 and (C) m-GDF15 protein relative expression in the parietal cortex from seven non-demented old subjects (NDO) and 11 AD patients (AD). The bars represent mean±SE. Student's *t* test was applied. Western blotting quantification was performed using ImageJ software and normalized to GAPDH expression.

SUPPLEMENTARY FIGURE S3
Relative transcript levels of **(A)** *GDF15* and **(B)** *IL-6* in DFs from five non-demented old subjects in the age range 73–78 (ND) and 3 AD patients (AD), considered separately, treated with scramble siRNA or GDF15 siRNA. The bars represent mean±SE. Student's *t* and one-way ANOVA tests with Bonferroni correction were applied.

References

Abulizi, P., Loganathan, N., Zhao, D., Mele, T., Zhang, Y., Zwiep, T., et al. (2017). Growth differentiation factor-15 deficiency augments inflammatory response and exacerbates septic heart and renal injury induced by lipopolysaccharide. *Sci. Rep.* 7:1037. doi: 10.1038/s41598-017-00902-5

Aguilar Diaz De Leon, J., and Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the Thiobarbituric acid reactive substances assay. *J. Vis. Exp.* 159:10.3791/61122. doi: 10.3791/61122

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®) [Internet]*. Washington, DC: American Psychiatric Publishing.

Andersson, J., Fall, T., Delicano, R., Wennberg, P., and Jansson, J. H. (2020). GDF-15 is associated with sudden cardiac death due to incident myocardial infarction. *Resuscitation* 152, 165–169. doi: 10.1016/j.resuscitation.2020.05.001

Baleriola, J., Walker, C. A., Jean, Y. Y., Crary, J. F., Troy, C. M., Nagy, P. L., et al. (2014). Axonally synthesized ATF4 transmits a neurodegenerative signal across brain regions. *Cells* 158, 1159–1172. doi: 10.1016/j.cell.2014.07.001

Barbato, S., Sgarbi, G., Gorini, G., Baracca, A., and Solaini, G. (2015). The inhibitor protein (IF1) of the F1F0-ATPase modulates human osteosarcoma cell bioenergetics. *J. Biol. Chem.* 290, 6338–6348. doi: 10.1074/jbc.M114.631788

Beck, J. S., Mufson, E. J., and Counts, S. E. (2016). Evidence for mitochondrial UPR gene activation in familial and sporadic Alzheimer's disease. *Curr. Alzheimer Res.* 13, 610–614. doi: 10.2174/1567205013666151221145445

Bootcov, M. R., Bauskin, A. R., Valenzuela, S. M., Moore, A. G., Bansal, M., He, X. Y., et al. (1997). MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc. Natl. Acad. Sci. U. S. A.* 94, 11514–11519. doi: 10.1073/pnas.94.21.11514

GeroScience (2023) 45:1557–1571
https://doi.org/10.1007/s11357-023-00746-0

ORIGINAL ARTICLE

Plasma microglial-derived extracellular vesicles are increased in frail patients with Mild Cognitive Impairment and exert a neurotoxic effect

C. Visconte · M.T. Golia · C. Fenoglio · M. Serpente · M. Gabrielli · M. Arcaro · F. Sorrentino · M. Busnelli · A. Arighi · G. Fumagalli · E. Rotondo · P. Rossi · B. Arosio · E. Scarpini · C. Verderio · D. Galimberti

Received: 17 October 2022 / Accepted: 26 January 2023 / Published online: 1 February 2023

Abstract Extracellular vesicles (EVs) are mediators of cellular communication that can be released by almost all cell types in both physiological and pathological conditions and are present in most biological fluids. Such characteristics make them attractive in the research of biomarkers for age-related pathological conditions. Based on this, the aim of the present study was to examine the changes in EV concentration and size in the context of frailty, a geriatric syndrome associated with a progressive physical and cognitive decline. Specifically, total EVs and neural and microglial-derived EVs (NDVs and MDVs respectively) were investigated in plasma of frail and non-frail controls (CTRL), mild cognitive impairment (MCI) subjects, and in Alzheimer's disease (AD) patients. Results provided evidence that AD patients displayed diminished NDV concentration ($3.61 \times 10^9 \pm 1.92 \times 10^9$ vs $7.16 \times 10^9 \pm 4.3 \times 10^9$ particles/ml) and showed high diagnostic performance. They are able to discriminate between AD and CTRL with an area under the curve of 0.80, a sensitivity of 78.95% and a specificity of 85.7%, considering the cut-off of 5.27×10^9 particles/ml. Importantly, we also found that MDV concentration was increased in frail MCI patients compared to CTRL ($5.89 \times 10^9 \pm 3.98 \times 10^9$ vs $3.16 \times 10^9 \pm 3.04 \times 10^9$ particles/ml, $P < 0.05$) and showed high neurotoxic effect on neurons. MDV concentration discriminate frail MCI vs non-frail CTRL (AUC = 0.76) with a sensitivity of 80% and a specificity of 70%, considering the cut-off of 2.69×10^9 particles/ml. Altogether, these results demonstrated an alteration in NDV and MDV release during cognitive decline, providing important insight into the role of EVs in frailty status.

Keywords Extracellular vesicles · Frailty · Mild cognitive impairment · Microglial derived extracellular vesicles · Neuronal derived extracellular vesicles

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11357-023-00746-0.

C. Visconte · F. Sorrentino · D. Galimberti
Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

M. Golia · M. Gabrielli · M. Busnelli · C. Verderio
CNR, Institute of Neuroscience, Vedano al Lambro, Monza and Brianza, Milan, Italy

C. Fenoglio (✉)
Department of Physiopathology and Transplantation, University of Milan, "Dino Ferrari" Center, Milan, Italy
e-mail: chiara.fenoglio@unimi.it

M. Serpente · M. Arcaro · A. Arighi · G. Fumagalli · E. Rotondo · P. Rossi · E. Scarpini · D. Galimberti
Fondazione, IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy

B. Arosio
Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

Journal of the Neurological Sciences 446 (2023) 120590

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

The Benson Complex Figure Test detects deficits in visuoconstruction and visual memory in symptomatic familial frontotemporal dementia: A GENFI study

Lize C. Jiskoot [a,b,*], Lucy L. Russell [b], Georgia Peakman [b], Rhian S. Convery [b], Caroline V. Greaves [b], Martina Bocchetta [b], Jackie M. Poos [a], Harro Seelaar [a], Lucia A.A. Giannini [a], John C. van Swieten [a], Rick van Minkelen [c], Yolande A.L. Pijnenburg [d], James B. Rowe [e], Barbara Borroni [f], Daniela Galimberti [g,h], Mario Masellis [i], Carmela Tartaglia [j], Elizabeth Finger [k], Chris R. Butler [l], Caroline Graff [m], Robert Laforce Jr [n], Raquel Sanchez-Valle [o], Alexandre de Mendonça [p], Fermin Moreno [q], Matthias Synofzik [r,s], Rik Vandenberghe [t], Simon Ducharme [u], Isabelle le Ber [v,w,x], Johannes Levin [y,z,aa], Markus Otto [ab], Florence Pasquier [ac,ad,ae], Isabel Santana [af], David M. Cash [b], David Thomas [b], Jonathan D. Rohrer [b], on behalf of Genetic Frontotemporal dementia Initiative (GENFI)[1]

[a] *Department of Neurology, Erasmus Medical Center, Rotterdam, the Netherlands*
[b] *Dementia Research Centre, University College London, London, UK*
[c] *Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands*
[d] *Alzheimer Center and Department of Neurology, Neuroscience Campus Amsterdam, Amsterdam, the Netherlands*
[e] *Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK*
[f] *Centre for Neurodegenerative Disorders, Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy*
[g] *University of Milan, Centro Dino Ferrari, Milan, Italy*
[h] *Fondazione IRCCS Ca' Granda, Ospedale Policlinico, Neurodegenerative Diseases Unit, Milan, Italy*
[i] *Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada*
[j] *Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada.*
[k] *Department of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada*
[l] *Department of Clinical Neurology, University of Oxford, Oxford, UK*
[m] *Department of Geriatric Medicine, Karolinska University Hospital-Huddinge, Stockholm, Sweden.*
[n] *Clinique Interdisciplinaire de Mémoire, Département des Sciences Neurologiques, Université Laval, Québec, Canada*
[o] *Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic de Barcelona, Institut d'Investigacións Biomèdiques August Pi I Sunyer, University of Barcelona, Barcelona, Spain*
[p] *Faculty of Medicine, University of Lisbon, Lisbon, Portugal*
[q] *Cognitive Disorders Unit, Department of Neurology, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain*
[r] *Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany*
[s] *German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany*
[t] *Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium*
[u] *Department of Psychiatry, McGill University Health Centre, McGill University, Montreal, Québec, Canada*
[v] *Paris Brain Institute - Institut du Cerveau - Hôpital Pitié-Salpêtrière, Sorbonne Université, Paris, France*
[w] *Centre de référence des démences rares ou précoces, IM2A, Département de Neurologie, Hôpital Pitié-Salpêtrière, Paris, France*
[x] *Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France*
[y] *Department of Neurology, Ludwig-Maximilians-University, Munich, Germany*

* Corresponding author at: Erasmus Medical Center, Department of Neurology, NF-331, Post box 2040, 3000 CA Rotterdam, the Netherlands.
E-mail addresses: l.c.jiskoot@erasmusmc.nl (L.C. Jiskoot), l.russell@ucl.ac.uk (L.L. Russell), georgia.peakman.18@alumni.ucl.ac.uk (G. Peakman), rhian.convery.16@ucl.ac.uk (R.S. Convery), caroline.greaves.14@ucl.ac.uk (C.V. Greaves), m.bocchetta@ucl.ac.uk (M. Bocchetta), j.m.poos@erasmusmc.nl (J.M. Poos), h.seelaar@erasmusmc.nl (H. Seelaar), l.a.a.giannini@erasmusmc.nl (L.A.A. Giannini), j.c.vanswieten@erasmusmc.nl (J.C. van Swieten), r.vanminkelen@erasmusmc.nl (R. van Minkelen), y.pijnenburg@vumc.nl (Y.A.L. Pijnenburg), james.rowe@mrc-cbu.cam.ac.uk (J.B. Rowe), daniela.galimberti@unimi.it (D. Galimberti), mario.masellis@sunnybrook.ca (M. Masellis), carmela.tartaglia@utoronto.ca (C. Tartaglia), elizabeth.finger@lhsc.on.ca (E. Finger), chris.butler@ndcn.ox.ac.uk (C.R. Butler), caroline.graff@ki.se (C. Graff), robert.laforce@fmed.ulaval.ca (R. Laforce), rsanchez@clinic.cat (R. Sanchez-Valle), mendonca@medicina.ulisboa.pt (A. de Mendonça), matthis.synofzik@uni-tuebingen.de (M. Synofzik), rik.vandenberghe@uzleuven.be (R. Vandenberghe), simon.ducharme@mcgill.ca (S. Ducharme), johannes.levin@med.uni-muenchen.de (J. Levin), markus.otto@uni-ulm.de (M. Otto), florence.pasquier@chru-lille.fr (F. Pasquier), isabelle.leber@upmc.fr (I. Santana), d.cash@ucl.ac.uk (D.M. Cash), d.thomas@ucl.ac.uk (D. Thomas), j.rohrer@ucl.ac.uk (J.D. Rohrer).

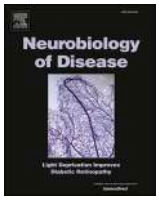
https://doi.org/10.1016/j.jns.2023.120590
Received 26 October 2022; Received in revised form 24 January 2023; Accepted 13 February 2023
Available online 16 February 2023

Neurobiology of Disease 179 (2023) 106068

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Early neurotransmitters changes in prodromal frontotemporal dementia: A GENFI study

Enrico Premi [a,1], Marta Pengo [b,c,1], Irene Mattioli [c], Valentina Cantoni [c], Juergen Dukart [d,e], Roberto Gasparotti [f], Emanuele Buratti [g], Alessandro Padovani [a,c], Martina Bocchetta [h], Emily G. Todd [h], Arabella Bouzigues [h], David M. Cash [h,i], Rhian S. Convery [h], Lucy L. Russell [h], David L. Thomas [h,j], John C. van Swieten [k], Lize C. Jiskoot [k], Harro Seelaar [k], Daniela Galimberti [l,m], Raquel Sanchez-Valle [n], Robert Laforce Jr [o], Fermin Moreno [p], Matthis Synofzik [q,r], Caroline Graff [s,t], Mario Masellis [u], Maria Carmela Tartaglia [v], James B. Rowe [w], Kamen A. Tsvetanov [w], Rik Vandenberghe [x], Elizabeth Finger [y], Fabrizio Tagliavini [z], Alexandre de Mendonça [aa], Isabel Santana [ab], Chris R. Butler [ac], Simon Ducharme [ad], Alexander Gerhard [ae,af], Adrian Danek [ag], Johannes Levin [ag], Markus Otto [ah], Sandro Sorbi [ai,aj], Isabelle Le Ber [ak,al,am,an], Florence Pasquier [ao,ap,1], Jonathan D. Rohrer [h], Barbara Borroni [a,c,*], on behalf of the Genetic Frontotemporal dementia Initiative (GENFI)

[a] *Stroke Unit, Neurology Unit, Department of Neurological and Vision Sciences, ASST Spedali Civili, Brescia, Italy*
[b] *Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy*
[c] *Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy*
[d] *Institute of Neuroscience and Medicine, Brain & Behaviour (INM-7), Research CentreJülich, Jülich, Germany*
[e] *Institute of Systems Neuroscience, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[f] *Neuroradiology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, Brescia, Italy*
[g] *ICGEB, Trieste, Italy*
[h] *Dementia Research Centre, Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom*
[i] *Centre for Medical Image Computing, Department of Medical Physics and Biomedical Engineering, University College London, London, United Kingdom*
[j] *Neuroradiological Academic Unit, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom*
[k] *Department of Neurology and Alzheimer center, Erasmus Medical Center Rotterdam, the Netherlands*
[l] *Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy*
[m] *Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy*
[n] *Neurology Department, Hospital Clinic, Institut d'Investigacions Biomèdiques, Barcelona, Spain*
[o] *Clinique Interdisciplinaire de Mémoire, Département des Sciences Neurologiques, CHU de Québec, Faculté de Médecine, Université Laval, Québec, Canada*
[p] *Hospital Universitario Donostia, San Sebastian, Spain*
[q] *Division Translational Genomics of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research (HIH), University of Tübingen, Tübingen, Germany*
[r] *German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany*
[s] *Karolinska Institutet, Department NVS, Division of Neurogeriatrics, Stockholm, Sweden*
[t] *Unit for Hereditray Dementia, Theme Aging, Karolinska University Hospital-Solna, Stockholm, Sweden*
[u] *Campbell Cognitive Neurology Research Unit, Sunnybrook Research Institute, Toronto, ON, Canada*
[v] *Toronto Western Hospital, Tanz Centre for Research in Neurodegenerative Disease, Toronto, ON, Canada*
[w] *Department of Clinical Neurosciences and Cambridge University Hospitals NHS Trust and Medical Research Council Cognition and brain Sciences Unit, University of Cambridge, Cambridge, United Kingdom*
[x] *Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium*
[y] *Department of Clinical Neurological Sciences, University of Western Ontario, London, ON, Canada*
[z] *Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Neurologico Carlo Besta, Milan, Italy*
[aa] *Faculty of Medicine, University of Lisbon, Lisbon, Portugal*
[ab] *Neurology Department, Centro Hospitalar e Universitário de Coimbra, Portugal*
[ac] *Department of Clinical Neurology, University of Oxford, Oxford, United Kingdom*
[ad] *Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada*
[ae] *Division of Neuroscience and Experimental Psychology, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, United Kingdom*
[af] *Departments of Geriatric Medicine and Nuclear Medicine, University of Duisburg-, Essen, Germany*

* Corresponding author at: Clinica Neurologica, Dipartimento Scienze Cliniche e Sperimentali, Università degli Studi di Brescia, P.le Spedali Civili 1, 25123 Brescia, Italy.
E-mail address: bborroni7@gmail.com (B. Borroni).

https://doi.org/10.1016/j.nbd.2023.106068
Received 5 February 2023; Received in revised form 1 March 2023; Accepted 4 March 2023
Available online 8 March 2023

Appendix A. Appendix

List of GENFI consortium authors:

Author	Affiliation
	Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK;
Aitana Sogorb Esteve	UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK
Carolin Heller	Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
Caroline V Greaves	Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
Henrik Zetterberg	UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK; Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
Imogen J Swift	Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK; UK Dementia Research Institute at University College London, UCL Queen Square Institute of Neurology, London, UK
Kiran Samra	Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
Rachelle Shafei	Department of Neurodegenerative Disease, Dementia Research Centre, UCL Queen Square Institute of Neurology, London, UK
Carolyn Timberlake	Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
Thomas Cope	Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK
Timothy Rittman	Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
Andrea Arighi	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy
Chiara Fenoglio	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy
Elio Scarpini	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy
Giorgio Fumagalli	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy
Vittoria Borracci	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
Giacomina Rossi	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
Giorgio Giaccone	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
Giuseppe Di Fede	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
Paola Caroppo	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
Pietro Tiraboschi	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
Sara Prioni	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
Veronica Redaelli	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
David Tang-Wai	The University Health Network, Krembil Research Institute, Toronto, Canada
Ekaterina Rogaeva	Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada
Miguel Castelo-Branco	Faculty of Medicine, University of Coimbra, Coimbra, Portugal
Morris Freedman	Baycrest Health Sciences, Rotman Research Institute, University of Toronto, Toronto, Canada
Ron Keren	The University Health Network, Toronto Rehabilitation Institute, Toronto, Canada
Sandra Black	Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada
Sara Mitchell	Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada
Christen Shoesmith	Department of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada
Robart Bartha	Department of Medical Biophysics, The University of Western Ontario, London, Ontario, Canada; Centre for Functional and Metabolic Mapping, Robarts Research Institute, The University of Western Ontario, London, Ontario, Canada
Rosa Rademakers	Center for Molecular Neurology, University of Antwerp
Jackie Poos	Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands
Janne M. Papma	Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands
Lucia Giannini	Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands
Rick van Minkelen	Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, Netherlands
Yolande Pijnenburg	Amsterdam University Medical Centre, Amsterdam VUmc, Amsterdam, Netherlands
Benedetta Nacmias	Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
Camilla Ferrari	Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
Cristina Polito	Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", Nuclear Medicine Unit, University of Florence, Florence, Italy
Gemma Lombardi	Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
Valentina Bessi	Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
Michele Veldsman	Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford, Oxford, UK
Christin Andersson	Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
Hakan Thonberg	Center for Alzheimer Research, Division of Neurogeriatrics, Karolinska Institutet, Stockholm, Sweden
Linn Öijerstedt	Center for Alzheimer Research, Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Solna, Sweden; Unit for Hereditary Dementias, Theme Aging, Karolinska University Hospital, Solna, Sweden
Vesna Jelic	Division of Clinical Geriatrics, Karolinska Institutet, Stockholm, Sweden
Paul Thompson	Division of Neuroscience and Experimental Psychology, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK
Tobias Langheinrich	Division of Neuroscience and Experimental Psychology, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK; Manchester Centre for Clinical Neurosciences, Department of Neurology, Salford Royal NHS Foundation Trust, Manchester, UK
Albert Lladó	Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Barcelona, Spain
Anna Antonell	Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Barcelona, Spain
Jaume Olives	Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Barcelona, Spain
Mircea Balasa	Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Barcelona, Spain
Nuria Bargalló	Imaging Diagnostic Center, Hospital Clínic, Barcelona, Spain
Sergi Borrego-Ecija	Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Barcelona, Spain
Ana Verdelho	Department of Neurosciences and Mental Health, Centro Hospitalar Lisboa Norte - Hospital de Santa Maria & Faculty of Medicine, University of Lisbon, Lisbon, Portugal
Carolina Maruta	Laboratory of Language Research, Centro de Estudos Egas Moniz, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
Catarina B. Ferreira	Laboratory of Neurosciences, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
Gabriel Miltenberger	Faculty of Medicine, University of Lisbon, Lisbon, Portugal
Frederico Simões do Couto	Faculdade de Medicina, Universidade Católica Portuguesa

(*continued on next page*)

BRAIN COMMUNICATIONS

Genetic forms of primary progressive aphasia within the GENetic Frontotemporal dementia Initiative (GENFI) cohort: comparison with sporadic primary progressive aphasia

Kiran Samra,[1] Amy M. MacDougall,[2] Arabella Bouzigues,[1] Martina Bocchetta,[1] David M. Cash,[1] Caroline V. Greaves,[1] Rhian S. Convery,[1] Chris Hardy,[1] John C. van Swieten,[3] Harro Seelaar,[3] Lize C. Jiskoot,[3] Fermin Moreno,[4,5] Raquel Sanchez-Valle,[6] Robert Laforce,[7] Caroline Graff,[8,9] Mario Masellis,[10] Maria Carmela Tartaglia,[11] James B. Rowe,[12] Barbara Borroni,[13] Elizabeth Finger,[14] Matthis Synofzik,[15,16] Daniela Galimberti,[17,18] Rik Vandenberghe,[19,20,21] Alexandre de Mendonça,[22] Chris R. Butler,[23,24] Alexander Gerhard,[25,26] Simon Ducharme,[27,28] Isabelle Le Ber,[29,30,31] Isabel Santana,[32,33] Florence Pasquier,[34,35,36] Johannes Levin,[37,38,39] Markus Otto,[40] Sandro Sorbi,[41,42] Jason D. Warren,[1] Jonathan D. Rohrer,[1]* and Lucy L. Russell[1]* GENetic Frontotemporal dementia Initiative (GENFI)

* These authors contributed equally to this work.

Primary progressive aphasia is most commonly a sporadic disorder, but in some cases, it can be genetic. This study aimed to understand the clinical, cognitive and imaging phenotype of the genetic forms of primary progressive aphasia in comparison to the canonical nonfluent, semantic and logopenic subtypes seen in sporadic disease. Participants with genetic primary progressive aphasia were recruited from the international multicentre GENetic Frontotemporal dementia Initiative study and compared with healthy controls as well as a cohort of people with sporadic primary progressive aphasia. Symptoms were assessed using the GENetic Frontotemporal dementia Initiative language, behavioural, neuropsychiatric and motor scales. Participants also underwent a cognitive assessment and 3 T volumetric T1-weighted MRI. One *C9orf72* (2%), 1 *MAPT* (6%) and 17 *GRN* (44%) symptomatic mutation carriers had a diagnosis of primary progressive aphasia. In the *GRN* cohort, 47% had a diagnosis of nonfluent variant primary progressive aphasia, and 53% had a primary progressive aphasia syndrome that did not fit diagnostic criteria for any of the three subtypes, called primary progressive aphasia-not otherwise specified here. The phenotype of the genetic nonfluent variant primary progressive aphasia group largely overlapped with that of sporadic nonfluent variant primary progressive aphasia, although the presence of an associated atypical parkinsonian syndrome was characteristic of sporadic and not genetic disease. The primary progressive aphasia -not otherwise specified group however was distinct from the sporadic subtypes with impaired grammar/syntax in the presence of relatively intact articulation, alongside other linguistic deficits. The pattern of atrophy seen on MRI in the genetic nonfluent variant primary progressive aphasia group overlapped with that of the sporadic nonfluent variant primary progressive aphasia cohort, although with more posterior cortical involvement, whilst the primary progressive aphasia-not otherwise specified group was strikingly asymmetrical with involvement particularly of the insula and dorsolateral prefrontal cortex but also atrophy of the orbitofrontal cortex and the medial temporal lobes. Whilst there are overlapping symptoms between genetic and sporadic primary progressive aphasia

Received March 04, 2022. Revised August 26, 2022. Accepted February 15, 2023. Advance access publication February 17, 2023

syndromes, there are also distinct features. Future iterations of the primary progressive aphasia consensus criteria should encompass such information with further research needed to understand the earliest features of these disorders, particularly during the prodromal period of genetic disease.

1 Dementia Research Centre, Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London, UK
2 Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK
3 Department of Neurology, Erasmus Medical Centre, Rotterdam, Netherlands
4 Cognitive Disorders Unit, Department of Neurology, Donostia Universitary Hospital, San Sebastian, Spain
5 Neuroscience Area, Biodonostia Health Research Institute, San Sebastian, Gipuzkoa, Spain
6 Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Institut d'Investigacións Biomèdiques August Pi I Sunyer, University of Barcelona, Barcelona, Spain
7 Clinique Interdisciplinaire de Mémoire, Département des Sciences Neurologiques, CHU de Québec, and Faculté de Médecine, Université Laval, Québec City, Canada
8 Center for Alzheimer Research, Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Solna, Sweden
9 Unit for Hereditary Dementias, Theme Aging, Karolinska University Hospital, Solna, Sweden
10 Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada
11 Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, ON, Canada
12 Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
13 Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
14 Department of Clinical Neurological Sciences, University of Western Ontario, London, ON, Canada
15 Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany
16 Centre for Neurodegenerative Diseases (DZNE), Tübingen, Germany
17 Fondazione Ca' Granda, IRCCS Ospedale Policlinico, Milan, Italy
18 University of Milan, Centro Dino Ferrari, Milan, Italy
19 Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium
20 Neurology Service, University Hospitals Leuven, Leuven, Belgium
21 Leuven Brain Institute, KU Leuven, Leuven, Belgium
22 Laboratory of Neurosciences, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
23 Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford, Oxford, UK
24 Department of Brain Sciences, Imperial College London, London, UK
25 Division of Neuroscience and Experimental Psychology, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK
26 Departments of Geriatric Medicine and Nuclear Medicine, University of Duisburg-Essen, Germany
27 Department of Psychiatry, McGill University Health Centre, McGill University, Montreal, Québec, Canada
28 McConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University, Montreal, Québec, Canada
29 Sorbonne Université, Paris Brain Institute – Institut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
30 Centre de référence des démences rares ou précoces, IM2A, Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
31 Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
32 University Hospital of Coimbra (HUC), Neurology Service, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
33 Center for Neuroscience and Cell Biology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
34 Univ Lille, Inserm 1172, Lille, France
35 Inserm 1172, Lille, France
36 CHU, CNR-MAJ, Labex Distalz, LiCEND Lille, France
37 Department of Neurology, Ludwig-Maximilians Universität München, Munich, Germany
38 German Centre for Neurodegenerative Diseases (DZNE), Munich, Germany
39 Munich Cluster of Systems Neurology (SyNergy), Munich, Germany
40 Department of Neurology, University of Ulm, Ulm, Germany
41 Department of Neurofarba, University of Florence, Florence, Italy
42 IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy

Correspondence to: Dr Lucy L. Russell
Dementia Research Centre, Department of Neurodegenerative Disease
UCL Institute of Neurology, Queen Square
London, UK, WC1N 3BG
E-mail: l.russell@ucl.ac.uk

Keywords: primary progressive aphasia; *GRN*; *c9orf72*; *MAPT*

Journal of Alzheimer's Disease 93 (2023) 1125–1134
DOI 10.3233/JAD-230095
IOS Press

Banks of the Superior Temporal Sulcus in Alzheimer's Disease: A Pilot Quantitative Susceptibility Mapping Study

Luca Sacchi[a], Valeria Elisa Contarino[b,*], Silvia Siggillino[b], Tiziana Carandini[c], Giorgio Giulio Fumagalli[d], Anna Margherita Pietroboni[c], Marina Arcaro[c], Chiara Fenoglio[e], Eva Orunesu[f], Massimo Castellani[f], Silvia Casale[b], Giorgio Conte[b], Chunlei Liu[g], Fabio Triulzi[b,e], Daniela Galimberti[a,c], Elio Scarpini[c,e] and Andrea Arighi[c]
[a]*Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy*
[b]*Department of Neuroradiology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
[c]*Neurodegenerative Diseases Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy*
[d]*Center For Mind/Brain Sciences-CIMeC, University of Trento, Rovereto, Italy*
[e]*Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy*
[f]*Nuclear Medicine Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy*
[g]*Department of Electrical Engineering and Computer Sciences, University of California, Berkeley, CA, USA*

Handling Associate Editor: Marco Bozzali

Accepted 28 March 2023
Pre-press 5 May 2023

Abstract.
Background: Brain iron homeostasis is disrupted in neurodegeneration and areas of iron overload partially overlap with regions of amyloid and tau burden in Alzheimer's disease (AD). Previous studies demonstrated alterations in brain iron accumulation in AD using quantitative susceptibility mapping (QSM).
Objective: Here, we investigate brain alterations of QSM values in AD and non-AD patients as compared to healthy controls (HC) in the superior temporal sulcus and its banks (BANKSSTS), one of the top AD-affected regions.
Methods: Thirty-four patients who underwent brain MRI including a multi-echo gradient-echo sequence were subdivided into AD ($n = 19$) and non-AD ($n = 15$) groups according to their clinical profile, CSF ($A\beta_{42/40}$) and/or amyloid-PET status. Ten HC were also included. QSM values were extracted from left and right BANKSSTS and compared among groups. Correlation and binomial regression analyses between QSM values and CSF-AD biomarkers were conducted.
Results: QSM in left BANKSSTS was significantly different among groups ($p = 0.003$, $H = 11.40$), being higher in AD. QSM values in left BANKSSTS were correlated with $A\beta_{42}$ (rho –0.55, $p = 0.005$), $A\beta_{42/40}$ (rho –0.66, $p < 0.001$), pTau (rho 0.63, $p < 0.001$), tTau (rho 0.56, $p = 0.005$), $tTau/A\beta_{42}$ (rho 0.68, $p < 0.001$) and $pTau/A\beta_{42}$ (rho 0.71, $p < 0.001$). No correlations between QSM values and amyloid-PET SUVR in the left BANKSSTS were found. QSM values in left BANKSSTS showed good accuracy in discriminating AD (AUC = 0.80, $CI_{95\%}$ [0.66–0.93]). Higher QSM values were independent predictors of $A\beta_{42}$ (B = 0.63, $p = 0.032$), $A\beta_{42/40}$ (B = 0.81, $p = 0.028$), pTau (B = 0.96, $p = 0.046$), tTau (B = 0.55, $p = 0.027$), and $tTau/A\beta_{42}$ (B = 1.13, $p = 0.042$) positivity.

*Correspondence to: Valeria Elisa Contarino, Fondazione IRCCS Ca' Granda Ospedale Policlinico, Via F. Sforza 35, 20122 Milan, Italy. Tel.: +39 0255038618; E-mail: valeria.contarino@policlinico.mi.it.

ISSN 1387-2877/$35.00

tiple correlations were not applied to control for family-wise error. However, the leading hypothesis was soundly grounded in previous literature and the number of statistical tests was rather low. Noteworthy, all the observed correlations between QSM values in the left BANKSSTS and CSF biomarkers would have survived Bonferroni correction considering the four independent biomarkers ($\alpha/4 = p\ 0.0125$). Overall, we consider this research rather preliminary and encourage replication preferably in multicentric setting.

The main strength of our study is that non-AD dementia patients were considered for group comparison and diagnostic accuracy analyses besides HC. Another strength is that all clinical diagnoses were supported by AD biomarkers. Moreover, data were highly homogeneous since all CSF analyses were performed in the same laboratory and all PET and MRI images were acquired with standardized protocols in single PET and MRI scanners.

In conclusion, we demonstrated that the automatic analysis of the QSM values in the BANKSSTS may provide non-invasive and user-independent measures able to discriminate AD from non-AD patients that deserve multicentric validation.

ACKNOWLEDGMENTS

The authors have no acknowledgments to report.

FUNDING

This research was supported by Centro Dino Ferrari (University of Milan), Fondazione Monzino, Fondazione Gigi & Pupa Ferrari Onlus, and the Italian Ministry of Health ("Ricerca Corrente" to ES and RCR-2022-23682285 to VEC). CL was supported in part by the National Institute of Aging of the National Institutes of Health under Award Number R01AG070826. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

CONFLICT OF INTEREST

DG is an Editorial Board Member of this journal but was not involved in the peer-review process nor had access to any information regarding its peer-review.

All other authors have no conflict of interest to report.

DATA AVAILABILITY

The datasets used in this study are available from the corresponding author upon reasonable request.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-230095.

REFERENCES

[1] Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* **82**, 239-259.

[2] Dubois B, Villain N, Frisoni GB, Rabinovici GD, Sabbagh M, Cappa S, Bejanin A, Bombois S, Epelbaum S, Teichmann M, Habert M-O, Nordberg A, Blennow K, Galasko D, Stern Y, Rowe CC, Salloway S, Schneider LS, Cummings JL, Feldman HH (2021) Clinical diagnosis of Alzheimer's disease: Recommendations of the International Working Group. *Lancet Neurol* **20**, 484-496.

[3] Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R, Elliott C, Masliah E, Ryan L, Silverberg N (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**, 535-562.

[4] Liu C, Li W, Tong KA, Yeom KW, Kuzminski S (2015) Susceptibility-weighted imaging and quantitative susceptibility mapping in the brain: Brain susceptibility imaging and mapping. *J Magn Reson Imaging* **42**, 23-41.

[5] Haacke EM, Liu S, Buch S, Zheng W, Wu D, Ye Y (2015) Quantitative susceptibility mapping: Current status and future directions. *Magn Reson Imaging* **33**, 1-25.

[6] Langkammer C, Schweser F, Krebs N, Deistung A, Goessler W, Scheurer E, Sommer K, Reishofer G, Yen K, Fazekas F, Ropele S, Reichenbach JR (2012) Quantitative susceptibility mapping (QSM) as a means to measure brain iron? A post mortem validation study. *Neuroimage* **62**, 1593-1599.

[7] Gong N-J, Dibb R, Bulk M, van der Weerd L, Liu C (2019) Imaging beta amyloid aggregation and iron accumulation in Alzheimer's disease using quantitative susceptibility mapping MRI. *Neuroimage* **191**, 176-185.

[8] Ke Y, Qian ZM (2003) Iron misregulation in the brain: A primary cause of neurodegenerative disorders. *Lancet Neurol* **2**, 246-253.

[9] van Duijn S, Bulk M, van Duinen SG, Nabuurs RJA, van Buchem MA, van der Weerd L, Natté R (2017) Cortical iron reflects severity of Alzheimer's disease. *J Alzheimers Dis* **60**, 1533-1545.

[10] Sayre LM, Perry G, Harris PLR, Liu Y, Schubert KA, Smith MA (2001) In situ oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: A central role for bound transition metals. *J Neurochem* **74**, 270-279.

[11] Everett J, Céspedes E, Shelford LR, Exley C, Collingwood JF, Dobson J, van der Laan G, Jenkins CA, Arenholz E, Telling ND (2014) Evidence of redox-active iron formation following aggregation of ferrihydrite and the Alzheimer's disease peptide β-amyloid. *Inorg Chem* **53**, 2803-2809.

Journal of the Neurological Sciences 451 (2023) 120711

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Prodromal language impairment in genetic frontotemporal dementia within the GENFI cohort

Kiran Samra [a], Amy M. MacDougall [b], Arabella Bouzigues [a], Martina Bocchetta [a], David M. Cash [a], Caroline V. Greaves [a], Rhian S. Convery [a], John C. van Swieten [c], Lize Jiskoot [c], Harro Seelaar [c], Fermin Moreno [d,e], Raquel Sanchez-Valle [f], Robert Laforce [g], Caroline Graff [h,i], Mario Masellis [j], Maria Carmela Tartaglia [k], James B. Rowe [l], Barbara Borroni [m], Elizabeth Finger [n], Matthis Synofzik [o,p], Daniela Galimberti [q,r], Rik Vandenberghe [s,t,u], Alexandre de Mendonça [v], Chris R. Butler [w,x], Alex Gerhard [y,z], Simon Ducharme [aa,ab], Isabelle Le Ber [ac,ad,ae,af], Pietro Tiraboschi [ag], Isabel Santana [ah,ai], Florence Pasquier [aj,ak,al], Johannes Levin [am,an,ao], Markus Otto [ap], Sandro Sorbi [aq,ar], Jonathan D. Rohrer [a,1], Lucy L. Russell [a,*,1], on behalf of the Genetic FTD Initiative (GENFI)

[a] Dementia Research Centre, Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London, UK
[b] Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK
[c] Department of Neurology, Erasmus Medical Centre, Rotterdam, Netherlands
[d] Cognitive Disorders Unit, Department of Neurology, Donostia Universitary Hospital, San Sebastian, Spain
[e] Neuroscience Area, Biodonostia Health Research Institute, San Sebastian, Gipuzkoa, Spain
[f] Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Institut d'Investigacións Biomèdiques August Pi I Sunyer, University of Barcelona, Barcelona, Spain
[g] Clinique Interdisciplinaire de Mémoire, Département des Sciences Neurologiques, CHU de Québec, and Faculté de Médecine, Université Laval, QC, Canada
[h] Center for Alzheimer Research, Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Solna, Sweden
[i] Unit for Hereditary Dementias, Theme Aging, Karolinska University Hospital, Solna, Sweden
[j] Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada
[k] Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, ON, Canada
[l] Department of Clinical Neurosciences, University of Cambridge, UK
[m] Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[n] Department of Clinical Neurological Sciences, University of Western Ontario, London, ON, Canada
[o] Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany
[p] Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
[q] Fondazione Ca' Granda, IRCCS Ospedale Policlinico, Milan, Italy
[r] University of Milan, Centro Dino Ferrari, Milan, Italy
[s] Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium
[t] Neurology Service, University Hospitals Leuven, Leuven, Belgium
[u] Leuven Brain Institute, KU Leuven, Leuven, Belgium
[v] Laboratory of Neurosciences, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
[w] Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford, Oxford, UK
[x] Department of Brain Sciences, Imperial College London, UK
[y] Division of Neuroscience and Experimental Psychology, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK
[z] Departments of Geriatric Medicine and Nuclear Medicine, University of Duisburg-Essen, Germany
[aa] Department of Psychiatry, McGill University Health Centre, McGill University, Montreal, Québec, Canada
[ab] McConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University, Montreal, Québec, Canada
[ac] Sorbonne Université, Paris Brain Institute – Institut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
[ad] Centre de Référence des Démences rares ou Précoces, IM2A, Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
[ae] Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
[af] Reference Network for Rare Neurological Diseases (ERN-RND)
[ag] Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
[ah] University Hospital of Coimbra (HUC), Neurology Service, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[ai] Center for Neuroscience and Cell Biology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

* Corresponding author at: Dementia Research Centre, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK.
E-mail address: l.russell@ucl.ac.uk (L.L. Russell).

https://doi.org/10.1016/j.jns.2023.120711
Received 30 October 2022; Received in revised form 1 June 2023; Accepted 8 June 2023
Available online 10 June 2023

Journal of Alzheimer's Disease 94 (2023) 1225–1231
DOI 10.3233/JAD-230322
IOS Press

Klotho Gene Expression Is Decreased in Peripheral Blood Mononuclear Cells in Patients with Alzheimer's Disease and Frontotemporal Dementia

Federica Sorrentino[a,1], Chiara Fenoglio[a,b,1,*], Luca Sacchi[a], Maria Serpente[b], Andrea Arighi[b], Tiziana Carandini[b], Beatrice Arosio[a], Evelyn Ferri[b], Marina Arcaro[b], Caterina Visconte[a], Emanuela Rotondo[b], Elio Scarpini[b] and Daniela Galimberti[a,b]
[a]*University of Milan, Milan, Italy*
[b]*Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy*

Accepted 26 May 2023
Pre-press 24 June 2023

Abstract.
Background: The longevity gene Klotho (*KL*) was recently associated with neurodegenerative diseases including Alzheimer's disease (AD). Its role in the brain has not been completely elucidated, although evidence suggests that *KL-VS* heterozygosity is associated with a reduced risk of AD in Apolipoprotein E ε4 carriers. Conversely, no data about genetic association with frontotemporal dementia (FTD) are available so far.
Objective: To investigate the involvement of KL in AD and FTD by the determination of the genetic frequency of *KL-VS* variant and the expression analysis of *KL* gene.
Methods: A population consisting of 438 patients and 240 age-matched controls was enrolled for the study. *KL-VS* and *APOE* genotypes were assessed by allelic discrimination through a QuantStudio 12K system. *KL* gene expression analysis was performed in a restricted cohort of patients consisting of 43 AD patients, 41 FTD patients and 19 controls. KL gene expression was assessed in peripheral blood mononuclear cells with specific TaqMan assay. Statistical analysis was performed using GraphPad 9 Prims software.
Results: *KL-VS* frequency was comparable to the ones found in literature and no differences were found in both allelic and genotypic frequencies between patients and controls were found. Conversely, KL expression levels were significantly lower in AD and FTD patients compared with controls (mean fold regulation –4.286 and –6.561 versus controls in AD and FTD, respectively, $p = 0.0037$).
Conclusion: This is the first study investigating KL in FTD. We showed a decreased expression of the gene in AD and FTD, independent of the genotype, suggesting a role of Klotho in common steps during neurodegeneration.

Keywords: Alzheimer's disease, expression, frontotemporal dementia, Klotho, neurodegeneration

[1]These authors contributed equally to this work.

[*]Correspondence to: Chiara Fenoglio, University of Milan, Milan, Italy. Tel.: +39 0255033858; E-mail: chiara.fenoglio@unimi.it.

ISSN 1387-2877/$35.00

expression. However, the inconsistency of our results with the literature could be due to the small size of the gene expression cohort.

We found a correlation between *KL* gene expression and CSF Aβ in AD patients: high expression levels were associated to higher Aβ levels. In this regard, studies in animal models revealed that overexpression of KL in the brain reduces Aβ burden [24, 25]. *KL* could therefore play a role in AD pathology and this corroborate its potential as therapeutic target.

To the best of our knowledge, this is the first study investigating *KL* in FTD patients. As already stated, we found low KL expression levels in FTD compared to control group. Its involvement in both AD and FTD could indicate a pleotropic effect of KL. Regarding controls, subjects were very well characterized in terms of frailty index and cognition, but we acknowledge that a few of them may have comorbidities such as main internal diseases (i.e., diabetes) which can influence KL expression [26, 27]. As proof of its pleiotropic nature, KL seems to be associated to several conditions. For instance, reduced KL gene expression levels or protein levels have been found in relapsing-remitting multiple sclerosis (RR-MS) [28], Parkinson's disease [29], and in schizophrenia, possibly acting together with other detrimental factors. Therefore, the broad-spectrum neuroprotection of KL is intriguing since it expands the landscape of its therapeutic applicability. To date, several approaches are indeed being studied to increase KL expression (reviewed by Hanson and colleagues [22]). In this scenario, further investigating KL mechanisms would help to sharpen its therapeutic potential.

ACKNOWLEDGMENTS

The authors have no acknowledgments to report.

FUNDING

This work was supported by grants from the Italian Ministry of Health (Ricerca Corrente and RF-2018-12365333), Dino Ferrari Center and Fondazione Gigi & Pupa Ferrari Onlus. MS is supported by the Italian Ministry of Health, grant GR-2019-12369100. FS is supported by the Italian Ministry of University and Research (Joint Program on Neurodegenerative Diseases 2019 – project "DIPPA-FTD").

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

[1] Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* **390**, 45-51.

[2] Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M (2006) Regulation of fibroblast growth factor-23 signaling by Klotho. *J Biol Chem* **281**, 6120-6123.

[3] Imura A, Tsuji Y, Murata M, Maeda R, Kubota K, Iwano A, Obuse C, Togashi K, Tominaga M, Kita N, Tomiyama KI, Iijima J, Nabeshima Y, Fujioka M, Asato R, Tanaka S, Kojima K, Ito J, Nozaki K, Hashimoto N, Ito T, Nishio T, Uchiyama T, Fujimori T, Nabeshima YI (2007) α-klotho as a regulator of calcium homeostasis. *Science* **316**, 1615-1618.

[4] Nagai T, Yamada K, Kim HC, Kim YS, Noda Y, Imura A, Nabeshima Yo-ichi, Nabeshima T (2003) Cognition impairment in the genetic model of aging klotho gene mutant mice: A role of oxidative stress. *FASEB J* **17**, 50-52.

[5] Dubal DB, Yokoyama JS, Zhu L, Broestl L, Worden K, Wang D, Sturm VE, Kim D, Klein E, Yu GQ, Ho K, Eilertson KE, Yu L, Kuro-o M, De Jager PL, Coppola G, Small GW, Bennett DA, Kramer JH, Abraham CR, Miller BL, Mucke L (2014) Life extension factor Klotho enhances cognition. *Cell Rep* **7**, 1065-1076.

[6] Shardell M, Semba RD, Rosano C, Kalyani RR, Bandinelli S, Chia CW, Ferrucci L (2016) Plasma Klotho and cognitive decline in older adults: Findings from the InCHIANTI Study. *J Gerontol A Biol Sci Med Sci* **71**, 677-682.

[7] Semba RD, Moghekar AR, Hu J, Sun K, Turner R, Ferrucci L, O'Brien R (2014) Klotho in the cerebrospinal fluid of adults with and without Alzheimer's disease. *Neurosci Lett* **558**, 37-40.

[8] Ho WY, Navakkode S, Liu F, Soong TW, Ling SC (2020) Deregulated expression of a longevity gene, Klotho, in the C9orf72 deletion mice with impaired synaptic plasticity and adult hippocampal neurogenesis. *Acta Neuropathol Commun* **8**, 155.

[9] Arking DE, Atzmon G, Arking A, Barzilai N, Dietz HC (2005) Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. *Circ Res* **96**, 412-418.

[10] Driscoll I, Ma Y, Gallagher CL, Johnson SC, Asthana S, Hermann BP, Sager MA, Blennow K, Zetterberg H, Carlsson CM, Engelman CD, Dubal DB, Okonkwo OC (2021) Age-related tau burden and cognitive deficits are attenuated in Klotho KL-vs heterozygotes. *J Alzheimers Dis* **79**, 1297-1305.

frontiers | Frontiers in Aging Neuroscience

TYPE Brief Research Report
PUBLISHED 20 July 2023
DOI 10.3389/fnagi.2023.1191714

OPEN ACCESS

EDITED BY
Jianpan Huang,
The University of Hong Kong, Hong Kong SAR, China

REVIEWED BY
Lorenzo Gaetani,
University of Perugia, Italy
Giuseppe Barisano,
Stanford University, United States

*CORRESPONDENCE
Luca Sacchi
✉ luca.sacchi1@unimi.it

RECEIVED 22 March 2023
ACCEPTED 06 July 2023
PUBLISHED 20 July 2023

CITATION
Sacchi L, Arcaro M, Carandini T, Pietroboni AM, Fumagalli GG, Fenoglio C, Serpente M, Sorrentino F, Visconte C, Pintus M, Conte G, Contarino VE, Scarpini E, Triulzi F, Galimberti D and Arighi A (2023) Association between enlarged perivascular spaces and cerebrospinal fluid aquaporin-4 and tau levels: report from a memory clinic. *Front. Aging Neurosci.* 15:1191714. doi: 10.3389/fnagi.2023.1191714

Association between enlarged perivascular spaces and cerebrospinal fluid aquaporin-4 and tau levels: report from a memory clinic

Luca Sacchi[1,2]*, Marina Arcaro[2], Tiziana Carandini[2], Anna Margherita Pietroboni[2], Giorgio Giulio Fumagalli[3], Chiara Fenoglio[1], Maria Serpente[2], Federica Sorrentino[1], Caterina Visconte[1], Manuela Pintus[4], Giorgio Conte[5,6], Valeria Elisa Contarino[6], Elio Scarpini[2], Fabio Triulzi[5,6], Daniela Galimberti[1,2] and Andrea Arighi[2]

[1]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy, [2]Neurodegenerative Diseases Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [3]Center for Mind/Brain Sciences (CIMeC), University of Trento, Rovereto, Italy, [4]Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy, [5]Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy, [6]Neuroradiology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Background: Perivascular spaces (PVS) are fluid-filled compartments that dilate in response to many different conditions. A high burden of enlarged PVS (EPVS) in the centrum semiovale (CSO) has been linked to neurodegeneration. Moreover, an increase in cerebrospinal fluid (CSF) levels of aquaporin-4 (AQP4), a water channel expressed on PVS-bounding astrocytes, has been described in patients with neurodegenerative dementia. Our aim was to investigate the relationship between neurodegenerative diseases and two putative glymphatic system biomarkers: AQP4 and EPVS.

Methods: We included 70 individuals, 54 patients with neurodegenerative diseases and 16 subjects with non-degenerative conditions. EPVS were visually quantified on MRI-scans applying Paradise's scale. All subjects underwent lumbar puncture for the measurement of AQP4 levels in the cerebrospinal fluid (CSF). CSF levels of amyloid-β-1-42, phosphorylated and total tau (tTau) were also measured. Linear regression analyses were adjusted for age, sex, education and disease duration, after excluding outliers.

Results: Cerebrospinal fluid (CSF)-AQP4 levels were independent predictors of total (β = 0.28, standard error [SE] = 0.08, p = 0.001), basal ganglia (β = 0.20, SE = 0.08, p = 0.009) and centrum semiovale EPVS (β = 0.37, SE = 0.12, p = 0.003). tTau levels predicted CSO-EPVS (β = 0.30, SE = 0.15, p = 0.046). Moreover, increased levels of AQP4 were strongly associated with higher levels of tTau in the CSF (β = 0.35, SE = 0.13, p = 0.008).

Conclusion: We provide evidence that CSO-EPVS and CSF-AQP4 might be clinically meaningful biomarkers of glymphatic dysfunction and associated neurodegeneration.

KEYWORDS

glymphatic system, aquaporin-4, cerebrospinal fluid, Alzheimer's disease, brain perivascular spaces

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Comitato Etico Area 2 Milano, approval N 859_2021, date 14.09.2021. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LS and AA designed the study, analyzed, and interpreted the data. LS drafted the manuscript. TC, MP, GF, and AP contributed to the analysis and interpretation of the data. MA, CF, MS, FS, and CV performed CSF analyses. GC and VC helped in the analysis of MRI data. FT acquired MRI data. ES and DG drafted and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Funding

This work was supported by grants from the Italian Ministry of Health (Ricerca Corrente), Dino Ferrari Center and Fondazione Gigi & Pupa Ferrari Onlus. MS is supported by the Italian Ministry of Health, grant GR-2019-12369100. FS is supported by the Italian Ministry of University and Research (Joint Program on Neurodegenerative Diseases 2019—project "DIPPA-FTD").

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Albargothy, N. J., Johnston, D. A., MacGregor-Sharp, M., Weller, R. O., Verma, A., Hawkes, C. A., et al. (2018). Convective influx/glymphatic system: tracers injected into the CSF enter and leave the brain along separate periarterial basement membrane pathways. *Acta Neuropathol.* 136, 139–152. doi: 10.1007/s00401-018-1862-7

Arighi, A., Arcaro, M., Fumagalli, G. G., Carandini, T., Pietroboni, A. M., Sacchi, L., et al. (2022). Aquaporin-4 cerebrospinal fluid levels are higher in neurodegenerative dementia: looking at glymphatic system dysregulation. *Alz. Res. Therapy* 14:135. doi: 10.1186/s13195-022-01077-6

Ballerini, L., Lovreglio, R., Valdés Hernández, M. D. C., Ramirez, J., MacIntosh, B. J., Black, S. E., et al. (2018). Perivascular spaces segmentation in brain MRI using optimal 3D Filtering. *Sci. Rep.* 8:2132. doi: 10.1038/s41598-018-19781-5

Barisano, G., Lynch, K. M., Sibilia, F., Lan, H., Shih, N.-C., Sepehrband, F., et al. (2022). Imaging perivascular space structure and function using brain MRI. *Neuroimage* 257:119329. doi: 10.1016/j.neuroimage.2022.119329

Boespflug, E. L., Simon, M. J., Leonard, E., Grafe, M., Woltjer, R., Silbert, L. C., et al. (2018). Targeted Assessment of Enlargement of the Perivascular Space in Alzheimer's disease and vascular dementia subtypes implicates astroglial involvement specific to Alzheimer's Disease. *JAD* 66, 1587–1597. doi: 10.3233/JAD-180367

Bown, C. W., Carare, R. O., Schrag, M. S., and Jefferson, A. L. (2022). Physiology and clinical relevance of enlarged perivascular spaces in the aging brain. *Neurology* 98, 107–117. doi: 10.1212/WNL.0000000000013077

Evans, T. E., Knol, M. J., Schwingenschuh, P., Wittfeld, K., Hilal, S., Ikram, M. A., et al. (2023). Determinants of perivascular spaces in the general population: A pooled cohort analysis of individual participant data. *Neurology* 100, e107–e122. doi: 10.1212/WNL.0000000000201349

Groeschel, S., Chong, W. K., Surtees, R., and Hanefeld, F. (2006). Virchow-Robin spaces on magnetic resonance images: normative data, their dilatation, and a review of the literature. *Neuroradiology* 48, 745–754. doi: 10.1007/s00234-006-0112-1

Hiraldo-González, L., Trillo-Contreras, J. L., García-Miranda, P., Pineda-Sánchez, R., Ramírez-Lorca, R., Rodrigo-Herrero, S., et al. (2021). Evaluation of aquaporins in the cerebrospinal fluid in patients with idiopathic normal pressure hydrocephalus. *PLoS One* 16:e0258165. doi: 10.1371/journal.pone.0258165

Iliff, J. J., Chen, M. J., Plog, B. A., Zeppenfeld, D. M., Soltero, M., Yang, L., et al. (2014). Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. *J. Neurosci.* 34, 16180–16193. doi: 10.1523/JNEUROSCI.3020-14.2014

Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., et al. (2012). A Paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci. Transl. Med.* 4:147ra111. doi: 10.1126/scitranslmed.3003748

Jeong, S. H., Cha, J., Park, M., Jung, J. H., Ye, B. S., Sohn, Y. H., et al. (2022). Association of enlarged perivascular spaces with amyloid burden and cognitive decline in Alzheimer Disease Continuum. *Neurology* [Epub ahead of print]. doi: 10.1212/WNL.0000000000200989

Kang, K. M., Byun, M. S., Yi, D., Lee, K. H., Kim, M. J., Ahn, H., et al. (2022). Enlarged perivascular spaces are associated with decreased brain tau deposition. *CNS Neurosci. Ther.* 29, 577–586. doi: 10.1111/cns.14040

Kim, H. J., Cho, H., Park, M., Kim, J. W., Ahn, S. J., Lyoo, C. H., et al. (2021). MRI-Visible perivascular spaces in the centrum semiovale are associated with brain amyloid deposition in patients with Alzheimer Disease–Related cognitive impairment. *AJNR Am. J. Neuroradiol.* 42, 1231–1238. doi: 10.3174/ajnr.A7155

Kress, B. T., Iliff, J. J., Xia, M., Wang, M., Wei, H. S., Zeppenfeld, D., et al. (2014). Impairment of paravascular clearance pathways in the aging brain: Paravascular Clearance. *Ann. Neurol.* 76, 845–861. doi: 10.1002/ana.24271

MacGregor Sharp, M., Bulters, D., Brandner, S., Holton, J., Verma, A., Werring, D. J., et al. (2019). The fine anatomy of the perivascular compartment in the human brain: relevance to dilated perivascular spaces in cerebral amyloid angiopathy. *Neuropathol. Appl. Neurobiol.* 45, 305–308. doi: 10.1111/nan.12480

Paradise, M., Crawford, J. D., Lam, B. C. P., Wen, W., Kochan, N. A., Makkar, S., et al. (2021). Association of dilated perivascular spaces with cognitive decline and incident dementia. *Neurology* 96, e1501–e1511. doi: 10.1212/WNL.0000000000011537

Paradise, M. B., Beaudoin, M. S., Dawes, L., Crawford, J. D., Wen, W., Brodaty, H., et al. (2020). Development and validation of a rating scale for perivascular spaces on 3T MRI. *J. Neurol. Sci.* 409:116621. doi: 10.1016/j.jns.2019.116621

International Journal of
Molecular Sciences

MDPI

Article

Altered Extracellular Vesicle miRNA Profile in Prodromal Alzheimer's Disease

Caterina Visconte [1], Chiara Fenoglio [1,2,*], Maria Serpente [2], Paola Muti [1,3], Andrea Sacconi [4], Marta Rigoni [1,3], Andrea Arighi [2], Vittoria Borracci [2], Marina Arcaro [2], Beatrice Arosio [5], Evelyn Ferri [6], Maria Teresa Golia [1,7], Elio Scarpini [2] and Daniela Galimberti [1,2]

[1] Department of Biomedical, Surgical and Dental Sciences, University of Milan, 20122 Milan, Italy; caterina.visconte@unimi.it (C.V.); paola.muti@unimi.it (P.M.); marta.rigoni@unimi.it (M.R.); mariateresagolia89@gmail.com (M.T.G.); daniela.galimberti@unimi.it (D.G.)
[2] Neurodegenerative Diseases Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, 20122 Milan, Italy; maria.serpente@policlinico.mi.it (M.S.); andrea.arighi@policlinico.mi.it (A.A.); vittoria.borracci@policlinico.mi.it (V.B.); marina.arcaro@policlinico.mi.it (M.A.); elio.scarpini@unimi.it (E.S.)
[3] Dental and Maxillo-Facial Surgery Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, 20122 Milan, Italy
[4] UOSD Clinical Trial Center, Biostatistics and Bioinformatics, Regina Elena National Cancer Institute—IRCCS, 00144 Rome, Italy; sacconiandrea@hotmail.com
[5] Department of Clinical Sciences and Community Health, University of Milan, 20122 Milan, Italy; beatrice.arosio@unimi.it
[6] Geriatric Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, 20122 Milan, Italy; evelyn.ferri@policlinico.mi.it
[7] National Research Council of Italy, Institute of Neuroscience, Via Raoul Follereau 3, 20854 Vedano al Lambro, Italy
* Correspondence: chiara.fenoglio@unimi.it

Abstract: Extracellular vesicles (EVs) are nanosized vesicles released by almost all body tissues, representing important mediators of cellular communication, and are thus promising candidate biomarkers for neurodegenerative diseases like Alzheimer's disease (AD). The aim of the present study was to isolate total EVs from plasma and characterize their microRNA (miRNA) contents in AD patients. We isolated total EVs from the plasma of all recruited subjects using ExoQuickULTRA exosome precipitation solution (SBI). Subsequently, circulating total EVs were characterized using Nanosight nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and Western blotting. A panel of 754 miRNAs was determined with RT-qPCR using TaqMan OpenArray technology in a QuantStudio 12K System (Thermo Fisher Scientific). The results demonstrated that plasma EVs showed widespread deregulation of specific miRNAs (miR-106a-5p, miR-16-5p, miR-17-5p, miR-195-5p, miR-19b-3p, miR-20a-5p, miR-223-3p, miR-25-3p, miR-296-5p, miR-30b-5p, miR-532-3p, miR-92a-3p, and miR-451a), some of which were already known to be associated with neurological pathologies. A further validation analysis also confirmed a significant upregulation of miR-16-5p, miR-25-3p, miR-92a-3p, and miR-451a in prodromal AD patients, suggesting these dysregulated miRNAs are involved in the early progression of AD.

Keywords: extracellular vesicles; Alzheimer's disease (AD); miRNA; biomarker

Citation: Visconte, C.; Fenoglio, C.; Serpente, M.; Muti, P.; Sacconi, A.; Rigoni, M.; Arighi, A.; Borracci, V.; Arcaro, M.; Arosio, B.; et al. Altered Extracellular Vesicle miRNA Profile in Prodromal Alzheimer's Disease. *Int. J. Mol. Sci.* **2023**, *24*, 14749. https://doi.org/10.3390/ijms241914749

Academic Editors: Giacinto Bagetta, Damiana Scuteri and Daniele Bano

Received: 1 September 2023
Revised: 22 September 2023
Accepted: 24 September 2023
Published: 29 September 2023

1. Introduction

Prompt clinical diagnosis of Alzheimer's disease (AD) in its early stage is still uncertain, and unfortunately most patients are diagnosed when they have already progressed to the moderate or severe stages of the disease [1]. At present, the use of the cerebrospinal fluid (CSF) biomarkers amyloid-β (Aβ), tau, and phosphorylated tau (Ptau) allows discrimination between Mild Cognitive Impairment (MCI) due to AD, i.e., prodromal AD, and MCI due to other causes (non-AD MCI) with very high accuracy (see [2] for a review), but

4.7. Target Prediction and Pathway Enrichment Analysis

The MiRNet (https://www.mirnet.ca/miRNet/home.xhtml, accessed on 20 July 2023) web tool was used to provide visual exploration and functional interpretation of the miRNA–target interaction network and a pathway enrichment analysis [34]. A functional enrichment analysis was performed using the KEGG database with two different algorithms implemented in the miRNet tool (hypergeometric tests and empirical sampling, as recently proposed [35]).

miRNet 2.0 supports four query types, two enrichment algorithms (hypergeometric tests and empirical sampling), and nine annotation libraries for functional enrichment analysis that include the following: gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and disease ontology databases. The miRNA set libraries are based on the TAM 2.0 database, which includes miRNA-function, miRNA-disease, miRNA-TF, miRNA-cluster, miRNA-family, and miRNA-tissue set libraries [38].

4.8. Statistical Analysis

Normalized Ct values of miRNAs were used to analyze differences between healthy subjects and patients. In the discovery phase, the statistical significance of miRNA modulation was assessed using the Wilcoxon rank sum test. The analysis was carried out with Matlab R2022a. Regarding the validation phase, the statistical analysis was performed using GraphPad Prism 9.0 Software (GraphPad Software Inc., San Diego, CA, USA). The data distribution was tested for normality with the Kolmogorov–Smirnov and Shapiro–Wilk tests. Multiple comparisons were performed using a one-way ANOVA followed by Tukey's test or the Kruskal–Wallis test followed by Dunn's Multiple Comparison test as post hoc tests. The Spearman test was applied for correlations between deregulated miRNAs and age or clinical variables and pathogenic CSF protein levels. Significance was defined at the 5% level, and all data are shown as means ± SEMs. Lastly, a chi-squared test was used for the gender distribution among the groups.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ijms241914749/s1.

Author Contributions: Conceptualization, M.S., C.F. and D.G.; data curation, M.S. and C.V.; formal analysis, M.A., P.M., A.S., M.R., M.T.G. and C.F.; funding acquisition, M.S, E.S. and D.G.; resources, A.A., B.A., E.F. and V.B.; writing—original draft, C.V., C.F. and D.G.; writing—review and editing, C.F. and D.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from the Italian Ministry of Health (Ricerca Corrente and RF-2018-12365333), Dino Ferrari Center, Fondazione Gigi, and Pupa Ferrari Onlus. MS was supported by the Italian Ministry of Health (grant GR-2019-12369100). MTG was supported by the Italian Ministry of University and Research (DIPPA-FTD study funded by JPND/JPco-fuND 2—grant number 825664).

Institutional Review Board Statement: This study was approved by the Institutional Review Board (IRB) of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy)—Comitato Etico area 2 Parere 857-2021.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study or their caregivers.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: Part of this work was carried out at NOLIMITS, an advanced imaging facility established by the Università degli Studi di Milano.

Conflicts of Interest: The authors declare no conflict of interest.

Molecular Neurodegeneration

RESEARCH ARTICLE Open Access

Altered plasma protein profiles in genetic FTD – a GENFI study

Abbe Ullgren[1,2,3†], Linn Öijerstedt[1,2,3†], Jennie Olofsson[1,4], Sofia Bergström[1,4], Julia Remnestål[1,4], John C. van Swieten[5], Lize C. Jiskoot[5], Harro Seelaar[5], Barbara Borroni[6], Raquel Sanchez-Valle[7], Fermin Moreno[8,9], Robert Laforce[10], Matthis Synofzik[11,12], Daniela Galimberti[13,14], James B. Rowe[15], Mario Masellis[16], Maria Carmela Tartaglia[17], Elizabeth Finger[18], Rik Vandenberghe[19,20,21], Alexandre de Mendonça[22], Pietro Tirabosch[23], Isabel Santana[24,25], Simon Ducharme[26,27], Chris R. Butler[28,29], Alexander Gerhard[30,31], Markus Otto[32], Arabella Bouzigues[33,34], Lucy Russell[33,34], Imogen J. Swift[33,34], Aitana Sogorb-Esteve[33,34], Carolin Heller[33,34], Jonathan D. Rohrer[33,34], Anna Månberg[1,4], Peter Nilsson[1,4], Caroline Graff[1,2,3*] and on behalf of the Genetic Frontotemporal Dementia Initiative (GENFI)

Abstract

Background Plasma biomarkers reflecting the pathology of frontotemporal dementia would add significant value to clinical practice, to the design and implementation of treatment trials as well as our understanding of disease mechanisms. The aim of this study was to explore the levels of multiple plasma proteins in individuals from families with genetic frontotemporal dementia.

Methods Blood samples from 693 participants in the GENetic Frontotemporal Dementia Initiative study were analysed using a multiplexed antibody array targeting 158 proteins.

Results We found 13 elevated proteins in symptomatic mutation carriers, when comparing plasma levels from people diagnosed with genetic FTD to healthy non-mutation controls and 10 proteins that were elevated compared to presymptomatic mutation carriers.

Conclusion We identified plasma proteins with altered levels in symptomatic mutation carriers compared to non-carrier controls as well as to presymptomatic mutation carriers. Further investigations are needed to elucidate their potential as fluid biomarkers of the disease process.

Keywords Frontotemporal dementia, Plasma biomarkers, *GRN*, *C9orf72*, *MAPT*, Neurodegeneration

Background

Frontotemporal dementia (FTD) is a group of neurodegenerative diseases where the most common phenotypes are behavioural variant FTD (bvFTD) and primary progressive aphasias (PPA). There is a great heterogeneity in FTD, both in terms of clinical symptoms, underlying genetic causes, and neuropathological findings. Over the past years, effort has been put into explaining the diversity by searching for fluid biomarkers that reflect different aspects of FTD [1]. Most efforts have focused on finding biomarkers in cerebrospinal fluid (CSF) and a few promising candidates have been found, such as neurofilament light chain (NEFL) and neuronal pentraxin 2 (NPTX2) [2, 3]. However, the use of CSF biomarkers is limited by the invasive nature of the sampling procedure and restricted availability. Therefore, a reliable blood-based biomarker

†Abbe Ullgren and Linn Öijerstedt shared first author.

*Correspondence:
Caroline Graff
caroline.graff@ki.se
Full list of author information is available at the end of the article

Table 3 Correlations between age and protein levels in mutation carriers

Protein	*p-value*	*β*
RPH3A	9.98×10^{-7}	0.019 (0.013—0.026)
IL1B	2.16×10^{-4}	0.012 (0.006—0.017)
RGS7BP	5.35×10^{-4}	0.012 (0.006—0.018)
TFEB	5.35×10^{-4}	0.013 (0.006—0.019)
S100A12	1.04×10^{-3}	0.013 (0.006—0.02)
GLA	4.18×10^{-3}	0.01 (0.004—0.016)
EIF4ENIF1	5.22×10^{-3}	0.008 (0.003—0.014)
APOE	5.22×10^{-3}	0.009 (0.003—0.015)
CHGA	5.22×10^{-3}	0.009 (0.003—0.015)
LRRFIP2	5.22×10^{-3}	0.011 (0.004—0.018)
ADAMTS1	5.25×10^{-3}	0.009 (0.003—0.015)
LAMA2	2.15×10^{-2}	0.008 (0.002—0.014)
APOC1	3.09×10^{-2}	0.006 (0.001—0.011)
XPO5	*1.19×10^{-1}*	*0.006 (-0.001—0.013)*
LCAT	*1.57×10^{-1}*	*0.005 (-0.002—0.012)*
NPTX2	*1.68×10^{-1}*	*0.005 (-0.002—0.013)*
C7	*3.73×10^{-1}*	*0.003 (-0.003—0.008)*

Correlations between protein levels and age in all mutation carriers (MC) including *p*-values and beta coefficients with 95% confidence intervals. All *p*-values are adjusted for multiple testing. Non-significant *p*-values are in italics

with other neurodegenerative diseases were not included in the analysis. Follow-up studies with comparisons to for example AD and ALS will elucidate the importance of altered plasma proteins in FTD in relation to other diseases as well as sporadic FTD. The suspension bead array technique is a method for analysing multiple proteins simultaneously, which is useful in an exploratory study like this. However, a high-throughput antibody-based single-binder assay can have reduced sensitivity, which may limit the detection of low abundant proteins and require further validation of antibody specificity. In addition, we used a targeted approach, and the protein analysis was thus limited by the protein selection as well as the availability of antibodies.

Conclusions

To our knowledge, this is the first large scale plasma protein profiling specifically in genetic FTD. A reliable fluid biomarker could aid for example in diagnosing FTD at an early stage or in selecting individuals for upcoming clinical trials. Blood-based biomarkers would have the advantage of being easy to access and widely available compared to CSF-biomarkers. Here, we have presented an exploratory study providing proteins, including a previous CSF-biomarker, that are of interest for future investigations as potential biomarkers.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13024-023-00677-6.

Additional file 1: Supplementary Table 1. Antibodies used in the suspension bead array plasma analysis. **Supplementary Table 2.** Proteins with different levels in PMC compared to NC. **Supplementary Figure 1.** Boxplots for the 13 proteins that differed between SMC and NC. **Supplementary Figure 2.** Boxplots for the 10 proteins that differed between SMC and PMC.

Acknowledgements

First, we would like to thank all the participants and their families for contributing to the study. We would also like to thank the GENFI research coordinators who helped with arranging the visits and the entire staff of the Human Protein Atlas for their efforts.

Genetic Frontotemporal Dementia Initiative (GENFI) collaboration group

Author	Affiliation
Sónia Afonso	Instituto Ciencias Nucleares Aplicadas a Saude. Universidade de Coimbra. Coimbra. Portugal
Maria Rosario Almeida	Faculty of Medicine. University of Coimbra. Coimbra. Portugal
Sarah Anderl-Straub	Department of Neurology. University of Ulm. Ulm. Germany
Christin Andersson	Department of Clinical Neuroscience. Karolinska Institutet. Stockholm. Sweden
Anna Antonell	Alzheimer's disease and Other Cognitive Disorders Unit. Neurology Service. Hospital Clínic. Barcelona. Spain
Andrea Arighi	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. Neurodegenerative Diseases Unit. Milan. Italy; University of Milan. Centro Dino Ferrari, Milan. Italy
Mircea Balasa	Alzheimer's disease and Other Cognitive Disorders Unit. Neurology Service. Hospital Clínic. Barcelona. Spain
Myriam Barandiaran	Cognitive Disorders Unit. Department of Neurology. Donostia University Hospital. San Sebastian. Gipuzkoa. Spain; Neuroscience Area. Biodonostia Health Research Insitute. San Sebastian. Gipuzkoa. Spain
Nuria Bargalló	Imaging Diagnostic Center. Hospital Clínic. Barcelona. Spain
Robart Bartha	Department of Medical Biophysics. The University of Western Ontario. London. Ontario. Canada; Centre for Functional and Metabolic Mapping. Robarts Research Institute. The University of Western Ontario. London. Ontario. Canada
Benjamin Bender	Department of Diagnostic and Interventional Neuroradiology. University of Tübingen. Tübingen. Germany
Emanuele Buratti	ICGEB Trieste, Italy
Luisa Benussi	Istituto di Ricovero e Cura a Carattere Scientifico Istituto Centro San Giovanni di Dio Fatebenefratelli. Brescia. Italy
Maxime Bertoux	Inserm 1172. Lille. France

BRAIN
ORIGINAL ARTICLE

Network structure and transcriptomic vulnerability shape atrophy in frontotemporal dementia

Golia Shafiei,[1,†] Vincent Bazinet,[1,†] Mahsa Dadar,[1,2] Ana L. Manera,[1] D. Louis Collins,[1] Alain Dagher,[1] Barbara Borroni,[3] Raquel Sanchez-Valle,[4] Fermin Moreno,[5,6] Robert Laforce Jr,[7] Caroline Graff,[8,9] Matthis Synofzik,[10,11] Daniela Galimberti,[12,13] James B. Rowe,[14] Mario Masellis,[15] Maria Carmela Tartaglia,[16] Elizabeth Finger,[17] Rik Vandenberghe,[18,19,20] Alexandre de Mendonça,[21] Fabrizio Tagliavini,[22] Isabel Santana,[23,24] Chris Butler,[25,26] Alex Gerhard,[27,28] Adrian Danek,[29] Johannes Levin,[29,30,31] Markus Otto,[32] Sandro Sorbi,[33,34] Lize C. Jiskoot,[35] Harro Seelaar,[35] John C. van Swieten,[35] Jonathan D. Rohrer,[36] Bratislav Misic,[1,†] Simon Ducharme,[1,37,†] Frontotemporal Lobar Degeneration Neuroimaging Initiative (FTLDNI) and GENetic Frontotemporal dementia Initiative (GENFI)

[†]These authors contributed equally to this work.

Connections among brain regions allow pathological perturbations to spread from a single source region to multiple regions. Patterns of neurodegeneration in multiple diseases, including behavioural variant of frontotemporal dementia (bvFTD), resemble the large-scale functional systems, but how bvFTD-related atrophy patterns relate to structural network organization remains unknown. Here we investigate whether neurodegeneration patterns in sporadic and genetic bvFTD are conditioned by connectome architecture. Regional atrophy patterns were estimated in both genetic bvFTD (75 patients, 247 controls) and sporadic bvFTD (70 patients, 123 controls). First, we identified distributed atrophy patterns in bvFTD, mainly targeting areas associated with the limbic intrinsic network and insular cytoarchitectonic class. Regional atrophy was significantly correlated with atrophy of structurally- and functionally-connected neighbours, demonstrating that network structure shapes atrophy patterns. The anterior insula was identified as the predominant group epicentre of brain atrophy using data-driven and simulation-based methods, with some secondary regions in frontal ventromedial and antero-medial temporal areas. We found that FTD-related genes, namely *C9orf72* and *TARDBP*, confer local transcriptomic vulnerability to the disease, modulating the propagation of pathology through the connectome. Collectively, our results demonstrate that atrophy patterns in sporadic and genetic bvFTD are jointly shaped by global connectome architecture and local transcriptomic vulnerability, providing an explanation as to how heterogenous pathological entities can lead to the same clinical syndrome.

1 McConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University, Montreal, QC, Canada
2 Radiology and Nuclear Medicine, Laval University, Quebec City, QC, Canada
3 Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

Received September 1, 2021. Revised December 14, 2021. Accepted January 30, 2022. Advance access publication February 22, 2022

4 Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Institut d'Investigacións Biomèdiques August Pi I Sunyer, University of Barcelona, Barcelona, Spain
5 Cognitive Disorders Unit, Department of Neurology, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain
6 Neuroscience Area, Biodonostia Health Research Institute, San Sebastian, Gipuzkoa, Spain
7 Clinique Interdisciplinaire de Mémoire, Département des Sciences Neurologiques, CHU de Québec, and Faculté de Médecine, Université Laval, Quebec, QC, Canada
8 Department of Geriatric Medicine, Karolinska University Hospital-Huddinge, Stockholm, Sweden
9 Unit for Hereditary Dementias, Theme Aging, Karolinska University Hospital, Solna, Sweden
10 Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany
11 Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
12 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy
13 Department of Biomedical, Surgical and Dental Sciences, University of Milan, Dino Ferrari Center, Milan, Italy
14 University of Cambridge, Department of Clinical Neurosciences, Cambridge University Hospitals NHS Trust, and MRC Cognition and Brain Sciences Unit, Cambridge, UK
15 Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, ON, Canada
16 Toronto Western Hospital, Tanz Centre for Research in Neurodegenerative Disease, Toronto, ON, Canada
17 Department of Clinical Neurological Sciences, University of Western Ontario, London, ON, Canada
18 Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium
19 Neurology Service, University Hospitals Leuven, Leuven, Belgium
20 Leuven Brain Institute, KU Leuven, Leuven, Belgium
21 Faculty of Medicine, University of Lisbon, Lisbon, Portugal
22 Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Istituto Neurologico Carlo Besta, Milan, Italy
23 Neurology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
24 Center for Neuroscience and Cell Biology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
25 Department of Clinical Neurology, University of Oxford, Oxford, UK
26 Department of Brain Sciences, Imperial College London, London, UK
27 Division of Neuroscience and Experimental Psychology, Faculty of Medicine, Biology and Health, University of Manchester, Manchester, UK
28 Department of Geriatric Medicine and Nuclear Medicine, University of Duisburg-Essen, Duisburg and Essen, Germany
29 Department of Neurology, Ludwig-Maximilians-Universität München, Munich, Germany
30 Clinical Research Unit, German Center for Neurodegenerative Diseases (DZNE), Munich, Germany
31 Munich Cluster of Systems Neurology (SyNergy), Munich, Germany
32 Department of Neurology, University Hospital Ulm, Ulm, Germany
33 Department of Neurofarba, University of Florence, Florence, Italy
34 IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy
35 Department of Neurology, Erasmus University Medical Centre, Rotterdam, The Netherlands
36 Department of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
37 Department of Psychiatry, Douglas Mental Health University Institute, McGill University, Montreal, QC, Canada

Correspondence to: Bratislav Misic
3801 Rue University
Webster 211, Montreal
QC H3A 2B4, Canada
E-mail: bratislav.misic@mcgill.ca

Keywords: connectome; frontotemporal dementia; disease epicentre; gene expression; network spreading

Introduction

Frontotemporal dementia (FTD) is one of the most common forms of early-onset dementia.[1,2] The behavioural variant of FTD (bvFTD), which presents with various combinations of behavioural (apathy, disinhibition, compulsions and stereotypies), personality (decreased empathy and sympathy, altered personal preferences) and cognitive (executive dysfunction and social cognitive deficits) changes, is the most common clinical variant of FTD.[2,3] Despite its distinctive clinical presentation, bvFTD is pathologically heterogenous, with the most common subtypes being related to the accumulation of hyperphosphorylated aggregates of either Tau or TDP-43.[4] This group of pathological proteinopathies causing FTD are classified under the frontotemporal lobar degeneration (FTLD) umbrella. Most cases are sporadic; however, around 20% are caused by an autosomal-dominant genetic mutation including hexanucleotide repeat expansions near *C9orf72*, *GRN* and *MAPT*, as the most common causative genes.[4]

FTLD pathology cause clinical bvFTD symptoms through their predominant localization in frontal and anterior temporal brain regions.[4] Clinically, this is reflected by progressive cortical atrophy, which is a crucial biomarker for the diagnosis.[5,6] While there is

Neurological Sciences (2023) 44:3885–3894
https://doi.org/10.1007/s10072-023-06903-9

ORIGINAL ARTICLE

Telemedicine for cognitive impairment: a telephone survey of patients' experiences with neurological video consultation

Fabiana Ruggiero[1] · Eleonora Zirone[1] · Maria Takeko Molisso[1] · Tiziana Carandini[1] · Giorgio Fumagalli[1] · Anna Pietroboni[1] · Roberta Ferrucci[2,5] · Edoardo Nicolò Aiello[3] · Barbara Poletti[2,3] · Vincenzo Silani[3,4] · Giacomo Comi[1,4] · Elio Scarpini[1,4] · Sergio Barbieri[1] · Andrea Arighi[1] · Francesca Mameli[1]

Received: 27 January 2023 / Accepted: 5 June 2023 / Published online: 27 June 2023

Abstract

Objective This study aimed to evaluate the experience with telemedicine in patients with cognitive impairments and their caregivers.

Methods We conducted a survey-based study of patients who completed neurological consultation via video link between January and April 2022.

Results A total of 62 eligible neurological video consultations were conducted for the following categories of patients: Alzheimer's disease (33.87%), amnesic mild cognitive impairment (24.19%), frontotemporal dementia (17.74%), Lewy body dementia (4.84%), mixed dementia (3.23%), subjective memory disorders (12.90%), non-amnesic mild cognitive impairment (1.61%), and multiple system atrophy (1.61%).

The survey was successfully completed by 87.10% of the caregivers and directly by the patients in 12.90% of cases. Our data showed positive feedback regarding the telemedicine experience; both caregivers and patients reported that they found neurological video consultation useful (caregivers: 87.04%, 'very useful'; patients: 87.50%, 'very useful') and were satisfied overall (caregivers: 90.74%, 'very satisfied'; patients: 100%, 'very satisfied'). Finally, all caregivers (100%) agreed that neurological video consultation was a useful tool to reduce their burden (Visual Analogue Scale mean ± SD: 8.56 ± 0.69).

Conclusions Telemedicine is well received by patients and their caregivers. However, successful delivery incorporates support from staff and care partners to navigate technologies. The exclusion of older adults with cognitive impairment in developing telemedicine systems may further exacerbate access to care in this population. Adapting technologies to the needs of patients and their caregivers is critical for the advancement of accessible dementia care through telemedicine.

Keywords Cognitive impairment · Dementia · Caregiver · COVID-19 · Telemedicine · Neurological video consultation

Introduction

The coronavirus disease (COVID-19) pandemic has rendered older adults more vulnerable to not receiving the healthcare needed. Furthermore, it has placed those living with dementia at an even increased risk of developing other mental health symptoms due to social isolation and loneliness [1]. Indeed, as the virus spreads, it has become necessary to introduce social distancing measures, such as quarantine within urban areas, prohibition of travel to and from certain countries, and suspension of a large range of clinical activities. Elective face-to-face consultations had to be rescheduled, and the need for health care during the pandemic required telehealth solutions. National initiatives have been launched to review and update previous restrictions

✉ Francesca Mameli
francesca.mameli@policlinico.mi.it

1 Department of Neuroscience and Mental Health, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

2 Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

3 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy

4 Department of Pathophysiology and Transplantation (DEPT), Dino Ferrari Centre, University of Milan, Neuroscience Section, Milan, Italy

5 ASST Santi Paolo e Carlo, Milan, Italy

frontiers | Frontiers in Cellular Neuroscience

TYPE Original Research
PUBLISHED 02 March 2023
DOI 10.3389/fncel.2023.1112405

OPEN ACCESS

EDITED BY
Tomomi Ichinose,
Wayne State University, United States

REVIEWED BY
Sokol V. Todi,
Wayne State University, United States
Katarzyna Gaweda-Walerych,
Mossakowski Medical Research Institute (PAN), Poland

*CORRESPONDENCE
Alfredo Iacoangeli
✉alfredo.iacoangeli@kcl.ac.uk

†These authors share senior authorship

SPECIALTY SECTION
This article was submitted to Cellular Neurophysiology, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 30 November 2022
ACCEPTED 06 February 2023
PUBLISHED 02 March 2023

CITATION
Adey BN, Cooper-Knock J, Al Khleifat A, Fogh I, van Damme P, Corcia P, Couratier P, Hardiman O, McLaughlin R, Gotkine M, Drory V, Silani V, Ticozzi N, Veldink JH, van den Berg LH, de Carvalho M, Pinto S, Mora Pardina JS, Povedano Panades M, Andersen PM, Weber M, Başak NA, Shaw CE, Shaw PJ, Morrison KE, Landers JE, Glass JD, Vourc'h P, Dobson RJB, Breen G, Al-Chalabi A, Jones AR and Iacoangeli A (2023) Large-scale analyses of CAV1 and CAV2 suggest their expression is higher in post-mortem ALS brain tissue and affects survival.
Front. Cell. Neurosci. 17:1112405.
doi: 10.3389/fncel.2023.1112405

Large-scale analyses of CAV1 and CAV2 suggest their expression is higher in post-mortem ALS brain tissue and affects survival

Brett N. Adey[1,2], Johnathan Cooper-Knock[3], Ahmad Al Khleifat[4], Isabella Fogh[4], Philip van Damme[5,6,7], Philippe Corcia[8,9], Philippe Couratier[10,11], Orla Hardiman[12], Russell McLaughlin[13], Marc Gotkine[14,15], Vivian Drory[16,17], Vincenzo Silani[18,19], Nicola Ticozzi[18,19], Jan H. Veldink[20], Leonard H. van den Berg[20], Mamede de Carvalho[21], Susana Pinto[21], Jesus S. Mora Pardina[22], Mónica Povedano Panades[23], Peter M. Andersen[24], Markus Weber[25], Nazli A. Başak[26], Christopher E. Shaw[4], Pamela J. Shaw[3], Karen E. Morrison[27], John E. Landers[28], Jonathan D. Glass[29], Patrick Vourc'h[7,30], Richard J. B. Dobson[2,31,32,33], Gerome Breen[1], Ammar Al-Chalabi[4,34], Ashley R. Jones[4†] and Alfredo Iacoangeli[2,4,31*†]

[1]Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, [2]Department of Biostatistics and Health Informatics, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, [3]Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, United Kingdom, [4]Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, [5]Department of Neurosciences, KU Leuven-University of Leuven, Experimental Neurology and Leuven Brain Institute (LBI), Leuven, Belgium, [6]VIB, Center for Brain and Disease Research, Leuven, Belgium , [7]Department of Neurology, University Hospitals Leuven, Leuven, Belgium, [8]UMR 1253, Université de Tours, Inserm, Tours, France, [9]Centre de référence sur la SLA, CHU de Tours, Tours, France, [10]Centre de référence sur la SLA, CHRU de Limoges, Limoges, France, [11]UMR 1094, Université de Limoges, Inserm, Limoges, France, [12]Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland, [13]Complex Trait Genomics Laboratory, Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland, [14]Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel, [15]Agnes Ginges Center for Human Neurogenetics, Department of Neurology, Hadassah Medical Center, Jerusalem, Israel, [16]Department of Neurology, Tel-Aviv Sourasky Medical Centre, Tel-Aviv, Israel, [17]Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel, [18]Department of Neurology and Laboratory of Neuroscience, Istituto Auxologico Italiano, IRCCS, Milan, Italy, [19]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy, [20]Department of Neurology, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht, Netherlands, [21]Instituto de Fisiologia, Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, [22]ALS Unit, Hospital San Rafael, Madrid, Spain, [23]Functional Unit of Amyotrophic Lateral Sclerosis (UFELA), Service of Neurology, Bellvitge University Hospital, L'Hospitalet de Llobregat, Barcelona, Spain, [24]Department of Clinical Science, Umeå University, Umeå, Sweden, [25]Neuromuscular Diseases Unit/ALS Clinic, St. Gallen, Switzerland, [26]Koc University School of Medicine, Translational Medicine Research Center, NDAL, Istanbul, Turkey, [27]School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom, [28]Department of Neurology, University of Massachusetts Medical School, Worcester, MA, United States, [29]Department of Neurology, Emory University School of Medicine, Atlanta, GA, United States, [30]Service de Biochimie et Biologie moléculaire, CHU de Tours, Tours, France, [31]National Institute for Health Research Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London, London, United Kingdom, [32]Institute of Health Informatics, University College London, London, United Kingdom, [33]NIHR Biomedical Research Centre at University College London Hospitals, NHS Foundation Trust, London, United Kingdom, [34]King's College Hospital, London, United Kingdom

RESEARCH ARTICLE OPEN ACCESS

Functional Connectivity From Disease Epicenters in Frontotemporal Dementia

Federica Agosta, MD, PhD, Edoardo Gioele Spinelli, MD, Silvia Basaia, PhD, Camilla Cividini, MSc, Francesco Falbo, MD, Costanza Pavone, MD, Nilo Riva, MD, PhD, Elisa Canu, PhD, Veronica Castelnovo, MSc, Giuseppe Magnani, MD, Francesca Caso, MD, PhD, Paola Caroppo, MD, PhD, Sara Prioni, MSc, Cristina Villa, PhD, Lucio Tremolizzo, MD, Ildebrando Appollonio, MD, Vincenzo Silani, MD, Keith A. Josephs, MD, MST, MSc, Jennifer Whitwell, PhD, and Massimo Filippi, MD

Correspondence
Dr. Agosta
agosta.federica@hsr.it

Neurology® 2023;100:e2290-e2303. doi:10.1212/WNL.0000000000207277

Abstract

Background and Objectives

MRI connectomics is an ideal tool to test a network-based model of pathologic propagation from a disease epicenter in neurodegenerative disorders. In this study, we used a novel graph theory–based MRI paradigm to explore functional connectivity reorganization, discerning between direct and indirect connections from disease epicenters, and its relationship with neurodegeneration across clinical presentations of the frontotemporal dementia (FTD) spectrum, including behavioral variant of FTD (bvFTD), nonfluent variant of primary progressive aphasia (nfvPPA), and semantic variant of primary progressive aphasia (svPPA).

Methods

In this observational cross-sectional study, disease epicenters were defined as the peaks of atrophy of a cohort of patients with high confidence of frontotemporal lobar degeneration pathology (Mayo Clinic). These were used as seed regions for stepwise functional connectivity (SFC) analyses in an independent (Milan) set of patients with FTD to assess connectivity in regions directly and indirectly connected to the epicenters. Correlations between SFC architecture in healthy conditions and atrophy patterns in patients with FTD were also tested.

Results

As defined by comparing the 42 Mayo Clinic patients with 15 controls, disease epicenters were the left anterior insula for bvFTD, left supplementary motor area for nfvPPA, and left inferior temporal gyrus (ITG) for svPPA. Compared with 94 age-matched controls, patients with bvFTD (n = 64) and nfvPPA (n = 34) of the Milan cohort showed widespread decreased SFC in bilateral cortical regions with direct/indirect connections with epicenters and increased SFC either in directly connected regions, physically close to the respective seed region, or in more distant cortical/cerebellar areas with indirect connections. Across all link steps, svPPA (n = 36) showed SFC decrease mostly within the temporal lobes, with co-occurrent SFC increase in cerebellar regions at indirect link steps. The average stepwise topological distance from the left ITG in a reference group of 50 young healthy controls correlated with regional gray matter volume in svPPA, consistent with network-based degeneration.

Discussion

Our findings demonstrate that each FTD syndrome is associated with a characteristic interplay of decreased and increased functional connectivity with the disease epicenter, affecting both

From the Neuroimaging Research Unit (F.A., E.G.S., S.B., C.C., F.F., C.P., E.C., V.C., M.F.), Division of Neuroscience, and Neurology Unit (F.A., E.G.S., G.M., F.C., M.F.), IRCCS San Raffaele Scientific Institute; Vita-Salute San Raffaele University (F.A., F.F., C.P., M.F.); Neurorehabilitation Unit (N.R., M.F.), IRCCS San Raffaele Scientific Institute; Experimental Neuropathology Unit (N.R.), Division of Neuroscience, IRCCS San Raffaele Scientific Institute; Unit of Neurology 5–Neuropathology (P.C., S.P., C.V.), Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan; Neurology Unit (L.T., I.A.), "San Gerardo" Hospital and University of Milano-Bicocca, Monza; Department of Neurology and Laboratory of Neuroscience (V.S.), IRCCS Istituto Auxologico Italiano, Milan; "Dino Ferrari" Center (V.S.), Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Italy; Department of Neurology (K.A.J.), and Department of Radiology (J.W.), Mayo Clinic, Rochester, MN; and Neurophysiology Service (M.F.), IRCCS San Raffaele Scientific Institute, Milan, Italy.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

The Article Processing Charge was funded by the authors.

Journal of Neurology
https://doi.org/10.1007/s00415-023-11749-4

SHORT COMMENTARY

Equating norms between the ALS Cognitive Behavioral Screen (ALS-CBS™) and the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) in non-demented ALS patients

Edoardo Nicolò Aiello[1,2] · Federica Solca[1] · Lucia Catherine Greco[3,4] · Silvia Torre[1] · Laura Carelli[1] · Claudia Morelli[1] · Alberto Doretti[1] · Eleonora Colombo[1] · Stefano Messina[1] · Debora Pain[5] · Alice Radici[5] · Andrea Lizio[3] · Jacopo Casiraghi[3] · Federica Cerri[3] · Susan Woolley[6] · Jennifer Murphy[7] · Lucio Tremolizzo[8] · Ildebrando Appollonio[8] · Federico Verde[1,9] · Valeria Ada Sansone[3,10] · Christian Lunetta[5] · Vincenzo Silani[1,9] · Nicola Ticozzi[1,9] · Barbara Poletti[1]

Received: 28 March 2023 / Revised: 22 April 2023 / Accepted: 26 April 2023

Abstract

Background The present study aimed at deriving equating norms to estimate scores on the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) based on those on the ALS Cognitive Behavioral Screen (ALS-CBS™) in an Italian cohort of non-demented ALS patients.

Methods ALS-CBS™ and ECAS scores of 293 ALS patients without frontotemporal dementia were retrospectively retrieved. Concurrent validity of the ALS-CBS™ towards the ECAS was tested by covarying for demographics, disease duration and severity, presence of *C9orf72* hexanucleotide repeat expansion and behavioural features. A linear-smoothing equipercentile equating (LSEE) model was employed to derive ALS-CBS™-to-ECAS cross-walks. Gaps in LSEE-based estimation were managed via a linear regression-based equating approach. Equivalence between empirical and derived ECAS scores was tested via a two-one-sided test (TOST) procedure for the dependent sample.

Results The ALS-CBS™ predicted the ECAS ($\beta = 0.75$), accounting for the vast majority of its variance (60% out of an $R^2 = 0.71$). Consistently, a strong, one-to-one linear association between ALS-CBS™ and ECAS scores was detected ($r = 0.84$; $R^2 = 0.73$). The LSEE was able to estimate conversions for the full range of the ALS-CBS™, except for raw scores equal to 1 and 6 – for whom a linear equating-based equation was derived. Empirical ECAS scores were equivalent to those derived with both methods.

Discussion Italian practitioners and researchers have been herewith provided with valid, straightforward cross-walks to estimate the ECAS based on ALS-CBS™ scores in non-demented ALS patients. Conversions herewith provided will help avoid cross-sectional/longitudinal inconsistencies in test adoption within research, and possibly clinical, settings.

Keywords ALS Cognitive Behavioral Screen · Edinburgh Cognitive and Behavioural ALS Screen · Amyotrophic lateral sclerosis · Frontotemporal degeneration · Equating

Background

Up to 50% of non-demented patients with amyotrophic lateral sclerosis (ALS) show cognitive deficits within the frontotemporal dementia (FTD) *spectrum* [1]—which, as negatively affecting their prognosis, need to be early screened for [2]. Moreover, cognitive screening measures are employed within observational/interventional studies addressing ALS [3].

To this aim, disease-specific cognitive screeners—i.e., (1) controlling for motor disabilities and (2) targeting those

Edoardo Nicolò Aiello and Federica Solca have contributed equally to this work.

Nicola Ticozzi and Barbara Poletti have contributed equally to this work.

✉ Barbara Poletti
b.poletti@auxologico.it

Extended author information available on the last page of the article

Lucia Catherine Greco
lucia.greco@centrocliniconemo.it

Silvia Torre
s.torre@auxologico.it

Laura Carelli
l.carelli@auxologico.it

Claudia Morelli
c.morelli@auxologico.it

Alberto Doretti
a.doretti@auxologico.it

Eleonora Colombo
e.colombo@auxologico.it

Stefano Messina
s.messina@auxologico.it

Debora Pain
debora.pain@icsmaugeri.it

Alice Radici
alice.radici@gmail.com

Andrea Lizio
andrea.lizio@centrocliniconemo.it

Jacopo Casiraghi
jacopo.casiraghi@centrocliniconemo.it

Federica Cerri
federica.cerri@centrocliniconemo.it

Susan Woolley
scwoolley@gmail.com

Jennifer Murphy
jennifermurphy66@me.com

Lucio Tremolizzo
lucio.tremolizzo@unimib.it

Ildebrando Appollonio
ildebrando.appollonio@unimib.it

Federico Verde
f.verde@auxologico.it

Valeria Ada Sansone
valeria.sansone@centrocliniconemo.it

Christian Lunetta
christian.lunetta@icsmaugeri.it

Vincenzo Silani
vincenzo.silani@unimi.it

Nicola Ticozzi
n.ticozzi@auxologico.it

[1] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Piazzale Brescia 20, 20149 Milan, MI, Italy

[2] PhD Program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

[3] Neuromuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milan, Italy

[4] NeMO Lab, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

[5] Istituti Clinici Scientifici Maugeri IRCCS, Neurorehabilitation Department of Milan Institute, Milan, Italy

[6] Syneos Health, Morrisville, NC, USA

[7] Biogen, Cambridge, MA, USA

[8] School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

[9] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

[10] Department of Biomedical Sciences of Health, University of Milan, Milan, Italy

frontiers | Frontiers in Aging Neuroscience

TYPE Brief Research Report
PUBLISHED 20 July 2023
DOI 10.3389/fnagi.2023.1217080

OPEN ACCESS

EDITED BY
Andrea Calvo,
University of Turin, Italy

REVIEWED BY
Nilo Riva,
San Raffaele Hospital (IRCCS), Italy
Umberto Manera,
University of Turin, Italy
Andrea Fortuna,
University Hospital of Padua, Italy

*CORRESPONDENCE
Barbara Poletti
b.poletti@auxologico.it

RECEIVED 04 May 2023
ACCEPTED 26 June 2023
PUBLISHED 20 July 2023

CITATION
Aiello EN, Solca F, Torre S, Patisso V, De Lorenzo A, Treddenti M, Colombo E, Maranzano A, Morelli C, Doretti A, Verde F, Silani V, Ticozzi N and Poletti B (2023) Bulbar involvement and cognitive features in amyotrophic lateral sclerosis: a retrospective study on 347 patients. *Front. Aging Neurosci.* 15:1217080. doi: 10.3389/fnagi.2023.1217080

Bulbar involvement and cognitive features in amyotrophic lateral sclerosis: a retrospective study on 347 patients

Edoardo Nicolò Aiello[1], Federica Solca[1], Silvia Torre[1], Valerio Patisso[2], Alberto De Lorenzo[2], Mauro Treddenti[2], Eleonora Colombo[1], Alessio Maranzano[1], Claudia Morelli[1], Alberto Doretti[1], Federico Verde[1,3], Vincenzo Silani[1,3], Nicola Ticozzi[1,3] and Barbara Poletti [1,4]*

[1]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [2]Neurology Residency Program, Università Degli Studi di Milano, Milan, Italy, [3]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università Degli Studi di Milano, Milan, Italy, [4]Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy

Background: This study aimed at clarifying the role of bulbar involvement (BI) as a risk factor for cognitive impairment (CI) in non-demented amyotrophic lateral sclerosis (ALS) patients.

Methods: Data on N = 347 patients were retrospectively collected. Cognition was assessed via the Edinburgh Cognitive and Behavioral ALS Screen (ECAS). On the basis of clinical records and ALS Functional Rating Scale-Revised (ALSFRS-R) scores, BI was characterized as follows: (1) BI at onset—from medical history; (2) BI at testing (an ALSFRS-R-Bulbar score $\leq$11); (3) dysarthria (a score $\leq$3 on item 1 of the ALSFRS-R); (4) severity of BI (the total score on the ALSFRS-R-Bulbar); and (5) progression rate of BI (computed as 12-ALSFRS-R-Bulbar/disease duration in months). Logistic regressions were run to predict a below- vs. above-cutoff performance on each ECAS measure based on BI-related features while accounting for sex, disease duration, severity and progression rate of respiratory and spinal involvement and ECAS response modality.

Results: No predictors yielded significance either on the ECAS-Total and -ALS-non-specific or on ECAS-Language/-Fluency or -Visuospatial subscales. BI at testing predicted a higher probability of an abnormal performance on the ECAS-ALS-specific (p = 0.035) and ECAS-Executive Functioning (p = 0.018). Lower ALSFRS-R-Bulbar scores were associated with a defective performance on the ECAS-Memory (p = 0.025). No other BI-related features affected other ECAS performances.

Discussion: In ALS, the occurrence of BI itself, while neither its specific features nor its presence at onset, might selectively represent a risk factor for executive impairment, whilst its severity might be associated with memory deficits.

KEYWORDS

bulbar, Frontotemporal Degeneration, cognition, neuropsychology, amyotrophic lateral sclerosis

frontiers | Frontiers in Psychology

TYPE Brief Research Report
PUBLISHED 20 January 2023
DOI 10.3389/fpsyg.2022.1107001

OPEN ACCESS

EDITED BY
Silvia Paola Caminiti,
San Raffaele Scientific Institute (IRCCS), Italy

REVIEWED BY
Fabiola De Marchi,
Azienda Ospedaliero Universitaria Maggiore della Carità, Italy
Weixi Kang,
Imperial College London, United Kingdom
Francesca Conca,
Neurological Institute Foundation Casimiro Mondino (IRCCS), Italy
Simona Raimo,
Magna Græcia University, Italy

*CORRESPONDENCE
Barbara Poletti
b.poletti@auxologico.it

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Neuropsychology, a section of the journal Frontiers in Psychology

RECEIVED 24 November 2022
ACCEPTED 22 December 2022
PUBLISHED 20 January 2023

CITATION
Aiello EN, Solca F, Greco LC, La Tona A, Torre S, Carelli L, Morelli C, Doretti A, Colombo E, Messina S, Pain D, Radici A, Lizio A, Casiraghi J, Cerri F, Brugnera A, Compare A, Woolley S, Murphy J, Tremolizzo L, Appollonio I, Verde F, Sansone VA, Lunetta C, Silani V, Ticozzi N and Poletti B (2023) Standardization of the Italian ALS-CBS™ Caregiver Behavioral Questionnaire.
Front. Psychol. 13:1107001.
doi: 10.3389/fpsyg.2022.1107001

Standardization of the Italian ALS-CBS™ Caregiver Behavioral Questionnaire

Edoardo Nicolò Aiello[1,2†], Federica Solca[1†], Lucia Catherine Greco[3,4], Antonino La Tona[5], Silvia Torre[1], Laura Carelli[1], Claudia Morelli[1], Alberto Doretti[1], Eleonora Colombo[1], Stefano Messina[1], Debora Pain[6], Alice Radici[6], Andrea Lizio[3], Jacopo Casiraghi[3], Federica Cerri[3], Agostino Brugnera[5], Angelo Compare[5], Susan Woolley[7], Jennifer Murphy[8], Lucio Tremolizzo[9], Ildebrando Appollonio[9], Federico Verde[1,10], Valeria Ada Sansone[3,11], Christian Lunetta[6], Vincenzo Silani[1,10], Nicola Ticozzi[1,10†] and Barbara Poletti [1*†]

[1]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [2]Ph.D. Program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy, [3]Neuromuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milan, Italy, [4]NeMO Lab, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [5]Department of Human and Social Sciences, University of Bergamo, Bergamo, Italy, [6]Istituti Clinici Scientifici Maugeri IRCCS, Department of Neurorehabilitation of Milan Institute, Milan, Italy, [7]Syneos Health, Morrisville, NC, United States, [8]Biogen Inc., Cambridge, MA, United States, [9]School of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy, [10]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy, [11]Department of Biomedical Sciences of Health, University of Milan, Milan, Italy

Background: The present investigation aimed at testing the psychometrics and diagnostics of the Italian version of the Caregiver Behavioral Questionnaire (CBQ) from the ALS Cognitive Behavioral Screen (ALS-CBS™), as well as its case–control discrimination, in a cohort of non-demented patients with ALS.

Methods: The caregivers of *N* = 265 non-demented patients with ALS and *N* = 99 healthy controls (HCs) were administered the CBQ and the Edinburgh Cognitive and Behavioural ALS Screen-Carer Interview (ECAS-CI). For *N* = 98 patients, an in-depth behavioural/psychopathological assessment *via* the Frontal Behavioural Inventory (FBI), the Dimensional Apathy Scale (DAS), the State and Trait Anxiety Inventory-Form Y (STAI-Y), and the Beck Depression Inventory (BDI) was also available. Factorial and construct validity, internal reliability, and diagnostics against an abnormal ECAS-CI score were tested in patients. Case–control discrimination was explored through logistic regression.

Results: The CBQ was internally reliable (McDonald's ω = 0.90) and underpinned by a simple, unidimensional structure; it converged with ECAS-CI, FBI, and DAS scores and diverged from STAI-Y and BDI ones. A cutoff of $\leq$ 33 accurately detected abnormal ECAS-CI scores (AUC = 0.85), yielding optimal error- and information-based diagnostics. The CBQ was independent of demographic and disease-related variables and discriminated patients from HCs ($p < 0.001$).

Discussion: The Italian version of the CBQ from the ALS-CBS™ is a valid, reliable, diagnostically sound, and feasible screener for detecting frontotemporal-like behavioural changes in non-demented patients with ALS. Its adoption is thus

Journal of Neural Transmission
https://doi.org/10.1007/s00702-023-02624-7

NEUROLOGY AND PRECLINICAL NEUROLOGICAL STUDIES - ORIGINAL ARTICLE

Clinimetrics and feasibility of the Italian version of the Frontal Assessment Battery (FAB) in non-demented Parkinson's disease patients

Edoardo Nicolò Aiello[1] · Alfonsina D'Iorio[2] · Federica Solca[1] · Silvia Torre[1] · Ruggero Bonetti[3] · Francesco Scheveger[3] · Eleonora Colombo[1] · Alessio Maranzano[1] · Luca Maderna[1] · Claudia Morelli[1] · Alberto Doretti[1] · Marianna Amboni[4,5] · Carmine Vitale[4,6] · Federico Verde[1,7] · Roberta Ferrucci[8,9] · Sergio Barbieri[9] · Eleonora Zirone[9] · Alberto Priori[10,11] · Gabriella Pravettoni[8,12] · Gabriella Santangelo[2] · Vincenzo Silani[1,7] · Nicola Ticozzi[1,7] · Andrea Ciammola[1] · Barbara Poletti[1]

Received: 20 February 2023 / Accepted: 18 March 2023

Abstract

Background This study aimed at assessing the cross-sectional and longitudinal clinimetrics and feasibility of the Frontal Assessment Battery (FAB) in non-demented Parkinson's disease (PD) patients.

Methods $N = 109$ PD patients underwent the FAB and the Montreal Cognitive Assessment (MoCA). A subsample of patients further underwent a thorough motor, functional and behavioral evaluation (the last including measures of anxiety, depression and apathy). A further subsample was administered a second-level cognitive battery tapping on attention, executive functioning, language, memory, praxis and visuo-spatial abilities. The following properties of the FAB were tested: (1) concurrent validity and diagnostics against the MoCA; (2) convergent validity against the second-level cognitive battery; (4) association with motor, functional and behavioral measures; (5) capability to discriminate patients from healthy controls (HCs; $N = 96$); (6) assessing its test–retest reliability, susceptibility to practice effects and predictive validity against the MoCA, as well as deriving reliable change indices (RCIs) for it, at a ≈ 6-month interval, within a subsample of patients ($N = 33$).

Results The FAB predicted MoCA scores at both T0 and T1, converged with the vast majority of second-level cognitive measures and was associated with functional independence and apathy. It accurately identified cognitive impairment (i.e., a below-cut-off MoCA score) in patients, also discriminating patients from HCs. The FAB was reliable at retest and free of practice effects; RCIs were derived according to a standardized regression-based approach.

Discussion The FAB is a clinimetrically sound and feasible screener for detecting dysexecutive-based cognitive impairment in non-demented PD patients.

Keywords Frontal assessment battery · Parkinson's disease · Cognitive screening · Dysexecutive · Neuropsychology

Background

In Parkinson's disease (PD) patients, executive functioning (EF) deficits represent an early, major driver of cognitive impairment (Kudlicka et al. 2011), detrimentally affect functional (Cahn et al. 1998; Puente et al. 2016; Vlagsma et al. 2017) and motor outcomes (Amboni et al. 2008; Smulders et al. 2013; Chung et al. 2021) and may predict incident dementia (Paulwoods and Tröster 2003). Hence, a timely detection of dysexecutive-based cognitive inefficiency via clinimetrically sound and feasible screeners is clinically pivotal in this population (Kudlicka et al. 2011; Rodriguez-Oroz et al. 2009). In addition, cognitive screening measures are often employed within clinical trials targeting both motor and non-motor features of PD (Chou et al. 2010; Litvan et al. 2018; Skorvanek et al. 2018).

Among performance-based screeners, the Frontal Assessment Battery (FAB) (Dubois et al. 2000) has proved

Edoardo Nicolò Aiello and Alfonsina D'Iorio have contributed equally to this work; Andrea Ciammola and Barbara Poletti have contributed equally as well.

✉ Barbara Poletti
b.poletti@auxologico.it

Extended author information available on the last page of the article

[1] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Piazzale Brescia 20, 20149 Milan, MI, Italy

[2] Department of Psychology, University of Campania "Luigi Vanvitelli", Caserta, Italy

[3] Neurology Residency Program, Università Degli Studi Di Milano, Milan, Italy

[4] Institute of Diagnosis and Health, IDC-Hermitage Capodimonte, Naples, Italy

[5] Department of Medicine, Surgery and Dentistry, University of Salerno, Salerno, Italy

[6] Department of Motor Sciences and Wellness, University "Parthenope", Naples, Italy

[7] Department of Pathophysiology and Transplantation, "Dino Ferrari Center", Università Degli Studi Di Milano, Milan, Italy

[8] Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

[9] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[10] ASST Santi Paolo e Carlo, San Paolo University Hospital, Milan, Italy

[11] Department of Health Sciences, "Aldo Ravelli" Center for Neurotechnology and Experimental Brain Therapeutics, University of Milan, Milan, Italy

[12] Applied Research Division for Cognitive and Psychological Science, IEO, European Institute of Oncology, IRCCS, Milan, Italy

Neurodegenerative Diseases

Brief Report

Neurodegener Dis 2023;22:159–163
DOI: 10.1159/000532115

Received: April 24, 2023
Accepted: July 6, 2023
Published online: July 21, 2023

Ecological Validity of the Montreal Cognitive Assessment in Non-Demented Parkinson's Disease Patients

Edoardo Nicolò Aiello[a] Alfonsina D'Iorio[b] Federica Solca[a] Silvia Torre[a] Eleonora Colombo[a] Alessio Maranzano[a] Alberto De Lorenzo[c] Valerio Patisso[c] Mauro Treddenti[c] Claudia Morelli[a] Alberto Doretti[a] Luca Maderna[a] Federico Verde[a, d] Roberta Ferrucci[e, g] Sergio Barbieri[f] Fabiana Ruggiero[f] Alberto Priori[g, h] Vincenzo Silani[a, d] Nicola Ticozzi[a, d] Gabriella Santangelo[b] Andrea Ciammola[a] Barbara Poletti[a, e]

[a]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milano, Italy; [b]Department of Psychology, University of Campania "Luigi Vanvitelli", Caserta, Italy; [c]Neurology Residency Program, Università degli Studi di Milano, Milano, Italy; [d]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milano, Italy; [e]Department of Oncology and Hemato-Oncology, University of Milan, Milano, Italy; [f]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy; [g]ASST Santi Paolo e Carlo, San Paolo University Hospital, Milano, Italy; [h]"Aldo Ravelli" Center for Neurotechnology and Experimental Brain Therapeutics, Department of Health Sciences, University of Milan, Milano, Italy

Keywords
Montreal Cognitive Assessment · Parkinson's disease · Ecological validity · Cognitive screening · Psychometrics

Abstract
Background: The ecological validity of performance-based cognitive screeners needs to be tested in order for them to be fully recommended for use within clinical practice and research. ***Objectives:*** The objective of this study was to examine, within an Italian cohort of non-demented Parkinson's disease (PD) patients, the ecological validity of the Montreal Cognitive Assessment (MoCA) by assessing its association with (1) functional independence (FI), (2) quality of life (QoL), and (3) behavioural-psychological (BP) outcomes. ***Methods:*** Seventy-four non-demented PD patients were administered the MoCA and underwent motor functional – i.e., Unified Parkinson's Disease Rating Scale (UPDRS), Modified Hoehn-Yahr Scale (HY), and Schwab and England Scale (SES) –, behavioural and psychological – i.e., State- and Trait-Anxiety Inventory-Form Y (STAI-Y1/-Y2), Beck Depression Inventory (BDI), and Dimensional Apathy Scale (DAS) – and QoL evaluations – i.e., MOS 36-Item Short Form Health Survey (SF-36). Associations of interest against FI, QoL, and BP outcomes were tested via Bonferroni-corrected Pearson's/Spearman's correlations while covarying for demographics, disease duration as well as UPDRS-III, UPDRS-IV, and HY scores. Intake of psychotropic drugs was also

Edoardo Nicolò Aiello, Alfonsina D'Iorio, Andrea Ciammola, and Barbara Poletti contributed equally to this work.

karger@karger.com
www.karger.com/ndd

Correspondence to:
Barbara Poletti, b.poletti@auxologico.it

Neurological Sciences
https://doi.org/10.1007/s10072-023-06643-w

ORIGINAL ARTICLE

Lower semantic fluency scores and a phonemic-over-semantic advantage predict abnormal CSF P-tau$_{181}$ levels in Aβ + patients within the Alzheimer's disease clinical spectrum

Edoardo Nicolò Aiello[1,2] · Federico Verde[1,3] · Federica Solca[1] · Ilaria Milone[1] · Eleonora Giacopuzzi Grigoli[4] · Antonella Dubini[5] · Antonia Ratti[1,6] · Roberta Ferrucci[7,8,9] · Erminio Torresani[5] · Alberto Priori[7,8] · Nicola Ticozzi[1,3] · Vincenzo Silani[1,3] · Barbara Poletti[1]

Received: 31 October 2022 / Accepted: 22 January 2023

Abstract

Background The present study aimed to determine whether patients with mild cognitive impairment (MCI) and dementia due to Alzheimer's disease (AD), semantic verbal fluency (SVF), and the semantic-phonemic discrepancy (SPD) could predict abnormal cerebrospinal fluid (CSF) phosphorylated tau (P-tau$_{181}$) and total tau (T-tau) levels.

Methods Phonemic verbal fluency (PVF) and SVF scores of $N = 116$ Aβ-positive patients with either MCI due to AD ($N = 39$) or probable AD dementia (ADD; $N = 77$) were retrospectively collected. The SPD was computed by subtracting PVF scores from SVF ones (positive and negative values corresponding to a semantic and phonemic advantage, respectively). Patients were cognitively phenotyped via a thorough test battery and profiled according to the amyloidosis/tauopathy/neurodegeneration (ATN) framework via CSF analyses. Two separate sets of logistic regressions were run to predict normal *vs.* abnormal P-tau$_{181}$ and T-tau levels by encompassing as predictors SVF + PVF and SPD and covarying for demographic, disease-related features, and cognitive profile.

Results Lower SVF, but not PVF, scores, as well as a greater phonemic advantage (i.e., negative SPD values), predicted abnormal CSF P-tau$_{181}$ levels ($p \leq .01$). Moreover, lower SVF scores were selectively predictive of abnormal CSF T-tau levels too ($p = .016$), while the SPD was not.

Discussion SVF and the SPD are able to predict tauopathy across the AD *spectrum*, thus supporting their status of valid, and sufficiently specific, cognitive markers of AD.

Keywords Verbal fluency · Semantic · Alzheimer's disease · Mild cognitive impairment · Cerebrospinal fluid · Tau

Background

Semantic verbal fluency (SVF) tasks have been historically proved effective in detecting and monitoring cognitive decline in mild cognitive impairment (MCI) and dementia due to Alzheimer's disease (AD) [1–3], as being able to capture temporal lobe-rooted, lexical-semantic deficits occurring as early as the preclinical stages [3–5], as well as to be sensitive to involutionary trends in cognition with advancing disease [6, 7]. Relevantly, associations have been reported across the AD *spectrum* between SVF and not only neuroradiological [8, 9] and cerebrospinal fluid (CSF) biomarkers [10–13], but also neuropathology [14]. A further, promising cognitive marker for AD-*spectrum* disorders has been identified in the loss of the "physiological" advantage of semantic over phonemic verbal fluency (PVF)—i.e., a task-difficulty effect whereby normotypical individuals retrieve, on average, a higher number of words within SVF than PVF [15]. A reversal of the semantic-phonemic discrepancy (SPD) indeed appears to be magnified within the AD *spectrum* as compared to the healthy aging [7, 16, 17], being rather specific to its cognitive phenotype [7, 18–20] also when compared to cognitive disorders of other etiologies (e.g., small vessel disease/vascular dementia

Edoardo Nicolò Aiello and Federico Verde contributed equally to this work; Vincenzo Silani and Barbara Poletti contributed equally as well.

✉ Barbara Poletti
b.poletti@auxologico.it

Extended author information available on the last page of the article

Authors and Affiliations

Edoardo Nicolò Aiello[1,2] · Federico Verde[1,3] · Federica Solca[1] · Ilaria Milone[1] · Eleonora Giacopuzzi Grigoli[4] · Antonella Dubini[5] · Antonia Ratti[1,6] · Roberta Ferrucci[7,8,9] · Erminio Torresani[5] · Alberto Priori[7,8] · Nicola Ticozzi[1,3] · Vincenzo Silani[1,3] · Barbara Poletti[1]

Edoardo Nicolò Aiello
e.aiello@auxologico.it

Federico Verde
f.verde@auxologico.it

Federica Solca
f.solca@auxologico.it

Ilaria Milone
ilaria.milone24@gmail.com

Eleonora Giacopuzzi Grigoli
eleonora.giacopuzzi@unimi.it

Antonella Dubini
antonella.dubini@auxologico.it

Antonia Ratti
antonia.ratti@unimi.it

Roberta Ferrucci
roberta.ferrucci@unimi.it

Erminio Torresani
e.torresani@auxologico.it

Alberto Priori
alberto.priori@unimi.it

Nicola Ticozzi
n.ticozzi@auxologico.it

Vincenzo Silani
vincenzo.silani@unimi.it

1 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Piazzale Brescia 20, 20149 Milan, Italy

2 PhD Program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

3 Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

4 Neurology Residency Program, Università Degli Studi Di Milano, Milan, Italy

5 Department of Laboratory Medicine, Laboratory of Clinical Chemistry, IRCCS Istituto Auxologico Italiano, Milan, Italy

6 Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy

7 Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics, Department of Health Sciences, International Medical School, University of Milan, Milan, Italy

8 ASST Santi Paolo E Carlo, San Paolo University Hospital, Milan, Italy

9 IRCCS Ca' Granda Foundation Maggiore Policlinico Hospital, Milan, Italy

Neurological Sciences
https://doi.org/10.1007/s10072-023-06791-z

ORIGINAL ARTICLE

Clinical usability of the Story-Based Empathy Task (SET) in non-demented ALS patients

Edoardo Nicolò Aiello[1] · Federica Solca[1] · Silvia Torre[1] · Eleonora Colombo[1] · Alessio Maranzano[1] · Marco Olivero[2] · Francesco Scheveger[2] · Claudia Morelli[1] · Alberto Doretti[1] · Federico Verde[1,3] · Roberta Ferrucci[4,5] · Sergio Barbieri[5] · Francesca Mameli[5] · Alberto Priori[6,7] · Vincenzo Silani[1,3] · Nicola Ticozzi[1,3] · Barbara Poletti[1]

Received: 25 February 2023 / Accepted: 31 March 2023

Abstract

Background This study aimed at assessing the clinical usability of the Story-Based Empathy Task (SET) in non-demented amyotrophic lateral sclerosis (ALS) patients.

Methods $N = 106$ non-demented ALS patients and $N = 101$ healthy controls (HCs) were administered the SET, which includes three subtests assessing Emotion Attribution (SET-EA), Intention Attribution (SET-IA) and causal inference (SET-CI) — the latter being a control task. Patients also underwent the Reading the Mind in the Eyes Test (RMET), the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) and a thorough behavioural and motor-functional evaluation. The diagnostics of the SET-EA and -IA were tested against a defective performance on the RMET. The association between SET subtests and cognitive/behavioural outcomes was examined net of demographic and motor-functional confounders. Case-control discrimination was explored for each SET subtest.

Results Demographically adjusted SET-EA and -IA scores accurately detected defective RMET performances at the optimal cutoffs of <3.04 (AUC = .84) and <3.61 (AUC = .88), respectively. By contrast, the SET-CI performed poorly in doing so (AUC = .58). The SET-EA converged with the RMET, as well as with ECAS-Executive and -Memory scores, whilst the SET-IA was unrelated to cognitive measures (including the RMET); the SET-CI was related to the ECAS-Language the ECAS-Executive. SET subscores were unrelated to behavioural outcomes. Only the SET-EA discriminated patients from HCs.

Conclusions The SET as a whole should not be addressed as a social-cognitive measure in this population. At variance, its subtest tapping on emotional processing — i.e., the SET-EA — is recommended for use as an estimate of social-cognitive abilities in non-demented ALS patients.

Keywords Social cognition · Story-Based Empathy Task · Amyotrophic lateral sclerosis · Frontotemporal degeneration · Neuropsychology

Background

The Story-Based Empathy Task (SET) is a non-verbal, second-level measure of social cognition that assesses both affective and cognitive Theory of Mind (ToM) facets — i.e., Emotion Attribution (SET-EA) and Intention Attribution (SET-IA), respectively — also including a control subtest targeting the ability to make Causal Inferences (SET-CI) [1].

At variance with the SET-CI, both the SET-EA and the SET-IA have been shown to validly detect social-cognitive dysfunctions in non-demented amyotrophic lateral sclerosis (ALS) patients [2–5] — whose cognitive phenotype is also featured by ToM deficits [6, 7] that are likely accountable for by fronto-temporal and limbic involvement [2, 3].

However, no study to date has focused on delivering an evaluation of the diagnostic properties of the SET-EA and -IA in this population — in spite of the diagnostic [8] and prognostic [9, 10] relevance of social-cognitive assessment in non-demented ALS patients. Relatedly, whilst the SET-EA has been systematically shown to discriminate such patients from healthy controls (HCs), evidence on the case-control discriminative capability of the SET-IA is conflicting

The authors Nicola Ticozzi and Barbara Poletti contributed equally.

✉ Barbara Poletti
b.poletti@auxologico.it

Extended author information available on the last page of the article

Authors and Affiliations

Edoardo Nicolò Aiello[1] · Federica Solca[1] · Silvia Torre[1] · Eleonora Colombo[1] · Alessio Maranzano[1] · Marco Olivero[2] · Francesco Scheveger[2] · Claudia Morelli[1] · Alberto Doretti[1] · Federico Verde[1,3] · Roberta Ferrucci[4,5] · Sergio Barbieri[5] · Francesca Mameli[5] · Alberto Priori[6,7] · Vincenzo Silani[1,3] · Nicola Ticozzi[1,3] · Barbara Poletti[1]

Edoardo Nicolò Aiello
e.aiello@auxologico.it

Federica Solca
f.solca@auxologico.it

Silvia Torre
s.torre@auxologico.it

Eleonora Colombo
e.colombo@auxologico.it

Alessio Maranzano
a.maranzano@auxologico.it

Marco Olivero
marco.olivero@unimi.it

Francesco Scheveger
francesco.scheveger@unimi.it

Claudia Morelli
c.morelli@auxologico.it

Alberto Doretti
a.doretti@auxologico.it

Federico Verde
f.verde@auxologico.it

Roberta Ferrucci
roberta.ferrucci@unimi.it

Sergio Barbieri
sergio.barbieri@policlinico.mi.it

Francesca Mameli
francesca.mameli@policlinico.mi.it

Alberto Priori
alberto.priori@unimi.it

Vincenzo Silani
vincenzo.silani@unimi.it

Nicola Ticozzi
n.ticozzi@auxologico.it

[1] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Piazzale Brescia 20, 20149 Milano, Italy

[2] Neurology Residency Program, Università degli Studi di Milano, Milano, Italy

[3] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milano, Italy

[4] Department of Oncology and Hemato-Oncology, University of Milan, Milano, Italy

[5] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy

[6] Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics, Department of Health Sciences, International Medical School, University of Milan, Milano, Italy

[7] ASST Santi Paolo e Carlo, San Paolo University Hospital, Milano, Italy

Neurological Sciences
https://doi.org/10.1007/s10072-023-07070-7

ORIGINAL ARTICLE

Validity, diagnostics and feasibility of the Italian version of the Montreal Cognitive Assessment (MoCA) in Huntington's disease

Edoardo Nicolò Aiello[1] · Federica Solca[1] · Silvia Torre[1] · Annalisa Lafronza[1] · Alessio Maranzano[1] · Ruggero Bonetti[2] · Francesco Scheveger[2] · Sabrina Maffi[3] · Consuelo Ceccarelli[4] · Marta Scocchia[4] · Melissa Casella[4] · Federico Verde[1,5] · Simone Migliore[3] · Vincenzo Silani[1,5] · Nicola Ticozzi[1,5] · Ferdinando Squitieri[3] · Andrea Ciammola[1] · Barbara Poletti[1,6]

Received: 5 June 2023 / Accepted: 8 September 2023

Abstract

Background This study is aimed at assessing the clinimetric properties and feasibility of the Italian version of the Montreal Cognitive Assessment (MoCA) in patients with Huntington's disease (HD).

Methods $N = 39$ motor-manifest HD patients, $N = 74$ Parkinson's disease (PD) patients and $N = 92$ matched HCs were administered the MoCA. HD patients further underwent the Unified Huntington's Disease Rating Scale (UHDRS), self-report questionnaires for anxiety and depression and a battery of first- and second-level cognitive tests. Construct validity was tested against cognitive and behavioural/psychiatric measures, whereas ecological validity against motor-functional subscales of the UHDRS. Sensitivity to disease severity was tested, via a logistic regression, by exploring whether the MoCA discriminated between patients in Shoulson-Fahn stage ≤ 2 *vs.* > 2. The same analysis was employed to test its ability to discriminate HD patients from HCs and PD patients.

Results The MoCA converged towards cognitive and behavioural measures but diverged from psychiatric ones, being also associated with motor/functional measures from the UHDRS. In identifying patients with cognitive impairment, adjusted MoCA scores were highly accurate (AUC = .92), yielding optimal diagnostics at the cut-off of < 19.945 ($J = .78$). The MoCA was able to discriminate patients in the middle-to-advanced from those in the early-to-middle stages of the disease ($p = .037$), as well as to differentiate HD patients from both HCs ($p < .001$) and PD patients ($p < .001$).

Conclusions The MoCA is a valid, diagnostically sound and feasible cognitive screener in motor-manifest HD patients, whose adoption is thus encouraged in clinical practice and research.

Keywords Montreal Cognitive Assessment · Huntington's disease · Cognitive screening · Dysexecutive · Diagnostics · Psychometrics

Introduction

Screening for cognitive dysfunctions in Huntington's disease (HD) patients is pivotal at both prognostic and interventional levels [1]. Moreover, cognitive screening measures are routinely employed as primary/secondary endpoints within epidemiological studies and clinical trials addressing this disorder [2, 3]. To such an aim, the Montreal Cognitive Assessment (MoCA) [4] has been listed amongst the "suggested" screeners by the Movement Disorders Society (MDS) [5], with recent meta-analytic evidence further availing its suitability for use in this population [6].

Nevertheless, it has been highlighted that disease-specific evidence on the diagnostic value of the MoCA in HD patients is seldom delivered—this similarly applying, albeit to a lesser extent, to its psychometrics (*e.g.* validity) and feasibility (*e.g.* its sensitivity to disease severity and its ability to discriminate this population from both normotypical individuals and patients with other brain disorders involving frontostriatal networks) [4–7]. Relevantly, the MDS itself has stressed out that such an unfortunate occurrence does lower the level of recommendation for a given cognitive

Edoardo Nicolò Aiello and Federica Solca contributed equally; Andrea Ciammola and Barbara Poletti contributed equally as well.

Extended author information available on the last page of the article

Authors and Affiliations

Edoardo Nicolò Aiello[1] · Federica Solca[1] · Silvia Torre[1] · Annalisa Lafronza[1] · Alessio Maranzano[1] · Ruggero Bonetti[2] · Francesco Scheveger[2] · Sabrina Maffi[3] · Consuelo Ceccarelli[4] · Marta Scocchia[4] · Melissa Casella[4] · Federico Verde[1,5] · Simone Migliore[3] · Vincenzo Silani[1,5] · Nicola Ticozzi[1,5] · Ferdinando Squitieri[3] · Andrea Ciammola[1] · Barbara Poletti[1,6]

✉ Barbara Poletti
b.poletti@auxologico.it

Edoardo Nicolò Aiello
e.aiello@auxologico.it

Federica Solca
f.solca@auxologico.it

Silvia Torre
s.torre@auxologico.it

Annalisa Lafronza
annalisa.lafronza@gmail.com

Alessio Maranzano
a.maranzano@auxologico.it

Ruggero Bonetti
ruggero.bonetti@unimi.it

Francesco Scheveger
francesco.scheveger@unimi.it

Consuelo Ceccarelli
consuelo.ceccarelli@lirh.it

Marta Scocchia
marta.scocchia@lirh.it

Melissa Casella
melissa.casella@lirh.it

Federico Verde
f.verde@auxologico.it

Simone Migliore
sim.migliore@gmail.com

Vincenzo Silani
vincenzo.silani@unimi.it

Nicola Ticozzi
n.ticozzi@auxologico.it

Ferdinando Squitieri
ferdinandosquitieri@yahoo.it

Andrea Ciammola
a.ciammola@auxologico.it

[1] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy

[2] Neurology Residency Program, Università degli Studi di Milano, Milan, Italy

[3] Huntington and Rare Diseases Unit, Fondazione IRCCS Casa Sollievo Della Sofferenza Research Hospital, San Giovanni Rotondo, Italy

[4] Italian League for Research On Huntington (LIRH) Foundation, Rome, Italy

[5] Department of Pathophysiology and Transplantation, "Dino Ferrari Center", Università degli Studi di Milano, Milan, Italy

[6] Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy

Neurological Sciences (2023) 44:1243–1249
https://doi.org/10.1007/s10072-022-06569-9

ORIGINAL ARTICLE

Clinimetrics of the cognitive section of the Italian ALS Cognitive Behavioral Screen (ALS-CBS™)

Edoardo Nicolò Aiello[1,2] · Lucia Catherine Greco[3,4] · Antonino La Tona[5] · Federica Solca[1] · Silvia Torre[1] · Laura Carelli[1] · Debora Pain[6] · Alice Radici[6] · Andrea Lizio[3] · Jacopo Casiraghi[3] · Federica Cerri[3] · Agostino Brugnera[5] · Angelo Compare[5] · Susan Woolley[7] · Jennifer Murphy[8] · Lucio Tremolizzo[9] · Ildebrando Appollonio[9] · Federico Verde[1,10] · Vincenzo Silani[1,10] · Nicola Ticozzi[1,10] · Christian Lunetta[6] · Valeria Ada Sansone[2,11] · Barbara Poletti[1]

Received: 22 November 2022 / Accepted: 15 December 2022 / Published online: 22 December 2022

Abstract

Background The present study aimed at (1) providing further validity and reliability evidence for the Italian version of the cognitive section of the ALS Cognitive Behavioral Screen (ALS-CBS™) and (2) testing its diagnostics within an Italian ALS cohort, as well as at (3) exploring its capability to discriminate patients from healthy controls (HCs).

Methods $N = 293$ non-demented ALS patients were administered the cognitive sections of the ALS-CBS™ and Edinburgh Cognitive and Behavioural ALS Screen (ECAS). $N = 96$ HCs demographically matched with $N = 96$ patients were also administered the cognitive section of the ALS-CBS™. In patients, factorial and construct validity, internal reliability, and diagnostics against a defective score on the cognitive section of the ECAS were tested. Case–control discrimination was assessed via a logistic regression.

Results ALS-CBS™ cognitive subscales were underpinned by a simple, unidimensional structure, internally reliable (McDonald's $\omega = 0.74$), and mostly related with ECAS *executive* and *fluency* scores ($r_s = 0.54$–0.71). Both raw and age- and education-adjusted scores on the cognitive section of the ALS-CBS™ accurately detected ECAS-defined cognitive impairment ($AUC = 0.80$ and .88, respectively), yielding optimal error-based, information-based and unitary diagnostics. A cut-off of < 15.374 was identified on adjusted scores. The test was able to discriminate patients from HCs ($p < 0.001$).

Discussion The cognitive section of the Italian ALS-CBS™ is a valid, reliable, and diagnostically sound ALS-specific screener for detecting frontotemporal, executive-/attentive-based cognitive inefficiency in non-demented ALS patients, being also able to discriminate them from normotypical individuals.

Keywords ALS Cognitive Behavioral Screen · Amyotrophic lateral sclerosis · Cognitive screening · Frontotemporal degeneration · Neuropsychology · Clinimetrics

Background

Cognitive deficits within the frontotemporal degeneration (FTD) *spectrum*—i.e., executive and language dysfunctions—affect up to 50% of non-demented amyotrophic lateral sclerosis (ALS) patients [1], negatively impacting on their prognosis and clinical management [2]. Early detecting FTD-*spectrum* cognitive impairment in this population is thereupon clinically pivotal [3]. Additionally, cognitive measures are addressed as outcomes within clinical trials addressing ALS [4].

To such an aim, disease-specific cognitive screeners—i.e., (1) sampling from those domains/functions typically involved in ALS and (2) controlling for motor disabilities possibly confounding cognitive performances—have been developed, namely the ALS Cognitive Behavioral Screen (ALS-CBS™) [5] and the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) [6]. The cognitive sections of

Edoardo Nicolò Aiello and Lucia Catherine Greco contributed equally; Valeria Ada Sansone and Barbara Poletti contributed equally as well.

✉ Barbara Poletti
b.poletti@auxologico.it

Extended author information available on the last page of the article

Authors and Affiliations

Edoardo Nicolò Aiello[1,2] · Lucia Catherine Greco[3,4] · Antonino La Tona[5] · Federica Solca[1] · Silvia Torre[1] · Laura Carelli[1] · Debora Pain[6] · Alice Radici[6] · Andrea Lizio[3] · Jacopo Casiraghi[3] · Federica Cerri[3] · Agostino Brugnera[5] · Angelo Compare[5] · Susan Woolley[7] · Jennifer Murphy[8] · Lucio Tremolizzo[9] · Ildebrando Appollonio[9] · Federico Verde[1,10] · Vincenzo Silani[1,10] · Nicola Ticozzi[1,10] · Christian Lunetta[6] · Valeria Ada Sansone[2,11] · Barbara Poletti[1]

Edoardo Nicolò Aiello
e.aiello@auxologico.it

Lucia Catherine Greco
lucia.greco@centrocliniconemo.it

Antonino La Tona
antonino.latona@unibg.it

Federica Solca
f.solca@auxologico.it

Silvia Torre
s.torre@auxologico.it

Laura Carelli
l.carelli@auxologico.it

Debora Pain
debora.pain@icsmaugeri.it

Alice Radici
alice.radici@gmail.com

Andrea Lizio
andrea.lizio@centrocliniconemo.it

Jacopo Casiraghi
jacopo.casiraghi@centrocliniconemo.it

Federica Cerri
federica.cerri@centrocliniconemo.it

Agostino Brugnera
agostino.brugnera@unibg.it

Angelo Compare
angelo.compare@unibg.it

Susan Woolley
scwoolley@gmail.com

Jennifer Murphy
jennifermurphy66@me.com

Lucio Tremolizzo
lucio.tremolizzo@unimib.it

Ildebrando Appollonio
ildebrando.appollonio@unimib.it

Federico Verde
f.verde@auxologico.it

Vincenzo Silani
vincenzo.silani@unimi.it

Nicola Ticozzi
n.ticozzi@auxologico.it

Christian Lunetta
christian.lunetta@icsmaugeri.it

Valeria Ada Sansone
valeria.sansone@centrocliniconemo.it

[1] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Piazzale Brescia 20, 20149 Milan, Italy

[2] PhD Program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

[3] Neuromuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milan, Italy

[4] NeMO Lab, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

[5] Department of Human and Social Sciences, University of Bergamo, Bergamo, Italy

[6] Istituti Clinici Scientifici Maugeri IRCCS, Neurorehabilitation Department of Milan Institute, Milan, Italy

[7] Syneos Health, Morrisville, NC, USA

[8] Biogen, Cambridge, MA, USA

[9] School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

[10] Department of Pathophysiology and Transplantation, Dino Ferrari Center", Università degli Studi di Milano, Milan, Italy

[11] Department of Biomedical Sciences of Health, University of Milan, Milan, Italy

Neurological Sciences
https://doi.org/10.1007/s10072-023-07074-3

ORIGINAL ARTICLE

Frontotemporal-spectrum disorders and functional independence in non-demented ALS patients

Edoardo Nicolò Aiello[1] · Federica Solca[1] · Silvia Torre[1] · Francesco Gentile[2] · Francesco Scheveger[2] · Marco Olivero[2] · Eleonora Colombo[1] · Alessio Maranzano[1] · Martina Manzoni[3] · Claudia Morelli[1] · Alberto Doretti[1] · Federico Verde[1,4] · Vincenzo Silani[1,4] · Nicola Ticozzi[1,4] · Barbara Poletti[1,5]

Received: 26 July 2023 / Accepted: 10 September 2023

Abstract

Background The present study aimed at determining whether, net of motor confounders, neuropsychological features affect functional independence (FI) in activities of daily living (ADLs) in non-demented amyotrophic lateral sclerosis (ALS) patients.

Methods $N = 88$ ALS patients without frontotemporal dementia were assessed for FI—Katz's Basic ADL Scale (BADL) and Lawton-Brody's Instrumental ADL Scale (IADL)—, cognition—Edinburgh Cognitive and Behavioural ALS Screen (ECAS)—and behaviour—Beaumont Behavioural Inventory and Dimensional Apathy Scale. The association between cognitive and behavioural measures and BADL/IADL scores was assessed by covarying for demographics, anxiety and depression levels, disease duration and motor confounders—i.e. ALS Functional Rating Scale-Revised (ALSFRS-R) scores, progression rate and both King's and Milano-Torino stages.

Results Higher scores on the ECAS-Language were associated with higher IADL scores ($p = 0.005$), whilst higher apathetic features—as measured by the Dimensional Apathy Scale (DAS)—were inversely related to the BADL ($p = 0.003$). Whilst IADL scores were related to all ECAS-Language tasks, the DAS-Initiation was the only subscale associated with BADL scores. Patients with abnormal ECAS-Language ($p = 0.023$) and DAS ($p = 0.008$) scores were more functionally dependent than those without.

Discussion Among non-motor features, language changes and apathetic features detrimentally affect FI in non-demented ALS patients.

Keywords Amyotrophic lateral sclerosis · Activities of daily living · Neuropsychology · Functional independence · Frontotemporal degeneration

Background

Frontotemporal-spectrum disorders (FTSDs) are acknowledged to detrimentally affect survival in non-demented amyotrophic lateral sclerosis (ALS) patients [1] by interfering with decision-making and adherence within care settings [2, 3].

However, little is known on the extent to which neuropsychological features impact on patients' functional independence (FI) in daily living—likely due to their physical disabilities representing a major confounder to the study of such a matter [4, 5]. Only two reports have indeed to this day addressed this topic—the first, by Mioshi et al. [4], showing that FI was dependent on both motor and behavioural features, and the second, by Kapustin et al. [5], failing to detect an association between cognitive/behavioural features and FI net of ALS severity. However, these studies either preceded the availability of [4], or did not employ [5], ALS-specific cognitive/behavioural measures [6]. Moreover, the only study [5] having explored the association between FI and a performance-based measure of cognition did not provide single domain-level information.

The above being said, assessing how neuropsychological features impact FI in both basic and instrumental activities of daily living (ADL) in this population is prognostically pivotal, as it would shed further light on the ecological relevance of FTSDs in ALS besides their already acknowledged impact on survival [1, 2]. Hence, by employing a

Extended author information available on the last page of the article

IADL Questionnaire©, a new tool to measure instrumental activities of daily living in dementia. Neuroepidemiology 41:35–41
59. Sikkes SA, Pijnenburg YA, Knol DL, de Lange-de Klerk ES, Scheltens P, Uitdehaag BM (2013) Assessment of instrumental activities of daily living in dementia: diagnostic value of the Amsterdam Instrumental Activities of Daily Living Questionnaire. J Geriatr Psychiatry Neurol 26:244–250
60. Koster N, Knol DL, Uitdehaag BM, Scheltens P, Sikkes SA (2015) The sensitivity to change over time of the Amsterdam IADL Questionnaire©. Alzheimers Dement 11:1231–1240
61. Jutten RJ, Peeters CF, Leijdesdorff SM et al (2017) Detecting functional decline from normal aging to dementia: development and validation of a short version of the Amsterdam IADL Questionnaire. Alzheimers Dement 8:26–35
62. McMillan CT, Wuu J, Rascovsky K et al (2022) Defining cognitive impairment in amyotrophic lateral sclerosis: an evaluation of empirical approaches. Amyotroph Lateral Scler Frontotemporal Degener 23:517–526
63. Aiello EN, Iazzolino B, Pain D et al (2022) The diagnostic value of the Italian version of the Edinburgh cognitive and behavioral ALS screen (ECAS). Amyotroph Lateral Scler Frontotemporal Degener 23:527–531
64. Aiello EN, Solca F, Torre S et al (2023) Reliable change indices for the Italian Edinburgh cognitive and behavioral ALS screen (ECAS). Amyotroph Lateral Scler Frontotemporal Degener 24:339–342

Authors and Affiliations

Edoardo Nicolò Aiello[1] · Federica Solca[1] · Silvia Torre[1] · Francesco Gentile[2] · Francesco Scheveger[2] · Marco Olivero[2] · Eleonora Colombo[1] · Alessio Maranzano[1] · Martina Manzoni[3] · Claudia Morelli[1] · Alberto Doretti[1] · Federico Verde[1,4] · Vincenzo Silani[1,4] · Nicola Ticozzi[1,4] · Barbara Poletti[1,5]

✉ Barbara Poletti
b.poletti@auxologico.it

Edoardo Nicolò Aiello
e.aiello@auxologico.it

Federica Solca
f.solca@auxologico.it

Silvia Torre
s.torre@auxologico.it

Francesco Gentile
francesco.gentile@unimi.it

Francesco Scheveger
francesco.scheveger@unimi.it

Marco Olivero
marco.olivero@unimi.it

Eleonora Colombo
e.colombo@auxologico.it

Alessio Maranzano
a.maranzano@auxologico.it

Martina Manzoni
martina.manzoni@lanostrafamiglia.it

Claudia Morelli
c.morelli@auxologico.it

Alberto Doretti
a.doretti@auxologico.it

Federico Verde
f.verde@auxologico.it

Vincenzo Silani
vincenzo.silani@unimi.it

Nicola Ticozzi
n.ticozzi@auxologico.it

[1] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Piazzale Brescia 20, 20149 Milan, MI, Italy

[2] Neurology Residency Program, Università Degli Studi Di Milano, Milan, Italy

[3] Child Psychopathology Unit, Scientific Institute, IRCCS E. Medea – La Nostra Famiglia, Bosisio Parini, Lecco, Italy

[4] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

[5] Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy

RESEARCH ARTICLE OPEN ACCESS

Incidence and Long-term Functional Outcome of Neurologic Disorders in Hospitalized Patients With COVID-19 Infected With Pre-Omicron Variants

Simone Beretta, MD, PhD, Viviana Cristillo, MD, Giorgia Camera, MD, Carlo Morotti Colleoni, MD, Gaia Pellitteri, MD, Beatrice Viti, MD, Elisa Bianchi, PhD, Stefano Gipponi, MD, Maria Grimoldi, MD, Mariarosaria Valente, MD, Susanna Guttmann, MD, Maria Sofia Cotelli, MD, Pasquale Palumbo, MD, Giorgio Gelosa, MD, PhD, Stefano Meletti, MD, PhD, Cristina Schenone, MD, Donatella Ottaviani, MD, PhD, Massimo Filippi, MD, Andrea Zini, MD, Paola Basilico, MD, Lucia Tancredi, MD, Pietro Cortelli, MD, PhD, Massimiliano Braga, MD, Valeria De Giuli, MD, Serenella Servidei, MD, Damiano Paolicelli, MD, Federico Verde, MD, Stefano Caproni, MD, Antonio Pisani, MD, PhD, Vincenzina Lo Re, MD, FEBN, Luca Massacesi, MD, Daria Valeria Roccatagliata, MD, Paolo Manganotti, MD, Daniele Spitaleri, MD, Anna Formenti, MD, Marta Piccoli, MD, Silvia Marino, MD, PhD, Paola Polverino, MD, Umberto Aguglia, MD, Raffaele Ornello, MD, PhD, Elisabetta Perego, MD, Gabriele Siciliano, MD, Paola Merlo, MD, Marco Capobianco, MD, Leonardo Pantoni, MD, PhD, Alessandra Lugaresi, MD, PhD, Stefania Angelocola, MD, Anna De Rosa, MD, Maria Sessa, MD, Ettore Beghi, MD, PhD, Elio Clemente Agostoni, MD, Salvatore Monaco, MD, Alessandro Padovani, MD, PhD, Alberto Priori, MD, PhD, Vincenzo Silani, MD, Gioacchino Tedeschi, MD, and Carlo Ferrarese, MD, PhD, for Neuro-COVID Italy

Correspondence
Dr. Beretta
simone.beretta@unimib.it

Neurology® 2023;101:e892-e903. doi:10.1212/WNL.0000000000207534

Abstract

Background and Objectives

A variety of neurologic disorders have been reported as presentations or complications of coronavirus disease 2019 (COVID-19) infection. The objective of this study was to determine their incidence dynamics and long-term functional outcome.

Methods

The Neuro-COVID Italy study was a multicenter, observational, cohort study with ambispective recruitment and prospective follow-up. Consecutive hospitalized patients presenting new neurologic disorders associated with COVID-19 infection (neuro-COVID), independently from respiratory severity, were systematically screened and actively recruited by neurology specialists in 38 centers in Italy and the Republic of San Marino. The primary outcomes were incidence of

From the Department of Neurology (S.B., C.M.C., C.F.), Fondazione IRCCS San Gerardo dei Tintori, Monza; Department of Medicine and Surgery (S.B., C.M.C., C.F.), University of Milano Bicocca; The Milan Center for Neuroscience (NeuroMI) (S.B., G.G., C.F.); Neurology Unit and Department of Clinical and Experimental Sciences (V.C., S. Gipponi, A. Padovani), University of Brescia; Unit of Neurology and Neurophysiology (G.C., M.G., M.S.), ASST PG23, Bergamo; Santa Maria della Misericordia University Hospital (G.P., M.V.), Udine, Italy; San Marino Neurological Unit (B.V., S. Guttmann), San Marino Hospital; The Mario Negri Institute for Pharmacological Research IRCCS (E. Bianchi, E. Beghi), Milan; Department of Medical Area (DAME) (M.V.), University of Udine; Neurology Unit (M.S.C.), ASST Valcamonica, Esine, Brescia; USL Centro Toscana (P. Palumbo), Neurology Unit, Nuovo Ospedale Santo Stefano, Prato; Department of Neurology and Stroke Unit (G.G., E.C.A.), Niguarda, Milan; Department of Neurology and Department of Clinical Neurophysiology AOU Modena (S. Meletti), University of Modena and Reggio Emilia; Department of Neuroscience, Rehabilitation, Ophtalmology, Genetics, Maternal and Child Health (C.S.), University of Genoa; Ospedale Santa Maria del Carmine di Rovereto (D.O.), Trento; Neurology Unit (M.F.), IRCCS San Raffaele Scientific Institute, Milan; Department of Neurology (A.Z.), Metropolitan Stroke Network, Ospedale Maggiore, Bologna; Department of Neurology (P.B.), Ospedale A. Manzoni ASST Lecco; University of Milan (L.T., L.P., A. Priori); Neurology Unit (L.T., A. Priori), ASST Santi Paolo e Carlo; Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics (L.T.), Milan; IRCCS Institute of Neurological Science of Bologna (P.C.); DIBINEM (P.C.), University of Bologna; UOC Neurology (M.B.), ASST Vimercate; Department of Neurology (V.D.G.), ASST Cremona; Neurophysiopathology Unit, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy; Department of Basic Medical Sciences, Neurosciences and Sense Organs (D.P.), University of Bari; Department of Neurology and Laboratory of Neuroscience (F.V., V.S.), IRCCS Istituto Auxologico Italiano; "Dino Ferrari" Center (F.V., V.S.), Department of Pathophysiology and Transplantation, Università degli Studi di Milano; Neurology Division (S.C.), "S. Maria" University Hospital, Terni; IRCCS Mondino Foundation (A. Pisani), Department of Brain and Behavioral Sciences, University of Pavia; Department of Diagnostic and Therapeutic Services (V.L.R.), IRCCS ISMETT, Palermo; Department of Neurology 2 (L.M.), Careggi University Hospital, Florence; Department of Neurology and Neurosurgery (D.V.R.), ASST di Mantova; Clinical Neurology Unit (P. Manganotti), Cattinara University Hospital, University of Trieste; Department of Neurology (D.L.A.S.), AORN S.Giovanni Moscati, Avellino; Neurology and Stroke Unit (A.F.), Neuroscience Department, ASST-Lecco, Merate; Department of Neurology (M.P.), Ospedale San Filippo Neri, Rome; IRCCS Centro Neurolesi Bonino-Pulejo (S. Marino), Messina; Department of Neurology (P. Polverino), IRCCS Humanitas Research Hospital, Rozzano, Milan; Department of Medical and Surgical Sciences (U.A.), Magna Graecia University of Catanzaro; Department of Biotechnological and Clinical Sciences (R.O.), University of L'Aquila; Department of Neurology (E.P.), Ospedale Valduce, Como; Neurological Clinic (G.S.), University of Pisa; Department of Neurology (P. Merlo), Humanitas Gavazzeni, Bergamo; Department of Neurology (M.C.), S. Luigi Gonzaga Hospital, Orbassano; Ospedale Luigi Sacco (L.P.), Milan; IRCCS Institute of Neurological Science of Bologna (A.L.), UOSI Multiple Sclerosis Rehabilitation; Department of Biomedical Science and Neuromotricity (A.L.), University of Bologna; Department of Neurology (S.A.), Fermo; Department of Neurosciences (A.D.R.), Federico II University, Naples; Neurology Unit and Department of Neurosciences (S. Monaco), University of Verona; IRCCS Fondazione Ospedale Maggiore Policlinico (A. Priori), Milan; and Department of Advanced Medical and Surgical Sciences (G.T.), University of Campania, Naples, Italy.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

The Article Processing Charge was funded by ULA.

Coinvestigators are listed at links.lww.com/WNL/C964.

frontiers | Frontiers in Aging Neuroscience

TYPE Original Research
PUBLISHED 27 November 2023
DOI 10.3389/fnagi.2023.1272135

OPEN ACCESS

EDITED BY
Ana I. Duarte,
University of Coimbra, Portugal

REVIEWED BY
Dhiraj Kumar,
National Eye Institute (NIH), United States
Katherine Athayde Teixeira de Carvalho,
Pelé Pequeno Príncipe Research Institute, Brazil
Danilo Bilches Medinas,
University of São Paulo, Brazil

*CORRESPONDENCE
Davide Gentilini
✉ d.gentilini@auxologico.it

†These authors have contributed equally to this work and share last authorship

RECEIVED 03 August 2023
ACCEPTED 09 November 2023
PUBLISHED 27 November 2023

CITATION
Brusati A, Peverelli S, Calzari L, Tiloca C, Casiraghi V, Sorce MN, Invernizzi S, Carbone E, Cavagnola R, Verde F, Silani V, Ticozzi N, Ratti A and Gentilini D (2023) Exploring epigenetic drift and rare epivariations in amyotrophic lateral sclerosis by epigenome-wide association study.
Front. Aging Neurosci. 15:1272135.
doi: 10.3389/fnagi.2023.1272135

Exploring epigenetic drift and rare epivariations in amyotrophic lateral sclerosis by epigenome-wide association study

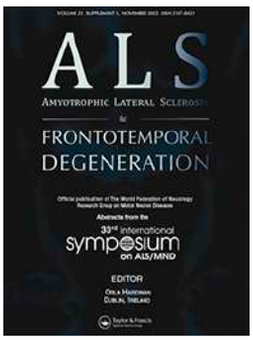
Alberto Brusati[1], Silvia Peverelli[2], Luciano Calzari[3], Cinzia Tiloca[2], Valeria Casiraghi[4], Marta Nice Sorce[2], Sabrina Invernizzi[2], Erika Carbone[5], Rebecca Cavagnola[1], Federico Verde[2,6], Vincenzo Silani[2,6], Nicola Ticozzi[2,6], Antonia Ratti[2,4†] and Davide Gentilini[1,3*†]

[1]Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy, [2]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [3]Bioinformatics and Statistical Genomics Unit, IRCCS Istituto Auxologico Italiano, Milan, Italy, [4]Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy, [5]Department of Endocrine and Metabolic Diseasesl, RCCS Istituto Auxologico Italiano, Milan, Italy, [6]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, University of Milan, Milan, Italy

During the last decades, our knowledge about the genetic architecture of sporadic amyotrophic lateral sclerosis (sALS) has significantly increased. However, besides the recognized genetic risk factors, also the environment is supposed to have a role in disease pathogenesis. Epigenetic modifications reflect the results of the interaction between environmental factors and genes and may play a role in the development and progression of ALS. A recent epigenome-wide association study (EWAS) in blood identified differentially methylated positions mapping to 42 genes involved in cholesterol biosynthesis and immune-related pathways. Here we performed a genome-wide DNA methylation analysis in the blood of an Italian cohort of 61 sALS patients and 61 healthy controls. Initially, a conventional genome-wide association analysis was performed, and results were subsequently integrated with the findings from the previous EWAS using a meta-analytical approach. To delve deeper into the significant outcomes, over-representation analysis (ORA) was employed. Moreover, the epigenetic signature obtained from the meta-analysis was examined to determine potential associations with chemical compounds, utilizing the Toxicogenomic Database. Expanding the scope of the epigenetic analysis, we explored both epigenetic drift and rare epivariations. Notably, we observed an elevated epigenetic drift in sALS patients compared to controls, both at a global and single gene level. Interestingly, epigenetic drift at a single gene level revealed an enrichment of genes related to the neurotrophin signaling pathway. Moreover, for the first time, we identified rare epivariations exclusively enriched in sALS cases associated with 153 genes, 88 of whom with a strong expression in cerebral areas. Overall, our study reinforces the evidence that epigenetics may contribute to the pathogenesis of ALS and that epigenetic drift may be a useful diagnostic marker. Moreover, this study suggests the potential role of epivariations in ALS.

KEYWORDS

ALS, epigenetics, bioinformatics, epivariations, EWAS, epigenetic-drift, SEMs, EML



Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iafd20

Italian reference values and brain correlates of verbal fluency index – *vs* standard verbal fluency test – to assess executive dysfunction in ALS

Elisa Canu, Veronica Castelnovo, Paola MV Rancoita, Michela Leocadi, Alessandra Lamanuzzi, Edoardo Gioele Spinelli, Silvia Basaia, Nilo Riva, Barbara Poletti, Federica Solca, Federico Verde, Nicola Ticozzi, Vincenzo Silani, Sharon Abrahams, Massimo Filippi & Federica Agosta

To cite this article: Elisa Canu, Veronica Castelnovo, Paola MV Rancoita, Michela Leocadi, Alessandra Lamanuzzi, Edoardo Gioele Spinelli, Silvia Basaia, Nilo Riva, Barbara Poletti, Federica Solca, Federico Verde, Nicola Ticozzi, Vincenzo Silani, Sharon Abrahams, Massimo Filippi & Federica Agosta (2023): Italian reference values and brain correlates of verbal fluency index – *vs* standard verbal fluency test – to assess executive dysfunction in ALS, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, DOI: 10.1080/21678421.2023.2167606

To link to this article: https://doi.org/10.1080/21678421.2023.2167606

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2023; 0: 1–9

RESEARCH ARTICLE

Italian reference values and brain correlates of verbal fluency index – *vs* standard verbal fluency test – to assess executive dysfunction in ALS

ELISA CANU[1], VERONICA CASTELNOVO[1], PAOLA MV RANCOITA[2], MICHELA LEOCADI[1,3], ALESSANDRA LAMANUZZI[1], EDOARDO GIOELE SPINELLI[1,4], SILVIA BASAIA[1], NILO RIVA[5], BARBARA POLETTI[6], FEDERICA SOLCA[6], FEDERICO VERDE[6,7], NICOLA TICOZZI[6,7], VINCENZO SILANI[6,7], SHARON ABRAHAMS[8,9], MASSIMO FILIPPI[1,3,4,5,10] & FEDERICA AGOSTA[1,3,4]

[1]*Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [2]*University Centre for Statistics in the Biomedical Sciences (CUSSB), Vita-Salute San Raffaele University, Milan, Italy,* [3]*Vita-Salute San Raffaele University, Milan, Italy,* [4]*Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [5]*Neurorehabilitation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [6]*Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy,* [7]*Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy,* [8]*Human Cognitive Neuroscience, Department of Psychology, University of Edinburgh, Edinburgh, UK,* [9]*Euan MacDonald Centre for MND Research, University of Edinburgh, Edinburgh, UK, and* [10]*Neurophysiology Service, IRCCS San Raffaele Scientific Institute, Milan, Italy*

Abstract

Objectives: In amyotrophic lateral sclerosis (ALS), verbal fluency index (V*fi*) is used to investigate fluency accounting for motor impairment. This study has three aims: (1) to provide V*fi* reference values from a cohort of Italian healthy subjects; (2) to assess the ability of V*fi* reference values (*vs* standard verbal fluency test [VFT]) in distinguishing ALS patients with and without executive dysfunction; and (3) to investigate the association between V*fi* and brain structural features of ALS patients. *Methods:* We included 180 healthy subjects and 157 ALS patients who underwent neuropsychological assessment, including VFT and V*fi*, and brain MRI. Healthy subjects were split into four subgroups according to sex and education. For each subgroup, we defined the 95th percentile of V*fi* as the cutoff. In ALS, the distributions of "abnormal" cases based on V*fi* and standard VFT cutoffs were compared using Fisher's exact test. Using quantile regressions in patients, we assessed the association between V*fi* and VFT scores, separately, with gray matter volumes and white matter (WM) tract integrity. *Results:* Applying V*fi* and VFT cutoffs, 9 and 13% of ALS cases, respectively, had abnormal scores ($p < 0.001$). In ALS, while higher V*fi* scores were associated with WM changes of callosal fibers linking supplementary motor area, lower VFT performances related to corticospinal tract alterations. *Discussion:* We provided Italian reference values for the spoken V*fi*. Compared to VFT, V*fi*s are critical to disentangle motor and cognitive deficits in ALS. In patients, abnormal V*fi*s were associated with damage to WM tracts specifically involved in ideational information processing.

Keywords: *Amyotrophic lateral sclerosis, cognitive classification, motor neuron disease, normative data, verbal fluency index*

Introduction

Amyotrophic lateral sclerosis (ALS), the most common form of motor neuron disease, is a progressive and fatal neurodegenerative disorder characterized by the degeneration of both the upper and lower motor neurons (1). In about 35–45% of ALS patients concomitant cognitive and/or behavioral deficits may occur, with 14% of these

Supplemental data for this article can be accessed online at https://doi.org/10.1080/21678421.2023.2167606.
Correspondence: Federica Agosta, Neuroimaging Research Unit, Division of Neuroscience, and Unit of Neurology, IRCCS San Raffaele Scientific Institute and Vita-Salute San Raffaele University, Via Olgettina, 60, 20132 Milan, Italy. Tel: #39-02-26433063; E-mail: agosta.federica@hsr.it
(Received 31 October 2022; revised 28 December 2022; accepted 9 January 2023)

ISSN 2167-8421 print/ISSN 2167-9223 online
DOI: 10.1080/21678421.2023.2167606

frontiers | Frontiers in Cellular Neuroscience

TYPE Original Research
PUBLISHED 05 December 2023
DOI 10.3389/fncel.2023.1285836

OPEN ACCESS

EDITED BY
Sara Salinas,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

REVIEWED BY
Larisa Ryskalin,
University of Pisa, Italy
Lara Gibellini,
University of Modena and Reggio Emilia, Italy

*CORRESPONDENCE
Mara Biasin
✉ mara.biasin@unimi.it

†These authors share senior authorship

RECEIVED 30 August 2023
ACCEPTED 17 November 2023
PUBLISHED 05 December 2023

CITATION
Cappelletti G, Colombrita C, Limanaqi F, Invernizzi S, Garziano M, Vanetti C, Moscheni C, Santangelo S, Zecchini S, Trabattoni D, Silani V, Clerici M, Ratti A and Biasin M (2023) Human motor neurons derived from induced pluripotent stem cells are susceptible to SARS-CoV-2 infection. *Front. Cell. Neurosci.* 17:1285836. doi: 10.3389/fncel.2023.1285836

Human motor neurons derived from induced pluripotent stem cells are susceptible to SARS-CoV-2 infection

Gioia Cappelletti[1], Claudia Colombrita[2], Fiona Limanaqi[1,3], Sabrina Invernizzi[2], Micaela Garziano[1,3], Claudia Vanetti[1], Claudia Moscheni[1], Serena Santangelo[4], Silvia Zecchini[1], Daria Trabattoni[1], Vincenzo Silani[2,5], Mario Clerici[3,6], Antonia Ratti[2,4†] and Mara Biasin[1*†]

[1]Laboratory of Immune-Biology, Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy, [2]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [3]Laboratory of Immunology, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy, [4]Department of Medical Biotechnology and Translational Medicine, Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics, University of Milan, Milan, Italy, [5]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, University of Milan, Milan, Italy, [6]Don C. Gnocchi Foundation, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Foundation, Milan, Italy

Introduction: COVID-19 typically causes Q7 respiratory disorders, but a high proportion of patients also reports neurological and neuromuscular symptoms during and after SARSCoV-2 infection. Despite a number of studies documenting SARS-CoV-2 infection of various neuronal cell populations, the impact of SARS-CoV-2 exposure on motor neuronal cells specifically has not been investigated so far.

Methods: Thus, by using human iPSC-derived motor neurons (iPSC-MNs) we assessed: (i) the expression of SARS-CoV-2 main receptors; (ii) iPSC-MN infectability by SARS-CoV-2; and (iii) the effect of SARS-CoV-2 exposure on iPSC-MN transcriptome.

Results: Gene expression profiling and immunofluorescence (IF) analysis of the main host cell receptors recognized by SARS-CoV-2 revealed that all of them are expressed in iPSC-MNs, with CD147 and NRP1 being the most represented ones. By analyzing SARS-CoV-2 N1 and N2 gene expression over time, we observed that human iPSC-MNs were productively infected by SARS-CoV-2 in the absence of cytopathic effect. Supernatants collected from SARS-CoV-2-infected iPSC-MNs were able to re-infect VeroE6 cells. Image analyses of SARS-CoV-2 nucleocapsid proteins by IF confirmed iPSC-MN infectability. Furthermore, SARS-CoV-2 infection in iPSCMNs significantly altered the expression of genes (IL-6, ANG, S1PR1, BCL2, BAX, Casp8, HLA-A, ERAP1, CD147, MX1) associated with cell survival and metabolism, as well as antiviral and inflammatory response.

Neurobiology of Disease 180 (2023) 106082

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

The contribution of Neanderthal introgression and natural selection to neurodegenerative diseases

Zhongbo Chen [a,b,c,1,**], Regina H. Reynolds [b,c,1], Antonio F. Pardiñas [d], Sarah A. Gagliano Taliun [e,f], Wouter van Rheenen [g], Kuang Lin [h], Aleksey Shatunov [i], Emil K. Gustavsson [b,c], Isabella Fogh [i], Ashley R. Jones [i], Wim Robberecht [j,k,l], Philippe Corcia [m], Adriano Chiò [n,o], Pamela J. Shaw [p], Karen E. Morrison [q], Jan H. Veldink [g], Leonard H. van den Berg [g], Christopher E. Shaw [i], John F. Powell [i], Vincenzo Silani [r,s], John A. Hardy [a,t,u,v,w], Henry Houlden [x], Michael J. Owen [d], Martin R. Turner [y], Mina Ryten [b,c], Ammar Al-Chalabi [i,*]

[a] Department of Neurodegenerative Disease, Queen Square Institute of Neurology, University College London (UCL), London, UK
[b] Department of Genetics and Genomic Medicine, Great Ormond Street Institute of Child Health, UCL, London, UK
[c] NIHR Great Ormond Street Hospital Biomedical Research Centre, UCL, London, UK
[d] MRC Centre for Neuropsychiatric Genetics and Genomics, Division of Psychological Medicine and Clinical Neurosciences, Cardiff University, Cardiff, UK
[e] Department of Medicine & Department of Neurosciences, Université de Montréal, Montréal, Québec, Canada
[f] Montréal Heart Institute, Montréal, Québec, Canada
[g] Department of Neurology and Neurosurgery, Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands
[h] Nuffield Department of Population Health, Oxford University, Oxford, UK
[i] Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK
[j] Department of Neurology, University Hospital Leuven, Leuven, Belgium
[k] Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease, Leuven, Belgium
[l] Vesalius Research Center, Laboratory of Neurobiology, Leuven, Belgium
[m] ALS Center, Department of Neurology, CHRU Bretonneau, Tours, France
[n] Rita Levi Montalcini Department of Neuroscience, ALS Centre, University of Torino, Turin, Italy
[o] Azienda Ospedaliera Universitaria Città della Salute e della Scienza, Torino, Italy
[p] Academic Neurology Unit, Department of Neuroscience, Faculty of Medicine, Dentistry and Health, University of Sheffield, Sheffield, UK
[q] School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, UK
[r] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milano, Italy
[s] Department of Pathophysiology and Transplantation, Dino Ferrari Center, Università degli Studi di Milano, 20122 Milano, Italy
[t] Reta Lila Weston Institute, Queen Square Institute of Neurology, UCL, London, UK
[u] UK Dementia Research Institute, Queen Square Institute of Neurology, UCL, London, UK
[v] NIHR University College London Hospitals Biomedical Research Centre, London, UK
[w] Institute for Advanced Study, The Hong Kong University of Science and Technology, Hong Kong, SAR, China
[x] Department of Neuromuscular Disease, Queen Square Institute of Neurology, UCL, London, UK
[y] Nuffield Department of Clinical Neurosciences, Oxford University, Oxford, UK

ARTICLE INFO

Keywords:
Neurodegenerative diseases

ABSTRACT

Humans are thought to be more susceptible to neurodegeneration than equivalently-aged primates. It is not known whether this vulnerability is specific to anatomically-modern humans or shared with other hominids. The

Abbreviations: SNPs, single nucleotide polymorphisms; ALS, amyotrophic lateral sclerosis; GWAS, genome-wide association studies; LDSC, linkage disequilibrium score regression; LD, linkage disequilibrium; FDR, False discovery rate.

* Corresponding author at: Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, King's College London, London SE5 9RX, UK.

** Corresponding author at: Department of Neurodgenerative Disease, Queen Square Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK.

E-mail addresses: zhongbo.chen@ucl.ac.uk (Z. Chen), ammar.al-chalabi@kcl.ac.uk (A. Al-Chalabi).

[1] ZC and RHR contributed equally to the work.

https://doi.org/10.1016/j.nbd.2023.106082
Received 15 October 2022; Received in revised form 10 March 2023; Accepted 13 March 2023
Available online 15 March 2023

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 26 September 2023
DOI 10.3389/fneur.2023.1249429

OPEN ACCESS

EDITED BY
Corrado Italo Angelini,
University of Padua, Italy

REVIEWED BY
Mauro Ceroni,
Neurological Institute Foundation Casimiro Mondino (IRCCS), Italy
Gianni Sorarù,
University of Padua, Italy
Zorica Dragisa Stevic,
University of Belgrade, Serbia

*CORRESPONDENCE
Nicola Ticozzi
✉ n.ticozzi@auxologico.it

RECEIVED 29 June 2023
ACCEPTED 13 September 2023
PUBLISHED 26 September 2023

CITATION
Colombo E, Gentile F, Maranzano A, Doretti A, Verde F, Olivero M, Gagliardi D, Faré M, Meneri M, Poletti B, Maderna L, Corti S, Corbo M, Morelli C, Silani V and Ticozzi N (2023) The impact of upper motor neuron involvement on clinical features, disease progression and prognosis in amyotrophic lateral sclerosis. *Front. Neurol.* 14:1249429. doi: 10.3389/fneur.2023.1249429

The impact of upper motor neuron involvement on clinical features, disease progression and prognosis in amyotrophic lateral sclerosis

Eleonora Colombo[1], Francesco Gentile[2], Alessio Maranzano[1], Alberto Doretti[1], Federico Verde[1,3], Marco Olivero[2], Delia Gagliardi[3,4], Matteo Faré[5,6], Megi Meneri[3,4], Barbara Poletti[1], Luca Maderna[1], Stefania Corti[3,4], Massimo Corbo[7], Claudia Morelli[1], Vincenzo Silani[1,3] and Nicola Ticozzi[1,3]*

[1]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [2]Neurology Residency Program, Università degli Studi di Milano, Milan, Italy, [3]"Dino Ferrari" Center, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy, [4]Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [5]Department of Neurology, San Gerardo Hospital ASST, Monza, Italy, [6]School of Medicine and Surgery, Università degli Studi di Milano-Bicocca, Milan, Italy, [7]Department of Neurorehabilitation Sciences, Casa di Cura Igea (CCI), Milan, Italy

Objectives: In amyotrophic lateral sclerosis (ALS) both upper (UMNs) and lower motor neurons (LMNs) are involved in the process of neurodegeneration, accounting for the great disease heterogeneity. We evaluated the associations of the burden of UMN impairment, assessed through the Penn Upper Motor Neuron Score (PUMNS), with demographic and clinical features of ALS patients to define the independent role of UMN involvement in generating disease heterogeneity, predicting disease progression and prognosis.

Methods: We collected the following clinical parameters on a cohort of 875 ALS patients: age and site of onset, survival, MRC scale, lower motor neuron score (LMNS), PUMNS, ALSFRS-R, change in ALSFRS-R over time (DFS), MITOS and King's staging systems (KSS). Transcranial magnetic stimulation was performed on a subgroup of patients and central motor conduction time (CMCT) and cortical silent period (CSP) were calculated.

Results: We observed that patients with an earlier age at onset and bulbar onset had higher PUMNS values. Higher values were also associated to lower ALSFRS-R and to higher DFS scores, as well as to higher MITOS and KSS, indicating that a greater UMN burden correlates with disease severity. Conversely, we did not appreciate any association between UMN involvement and survival or markers of LMN impairment. Moreover, PUMNS values showed a positive association with CMCT and a negative one with CSP values.

Interpretation: Our results suggest that the burden of UMN pathology, assessed through PUMNS, has an important independent role in defining clinical characteristics, functional disability, disease progression and prognosis in ALS patients. We also support the role of TMS in defining severity of UMN involvement.

Journal of Biomedical Informatics 148 (2023) 104557

Contents lists available at ScienceDirect

Journal of Biomedical Informatics

journal homepage: www.elsevier.com/locate/yjbin

Original Research

Advancing Italian biomedical information extraction with transformers-based models: Methodological insights and multicenter practical application

Claudio Crema [a,*], Tommaso Mario Buonocore [b], Silvia Fostinelli [c], Enea Parimbelli [b], Federico Verde [d,e], Cira Fundarò [f], Marina Manera [g], Matteo Cotta Ramusino [h], Marco Capelli [h], Alfredo Costa [h], Giuliano Binetti [c], Riccardo Bellazzi [b], Alberto Redolfi [a]

[a] Laboratory of Neuroinformatics, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy
[b] Dept. of Electrical, Computer and Biomedical Engineering, University of Pavia, Pavia, Italy
[c] Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy
[d] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy
[e] Department of Pathophysiology and Transplantation, Dino Ferrari Center, Università degli Studi di Milano, Milan, Italy
[f] Neurophysiopatology Unit, IRCCS Istituti Clinici Scientifici Maugeri, Pavia, Italy
[g] Psychology Unit, IRCCS Istituti Clinici Scientifici Maugeri, Pavia, Italy
[h] Unit of Behavioral Neurology, IRCCS Mondino Foundation Pavia, and Dept. of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

ARTICLE INFO

Keywords:
Natural language processing
Deep learning
Biomedical text mining
Language model
Transformer

ABSTRACT

The introduction of computerized medical records in hospitals has reduced burdensome activities like manual writing and information fetching. However, the data contained in medical records are still far underutilized, primarily because extracting data from unstructured textual medical records takes time and effort. Information Extraction, a subfield of Natural Language Processing, can help clinical practitioners overcome this limitation by using automated text-mining pipelines. In this work, we created the first Italian neuropsychiatric Named Entity Recognition dataset, PsyNIT, and used it to develop a Transformers-based model. Moreover, we collected and leveraged three external independent datasets to implement an effective multicenter model, with overall F1-score 84.77 %, Precision 83.16 %, Recall 86.44 %. The lessons learned are: (i) the crucial role of a consistent annotation process and (ii) a fine-tuning strategy that combines classical methods with a "low-resource" approach. This allowed us to establish methodological guidelines that pave the way for Natural Language Processing studies in less-resourced languages.

1. Introduction

The ubiquity of digital technologies is increasingly encompassing every aspect of our lives, and healthcare is no exception. In the last years there has been a rapid adoption of digital health tools [1]. This new technological paradigm has led to a dramatic increase in digitized medical text data in the everyday medical routine of healthcare institutions (e.g., discharge letters, examination results, medical notes) [2]. These documents, while very informative, are unstructured and not harmonized, creating a barrier that leads to insufficient use and under-exploitation. This lowers the efficiency of the clinical and research environments, since the extraction of such information into structured databases is time-consuming: physicians spend about 35 % of their time documenting patient data [3].

Artificial Intelligence (AI), and in particular Natural Language Processing (NLP), could provide useful tools to overcome these limitations. NLP is a collection of techniques and tools for processing human language written texts. Some examples of NLP tasks are: Named Entity Recognition (NER), which assigns words to predefined categories (e.g., person, location); Relation Extraction (RE), which connects named

* Corresponding author.
E-mail addresses: ccrema@fatebenefratelli.eu (C. Crema), buonocore.tms@gmail.com (T.M. Buonocore), sfostinelli@fatebenefratelli.eu (S. Fostinelli), enea.parimbelli@unipv.it (E. Parimbelli), f.verde@auxologico.it (F. Verde), cira.fundaro@icsmaugeri.it (C. Fundarò), marina.manera@icsmaugeri.it (M. Manera), matteo.cottaramusino@mondino.it (M.C. Ramusino), marco.capelli@mondino.it (M. Capelli), alfredo.costa@mondino.it (A. Costa), gbinetti@fatebenefratelli.eu (G. Binetti), riccardo.bellazzi@unipv.it (R. Bellazzi), aredolfi@fatebenefratelli.eu (A. Redolfi).

https://doi.org/10.1016/j.jbi.2023.104557
Received 29 June 2023; Received in revised form 26 October 2023; Accepted 24 November 2023
Available online 25 November 2023

Journal of Neural Transmission
https://doi.org/10.1007/s00702-023-02663-0

NEUROLOGY AND PRECLINICAL NEUROLOGICAL STUDIES - ORIGINAL ARTICLE

Clinimetrics of the Italian version of the Montreal Cognitive Assessment (MoCA) in adult-onset idiopathic focal dystonia

Alfonsina D'Iorio[1] · Edoardo Nicolò Aiello[2] · Assunta Trinchillo[4] · Vincenzo Silani[2,5] · Nicola Ticozzi[2,5] · Andrea Ciammola[2] · Barbara Poletti[2] · Marcello Esposito[3] · Gabriella Santangelo[1]

Received: 4 April 2023 / Accepted: 6 June 2023

Abstract

This study aimed at assessing the clinimetrics of the Montreal Cognitive Assessment (MoCA) in an Italian cohort of patients with adult-onset idiopathic focal dystonia (AOIFD). $N = 86$ AOIFD patients and $N = 92$ healthy controls (HCs) were administered the MoCA. Patients further underwent the Trail-Making Test (TMT) and Babcock Memory Test (BMT), being also screened via the Beck Depression Inventory-II (BDI-II) and the Dimensional Apathy Scale (DAS). Factorial structure and internal consistency were assessed. Construct validity was tested against TMT, BMT, BDI-II and DAS scores, whilst diagnostics against the co-occurrence of a defective performance on at least one TMT measure and on the BMT. Case–control discrimination was examined. The association between MoCA scores and motor-functional measures was explored. The MoCA was underpinned by a mono-component structure and acceptably reliable at an internal level. It converged towards TMT and BMT scores, as well as with the DAS, whilst diverging from the BDI-II. Its adjusted scores accurately detected cognitive impairment (AUC = .86) at a cut-off of < 17.212. The MoCA discriminated patients from HCs ($p < .001$). Finally, it was unrelated to disease duration and severity, as well as to motor phenotypes. The Italian MoCA is a valid, diagnostically sound and feasible cognitive screener in AOIFD patients.

Keywords Montreal Cognitive Assessment · Dystonia · Cognitive screening · Neuropsychology · Movement disorders · Hyperkinetic

Alfonsina D'Iorio and Edoardo Nicolò Aiello have contributed equally. Marcello Esposito and Gabriella Santangelo have contributed equally as well.

✉ Alfonsina D'Iorio
alfonsina.diorio@unicampania.it

Edoardo Nicolò Aiello
e.aiello@auxologico.it

Assunta Trinchillo
assuntatrinchillo94@gmail.com

Vincenzo Silani
vincenzo.silani@unimi.it

Nicola Ticozzi
n.ticozzi@auxologico.it

Andrea Ciammola
a.ciammola@auxologico.it

Barbara Poletti
b.poletti@auxologico.it

Marcello Esposito
marcelloesposito@live.it

Gabriella Santangelo
gabriella.santangelo@unicampania.it

[1] Department of Psychology, University of Campania "Luigi Vanvitelli", Caserta, Italy

[2] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy

[3] Clinical Neurophysiology Unit, Cardarelli Hospital, Naples, Italy

[4] Department of Neurosciences, Reproductive Sciences and Odontostomatology, University of Naples Federico II, Naples, Italy

[5] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università Degli Studi di Milano, Milan, Italy

Published online: 12 June 2023

Aging Clinical and Experimental Research (2023) 35:2157–2163
https://doi.org/10.1007/s40520-023-02493-w

ORIGINAL ARTICLE

Validity and diagnostics of the Italian version of the Montreal Cognitive Assessment (MoCA) in non-demented Parkinson's disease patients

Alfonsina D'Iorio[1] · Edoardo Nicolò Aiello[2] · Marianna Amboni[3,4] · Carmine Vitale[3,5] · Federico Verde[2,6] · Vincenzo Silani[2,6] · Nicola Ticozzi[2,6] · Andrea Ciammola[2] · Barbara Poletti[2,7] · Gabriella Santangelo[1]

Received: 20 February 2023 / Accepted: 3 July 2023 / Published online: 22 July 2023

Abstract

Background This study aimed at: (1) assessing, in an Italian cohort of non-demented Parkinson's disease (PD) patients, the construct validity of the Montreal Cognitive Assessment (MoCA) against both first- and second-level cognitive measures; (2) delivering an exhaustive and updated evaluation of its diagnostic properties.

Methods A retrospective cohort of $N = 237$ non-demented PD patients having been administered the MoCA was addressed, of whom $N = 169$ further underwent the Mini-Mental State Examination (MMSE) and $N = 68$ the Parkinson's Disease Cognitive Rating Scale (PD-CRS). A subsample ($N = 60$) also underwent a second-level cognitive battery encompassing measures of attention/executive functioning, language, memory, praxis and visuo-spatial abilities. Construct validity was assessed against both the PD-CRS and the second-level cognitive battery. Diagnostics were tested via receiver-operating characteristics analyses against a below-cut-off MMSE score.

Results The MoCA was associated with both PD-CRS scores ($p < .001$) and the vast majority of second-level cognitive measures ($ps < .003$). Both raw and adjusted MoCA scores proved to be highly accurate to the aim of identifying patients with MMSE-confirmed cognitive dysfunctions. A MoCA score adjusted for age and education according to the most recent normative dataset and < 19.015 is herewith suggested as indexing cognitive impairment in this population (AUC = .92; sensitivity = .92; specificity = .80).

Discussion The Italian MoCA is a valid and diagnostically sound screener for global cognitive inefficiency in non-demented PD patients. Further studies are nevertheless needed that confirm its diagnostic values against a measure other than the MMSE.

Keywords Montreal Cognitive Assessment · Parkinson's disease · Cognitive screening · Neuropsychology

Background

Up to 40% of non-demented patients with Parkinson's disease (PD) present with cognitive impairment [1] within both non-instrumental functions—*i.e.* attention and executive

Alfonsina D'Iorio and Edoardo Nicolò Aiello contributed equally; Barbara Poletti and Gabriella Santangelo contributed equally as well.

✉ Alfonsina D'Iorio
alfonsina.diorio@unicampania.it

[1] Department of Psychology, University of Campania "Luigi Vanvitelli", Caserta, Italy

[2] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy

[3] Institute of Diagnosis and Health, IDC-Hermitage Capodimonte, Naples, Italy

[4] Department of Medicine, Surgery and Dentistry, University of Salerno, Salerno, Italy

[5] Department of Motor Sciences and Wellness, University "Parthenope", Naples, Italy

[6] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

[7] Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy

Archives of Clinical Neuropsychology, 2023, **00**, 1–5
https://doi.org/10.1093/arclin/acad087
Original Empirical Article

Early Detection of Depression in Parkinson's Disease: Psychometrics and Diagnostics of the Spanish Version of the Beck Depression Inventory

Alfonsina D'Iorio[1], Gianpaolo Maggi[1], Pasqualina Guida[2,3], Edoardo Nicolò Aiello[4], Barbara Poletti[4], Vincenzo Silani[4,5], Nicola Ticozzi[4,5], Gabriella Santangelo[1,†], Ignacio Obeso[2,6,*,†]

[1]Department of Psychology, University of Campania Luigi Vanvitelli, Caserta, Italy
[2]Control and Habit Laboratory, HM CINAC (Centro Integral en Neurociencias), University Hospital HM Puerta del Sur; CEU San Pablo University, Madrid, Spain
[3]Control and Habit Laboratory, Network Center for Biomedical Research on Neurodegenerative Diseases, Carlos III Institute, Madrid, Spain
[4]Department of Neurology and Laboratory of Neuroscience, IRCCS, Istituto Auxologico Italiano, Milano, Italy
[5]Department of Pathophysiology and Transplantation, "Dino Ferrari Center", Università degli Studi di Milano, Milano, Italy
[6]Department of Psychobiology, Universidad Complutense de Madrid, Madrid, Spain

[*]Corresponding author at: Department of Psychobiology and Methods on Behavioural Sciences, Campus Somosaguas s/n, 28224, Complutense University of Madrid; HM CINAC, Centro Integral de Neurociencias AC; Hospital Universitario HM Puerta del Sur, HM Hospitales, Avda. Carlos V, 70. 28938, Madrid, Spain.
E-mail address: i.obesomartin@gmail.com (I. Obeso).

[†]These authors equally contributed

ABSTRACT

Objective: Depression is one of the most disabling non-motor symptoms in Parkinson's disease (PD) and requires proper diagnosis as it negatively impacts patients' and their relatives quality of life. The present study aimed to examine the psychometric and diagnostic properties of the Beck Depression Inventory-I (BDI-I) in a Spanish PD cohort.

Method: Consecutive PD outpatients completed the Spanish version of the BDI-I and other questionnaires assessing anxiety and apathy. Patients' caregivers completed the depression/dysphoria domain of the Neuropsychiatric Inventory (NPI-D). The internal consistency, convergent and divergent validity and the factorial structure of BDI-I were evaluated, and an optimal cut-off was defined by means of the Youden index.

Results: The BDI-I proved to have a good internal consistency and was underpinned by a mono-component structure. Regarding construct validity, the BDI-I was substantially related to anxiety and apathy measures in PD. Furthermore, the BDI-I overall showed good accuracy with adequate sensitivity and specificity. The optimal cut-off point was defined at 10.

Conclusions: We provided evidence of the psychometric and diagnostic properties of the Spanish version of the BDI-I as a screening tool for depression in Spanish speaking PD patients, suggesting its usefulness in clinical research and practice.

Keywords: Depression; Parkinson's disease; Assessment; Norms/normative studies

INTRODUCTION

Depression is one of the most common non-motor symptoms in Parkinson's disease (PD), with an average prevalence of 22.9% (Goodarzi et al., 2016), with great impact on quality of life (QoL) (Balestrino & Martinez-Martin, 2017). In recent years, the occurrence of depression in Spain has become an important problem of public health, which is the cause of heavy government healthcare spending. A review by Cardila and colleagues (2015) estimated that the prevalence rate for depression in the general population in Spain was 8.56% while, for Spanish PD patients, the prevalence of depression was 32.63% (Chuquilín-Arista et al., 2020). Accordingly, depressive symptoms should be identified early in PD patients to provide timely interventions (e.g., medication changes or psychotherapeutic support).

The Beck Depression Inventory-I (BDI-I) (Beck & Steer, 1987) is among the most widely used questionnaires to assess the occurrence and the severity of self-reported depressive symptoms in both research and clinical settings. The BDI-I, like other depression scales, includes somatic or movement-related and sexual activities often diminished or reduced by the disease itself, which could decrease the psychometric properties of the

Received: May 30, 2023. **Revised:** September 20, 2023. **Accepted:** October 10, 2023

Fortunato *et al.*
Orphanet Journal of Rare Diseases (2023) 18:196
https://doi.org/10.1186/s13023-023-02776-5

Orphanet Journal of Rare Diseases

RESEARCH

Open Access

Digital health and Clinical Patient Management System (CPMS) platform utility for data sharing of neuromuscular patients: the Italian EURO-NMD experience

Fernanda Fortunato[1,2†], Francesca Bianchi[3†], Giulia Ricci[3], Francesca Torri[3], Francesca Gualandi[2], Marcella Neri[2], Marianna Farnè[1,2], Fabio Giannini[4], Alessandro Malandrini[4], Nila Volpi[4], Diego Lopergolo[4], Vincenzo Silani[5,6], Nicola Ticozzi[5,6], Federico Verde[5,6], Davide Pareyson[7], Silvia Fenu[7], Silvia Bonanno[8], Vincenzo Nigro[9,10], Cristina Peduto[9], Paola D'Ambrosio[9], Roberta Zeuli[9], Mariateresa Zanobio[9], Esther Picillo[9], Serenella Servidei[11], Guido Primiano[11], Cristina Sancricca[11], Monica Sciacco[12], Roberta Brusa[12], Massimiliano Filosto[13,14,15], Stefano Cotti Piccinelli[13,14,15], Elena Pegoraro[16], Tiziana Mongini[17], Luca Solero[17], Giulio Gadaleta[17], Chiara Brusa[17], Carlo Minetti[18], Claudio Bruno[19], Chiara Panicucci[19], Valeria A. Sansone[20,21], Christian Lunetta[20], Alice Zanolini[20], Antonio Toscano[22,23], Alessia Pugliese[22], Giulia Nicocia[22], Enrico Bertini[24], Michela Catteruccia[24], Daria Diodato[24], Antonio Atalaia[25], Teresinha Evangelista[26], Gabriele Siciliano[3] and Alessandra Ferlini[1,2*]

Abstract

Background The development of e-health technologies for teleconsultation and exchange of knowledge is one of the core purposes of European Reference Networks (ERNs), including the ERN EURO-NMD for rare neuromuscular diseases. Within ERNs, the Clinical Patient Management System (CPMS) is a web-based platform that seeks to boost active collaboration within and across the network, implementing data sharing. Through CPMS, it is possible to both discuss patient cases and to make patients' data available for registries and databases in a secure way. In this view, CPMS may be considered a sort of a temporary storage for patients' data and an effective tool for data sharing; it facilitates specialists' consultation since rare diseases (RDs) require multidisciplinary skills, specific, and outstanding clinical experience.

Following European Union (EU) recommendation, and to promote the use of CPMS platform among EURO-NMD members, a twelve-month pilot project was set up to train the 15 Italian Health Care Providers (HCPs). In this paper, we report the structure, methods, and results of the teaching course, showing that tailored, ERN-oriented, training can significantly enhance the profitable use of the CPMS.

†Fernanda Fortunato and Francesca Bianchi have equal contribution.

*Correspondence:
Alessandra Ferlini
fla@unife.it
Full list of author information is available at the end of the article

Funding
Sarepta grant has funded this twelve-months project. This project has been supported by ERN EURO-NMD, which is partly cofinanced by the European Union within the framework of the Third Health Programme "ERN- 2016—Framework Partnership Agreement 2017–2021".

Availability of data and materials
The datasets generated during the current study are not publicly available due to CPMS restricted access to ERN members, but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All patients have signed a specific consent to have data uploaded in CPMS platform, to have data available for databases and registries and to have data available for researches. This consent process was established by DG SANTE in collaboration with the legal and ethical working group of the ERNs. A common, information and consent form, complying with GDPR, was translated in 24 EU languages and is available to be used across all ERNs.

Consent for publication
Not applicable.

Competing interests
Not applicable.

Author details
[1]Medical Genetics Unit, Department of Medical Sciences, University of Ferrara, Ferrara, Italy. [2]Medical Genetics Unit, Department of Mother and Child, Sant'Anna University Hospital of Ferrara, Ferrara, Italy. [3]Department of Clinical and Experimental Medicine, Neurological Clinic, University of Pisa, Pisa, Italy. [4]Department of Medical, Surgical and Neurological Sciences, University of Siena, Siena, Italy. [5]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy. [6]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università Degli Studi Di Milano, Milan, Italy. [7]Unit of Rare Neurodegenerative and Neurometabolic Diseases, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy. [8]Neuroimmunology and Neuromuscular Diseases Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy. [9]Department of Precision Medicine, University of Campania "L. Vanvitelli", Naples, Italy. [10]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Naples, Italy. [11]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy. [12]Neuromuscular and Rare Disease Unit, Department of Neuroscience, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. [13]Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy. [14]ASST Spedali Civili Di Brescia, Brescia, Italy. [15]NeMO-Brescia Clinical Center for Neuromuscular Diseases, Brescia, Italy. [16]Department of Neuroscience, University of Padova, Padua, Italy. [17]Department of Neurosciences "Rita Levi Montalcini", University of Turin, Turin, Italy. [18]Pediatric Neurology Unit and Muscle Unit, IRCCS Istituto Giannina Gaslini, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy. [19]Center of Translational and Experimental Myology, IRCCS Istituto Giannina Gaslini, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy. [20]The NEMO (NEuroMuscular Omniservice) Clinical Center, Milan, Italy. [21]Neurorehabilitation Unit, University of Milan, Milan, Italy. [22]Neurology and Neuromuscular Diseases Unit, Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy. [23]ERN-NMD Center of Messina, Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy. [24]Unit of Neuromuscular and Neurodegenerative Disorders, Department of Neurosciences, Bambino Gesù Children's Hospital IRCCS, Rome, Italy. [25]Service of Neuromyology, APHP-GH Pitié-Salpêtrière, Sorbonne Université, Paris, France. [26]Neuromuscular Morphology Unit, Institute of Myology, GHU Pitié-Salpêtrière, Sorbonne Université, Paris, France.

Received: 15 September 2022 Accepted: 18 June 2023
Published online: 21 July 2023

References

1. Website of the European Commission https://ec.europa.eu/health/non_communicable_diseases/rare_diseases_en. Accessed 15 July 2022
2. Héon-Klin V. European Reference networks for rare diseases: what is the conceptual framework? Orphanet J Rare Dis. 2017;12:137. https://doi.org/10.1186/s13023-017-0676-3.
3. Website of the European Commission https://ec.europa.eu/health/ern_en. Accessed 15 July 2022
4. Website of ERN EURO-NMD. https://ern-euro-nmd.eu/. Accessed 21 March 2023
5. Deenen JC, Horlings CG, Verschuuren JJ, Verbeek AL, van Engelen BG. The epidemiology of neuromuscular disorders: A comprehensive overview of the literature. J Neuromuscul Dis. 2015;2:73–85.
6. Teoli D, Aeddula NR. Telemedicine. NCBI Books https://www.ncbi.nlm.nih.gov/books/NBK535343/ Accessed 15 July 2022
7. Chirra M, Marsili L, Wattley L, Sokol LL, Keeling E, Maule S, Sobrero G, Artusi CA, Romagnolo A, Zibetti M, Lopiano L, Espay AJ, Obeidat AZ, Merola A. Telemedicine in nurological disorders: opportunities and challenges. Telemed J E Health. 2019;25:541–50. https://doi.org/10.1089/tmj.2018.0101.
8. Smith M, Alexander E, Marcinkute R, Dan D, Rawson M, Banka S, Gavin J, Mina H, Hennessy C, Riccardi F, Radio FC, Havlovicova M, Cassina M, Emandi AC, Fradin M, Gompertz L, Nordgren A, Traberg R, Rossi M, Trimouille A, Sowmyalakshmi R, Dallapiccola B, Renieri A, Faivre L, Kerr B, Verloes A, Clayton-Smith J, Douzgou S. ERN ITHACA. Telemedicine strategy of the European Reference Network ITHACA for the diagnosis and management of patients with rare developmental disorders. Orphanet J Rare Dis. 2020;15:103. https://doi.org/10.1186/s13023-020-1349-1.
9. Smith CIE, Bergman P, Hagey DW. Estimating the number of diseases—the concept of rare, ultra-rare, and hyper-rare. iScience. 2022;25:104698. https://doi.org/10.1016/j.isci.2022.104698.
10. ERN CPMS https://cpms.ern-net.eu/login. Accessed 15 July 2022
11. ERN CPMS https://ern-euro-nmd.eu/accessing-and-using-the-cpms/. Accessed 15 July 2022
12. Anderson CL, Munawar S, Reilly L, Kamp TJ, January CT, Delisle BP, Eckhardt LL. How functional genomics can keep pace with VUS identification. Front Cardiovasc Med. 2022;9:900431. https://doi.org/10.3389/fcvm.2022.900431.
13. Monaghesh E, Hajizadeh A. The role of telehealth during COVID-19 outbreak: a systematic review based on current evidence. BMC Public Health. 2020;20:1193. https://doi.org/10.1186/s12889-020-09301-4.
14. Lepida platform https://www.lepida.net/. Accessed 15 July 2022
15. Mönig I, Steenvoorden D, de Graaf JP, Ahmed SF, Taruscio D, Beun JG, Johannsen TH, Juul A, Hiort O, Pereira AM. CPMS-improving patient care in Europe via virtual case discussions. Endocrine. 2021;71:549–54. https://doi.org/10.1007/s12020-021-02628-x.

Publisher's Note

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 17 May 2023
DOI 10.3389/fneur.2023.1169689

OPEN ACCESS

EDITED BY
Marka van Blitterswijk,
Mayo Clinic Florida, United States

REVIEWED BY
Melissa Nel,
University of Cape Town, South Africa
Philippe Corcia,
Université de Tours, France

*CORRESPONDENCE
Stefania Corti
✉ stefania.corti@unimi.it

†These authors have contributed equally to this work

RECEIVED 19 February 2023
ACCEPTED 19 April 2023
PUBLISHED 17 May 2023

CITATION
Gagliardi D, Ripellino P, Meneri M, Del Bo R, Antognozzi S, Comi GP, Gobbi C, Ratti A, Ticozzi N, Silani V, Ronchi D and Corti S (2023) Clinical and molecular features of patients with amyotrophic lateral sclerosis and *SOD1* mutations: a monocentric study.
Front. Neurol. 14:1169689.
doi: 10.3389/fneur.2023.1169689

Clinical and molecular features of patients with amyotrophic lateral sclerosis and *SOD1* mutations: a monocentric study

Delia Gagliardi[1,2†], Paolo Ripellino[3†], Megi Meneri[1,2], Roberto Del Bo[1], Sara Antognozzi[1], Giacomo Pietro Comi[1,4], Claudio Gobbi[3,5], Antonia Ratti[6,7], Nicola Ticozzi[1,6], Vincenzo Silani[1,6], Dario Ronchi[1] and Stefania Corti[1,2*]

[1]Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), Dino Ferrari Centre, University of Milan, Milan, Italy, [2]Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [3]Department of Neurology, Neurocenter of Southern Switzerland EOC, Lugano, Switzerland, [4]Neuromuscular and Rare Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [5]Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano, Switzerland, [6]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [7]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy

Introduction: *SOD1* was the first gene associated with both familial and sporadic forms of amyotrophic lateral sclerosis (ALS) and is the second most mutated gene in Caucasian ALS patients. Given their high clinical and molecular heterogeneity, a detailed characterization of *SOD1*-ALS patients could improve knowledge about the natural history of this disease. Here, the authors aimed to provide a clinical and molecular description of a monocentric cohort of *SOD1*-ALS patients.

Methods: Amyotrophic lateral sclerosis (ALS) patients referring to the neurology unit of our center between 2008 and 2021 were clinically assessed and underwent molecular testing for *SOD1*. Segregation studies in available family members and *in silico* analysis were performed to sustain the pathogenicity of the identified *SOD1* variants.

Results: Among the 576 patients in our cohort, we identified 19 individuals harboring a mutation in *SOD1* (3.3%), including 15 (78.9%) with a familial and four (21.1%) with a sporadic form. The spinal onset of the disease was observed in all patients, and survival was extremely variable, ranging from 8months to over 30years. Twelve different *SOD1* missense variants were identified in our cohort, including one novel mutation (p.Pro67Leu).

Discussion: In the present series, we provided the first description of an Italian monocentric cohort of *SOD1*-ALS patients, and we expanded the repertoire of *SOD1* mutations. Our cohort presents several remarkable features, including variable expressivity in the same family, atypical presentation (ataxia, cognitive impairment, and other extra-motor symptoms), and different modes of inheritance of a given mutation in the same family. Given the recent authorization of *SOD1*-directed antisense oligonucleotide for use in *SOD1*-ALS patients, we recommend prompt screening for *SOD1* mutations in novel ALS patients with familiar or sporadic presentations.

KEYWORDS

amyotrophic lateral sclerosis, superoxide dismutase, *SOD1*-ALS, cohort, *SOD1* variants

Journal of Neurology
https://doi.org/10.1007/s00415-023-12015-3

ORIGINAL COMMUNICATION

The value of routine blood work-up in clinical stratification and prognosis of patients with amyotrophic lateral sclerosis

Francesco Gentile[1] · Alessio Maranzano[2] · Federico Verde[2,3] · Veronica Bettoni[4] · Eleonora Colombo[2] · Alberto Doretti[2] · Marco Olivero[1] · Francesco Scheveger[1] · Claudia Colombrita[5] · Ilaria Bulgarelli[5] · Edoardo Gioele Spinelli[6,7,8] · Erminio Torresani[5] · Stefano Messina[2] · Luca Maderna[2] · Federica Agosta[6,7,8] · Claudia Morelli[2] · Massimo Filippi[6,7,8,9,10] · Vincenzo Silani[2,3] · Nicola Ticozzi[2,3]

Received: 19 July 2023 / Revised: 18 September 2023 / Accepted: 19 September 2023

Abstract

Background There is an unmet need in amyotrophic lateral sclerosis (ALS) to provide specific biomarkers for the disease. Due to their easy availability, we aimed to investigate whether routine blood parameters provide useful clues for phenotypic classification and disease prognosis.

Methods We analyzed a large inpatient cohort of 836 ALS patients who underwent deep phenotyping with evaluation of the clinical and neurophysiological burden of upper (UMN) and lower (LMN) motor neuron signs. Disability and progression rate were measured through the revised ALS Functional Rating Scale (ALSFRS-R) and its changes during time. Cox regression analysis was performed to assess survival associations.

Results Creatinine significantly correlated with LMN damage ($r=0.38$), active ($r=0.18$) and chronic ($r=0.24$) denervation and baseline ALSFRS-R ($r=0.33$). Creatine kinase (CK), alanine (ALT) and aspartate (AST) transaminases correlated with active ($r=0.35$, $r=0.27$, $r=0.24$) and chronic ($r=0.37$, $r=0.20$, $r=0.19$) denervation, while albumin and C-reactive protein significantly correlated with LMN score ($r=0.20$ and $r=0.17$). Disease progression rate showed correlations with chloride ($r=-0.19$) and potassium levels ($r=-0.16$). After adjustment for known prognostic factors, total protein [HR 0.70 (95% CI 0.57–0.86)], creatinine [HR 0.86 (95% CI 0.81–0.92)], chloride [HR 0.95 (95% CI 0.92–0.99)], lactate dehydrogenase [HR 0.99 (95% CI 0.99–0.99)], and AST [HR 1.02 (95% CI 1.01–1.02)] were independently associated with survival.

Conclusions Creatinine is a reliable biomarker for ALS, associated with clinical features, disability and survival. Markers of nutrition/inflammation may offer additional prognostic information and partially correlate with clinical features. AST and chloride could further assist in predicting progression rate and survival.

Keywords Amyotrophic lateral sclerosis · Blood · Biomarkers · Survival

✉ Nicola Ticozzi
n.ticozzi@auxologico.it

[1] Neurology Residency Program, Università degli Studi di Milano, Milan, Italy

[2] Department of Neurology, IRCCS Istituto Auxologico Italiano, P. Le Brescia 20, 20149 Milan, Italy

[3] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

[4] Department of Brain and Behavioral Sciences, IRCCS Mondino Foundation, Università degli Studi di Pavia, Pavia, Italy

[5] Department of Laboratory Medicine, Laboratory of Clinical Chemistry and Microbiology, IRCCS Istituto Auxologico Italiano, Milan, Italy

[6] Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

[7] Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

[8] Vita-Salute San Raffaele University, Milan, Italy

[9] Neurorehabilitation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

[10] Neurophysiology Service, IRCCS San Raffaele Scientific Institute, Milan, Italy

Published online: 06 October 2023

biomedicines

MDPI

Review

Modeling Electric Fields in Transcutaneous Spinal Direct Current Stimulation: A Clinical Perspective

Matteo Guidetti [1,2], Stefano Giannoni-Luza [3], Tommaso Bocci [1,4], Kevin Pacheco-Barrios [5,6], Anna Maria Bianchi [2], Marta Parazzini [7], Silvio Ionta [3], Roberta Ferrucci [4,8], Natale Vincenzo Maiorana [1], Federico Verde [9,10], Nicola Ticozzi [9,10], Vincenzo Silani [9,10] and Alberto Priori [1,4,*]

1 Aldo Ravelli Research Center for Neurotechnology and Experimental Neurotherapeutics, Department of Health Sciences, University of Milan, 20142 Milan, Italy; matteo.guidetti@unimi.it (M.G.); tommaso.bocci@unimi.it (T.B.); natale.maiorana@unimi.it (N.V.M.)
2 Department of Electronics, Information and Bioengineering, Politecnico di Milano, 20133 Milan, Italy; annamaria.bianchi@polimi.it
3 Sensory-Motor Lab (SeMoLa), Department of Ophthalmology—University of Lausanne, Jules Gonin Eye Hospital/Fondation Asile des Aveugles, 1015 Lausanne, Switzerland; sgiannonil@gmail.com (S.G.-L.); ionta.silvio@gmail.com (S.I.)
4 III Neurology Clinic, ASST-Santi Paolo e Carlo University Hospital, 20142 Milan, Italy; roberta.ferrucci@unimi.it
5 Neuromodulation Center and Center for Clinical Research Learning, Spaulding Rehabilitation Hospital and Massachusetts General Hospital, Boston, MA 02129, USA; kevin.pacheco.barrios@gmail.com
6 Unidad de Investigación para la Generación y Síntesis de Evidencias en Salud, Universidad San Ignacio de Loyola, Vicerrectorado de Investigación, Lima 15024, Peru
7 Istituto di Elettronica e di Ingegneria Dell'Informazione e delle Telecomunicazioni (IEIIT), Consiglio Nazionale delle Ricerche (CNR), 10129 Milan, Italy; marta.parazzini@ieiit.cnr.it
8 Department of Oncology and Hematology, University of Milan, 20122 Milan, Italy
9 Department of Neurology, Istituto Auxologico Italiano IRCCS, 20149 Milan, Italy; federico.verde@unimi.it (F.V.); nicola.ticozzi@unimi.it (N.T.); vincenzo.silani@unimi.it (V.S.)
10 Department of Pathophysiology and Transplantation, 'Dino Ferrari' Center, Università degli Studi di Milano, 20122 Milan, Italy
* Correspondence: alberto.priori@unimi.it

Abstract: Clinical findings suggest that transcutaneous spinal direct current stimulation (tsDCS) can modulate ascending sensitive, descending corticospinal, and segmental pathways in the spinal cord (SC). However, several aspects of the stimulation have not been completely understood, and realistic computational models based on MRI are the gold standard to predict the interaction between tsDCS-induced electric fields and anatomy. Here, we review the electric fields distribution in the SC during tsDCS as predicted by MRI-based realistic models, compare such knowledge with clinical findings, and define the role of computational knowledge in optimizing tsDCS protocols. tsDCS-induced electric fields are predicted to be safe and induce both transient and neuroplastic changes. This could support the possibility to explore new clinical applications, such as spinal cord injury. For the most applied protocol (2–3 mA for 20–30 min, active electrode over T10–T12 and the reference on the right shoulder), similar electric field intensities are generated in both ventral and dorsal horns of the SC at the same height. This was confirmed by human studies, in which both motor and sensitive effects were found. Lastly, electric fields are strongly dependent on anatomy and electrodes' placement. Regardless of the montage, inter-individual hotspots of higher values of electric fields were predicted, which could change when the subjects move from a position to another (e.g., from the supine to the lateral position). These characteristics underlines the need for individualized and patient-tailored MRI-based computational models to optimize the stimulation protocol. A detailed modeling approach of the electric field distribution might contribute to optimizing stimulation protocols, tailoring electrodes' configuration, intensities, and duration to the clinical outcome.

Keywords: non-invasive brain stimulation; neuromodulation; transcutaneous spinal direct current stimulation; electric fields; computational models; clinical study

Citation: Guidetti, M.; Giannoni-Luza, S.; Bocci, T.; Pacheco-Barrios, K.; Bianchi, A.M.; Parazzini, M.; Ionta, S.; Ferrucci, R.; Maiorana, N.V.; Verde, F.; et al. Modeling Electric Fields in Transcutaneous Spinal Direct Current Stimulation: A Clinical Perspective. *Biomedicines* **2023**, *11*, 1283. https://doi.org/10.3390/biomedicines11051283

Academic Editors: Felipe Fregni, Kevin Pacheco-Barrios and Aurore Thibaut

Received: 28 February 2023
Revised: 12 April 2023
Accepted: 21 April 2023
Published: 26 April 2023

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iafd20

Optineurin in patients with Amyotrophic Lateral Sclerosis associated to atypical Parkinsonism in Tunisian population

I. Kacem, I. Sghaier, S. Peverelli, Y. Abida, H. Ben Brahim, A. Ratti, A. Nasri, N. Ticozzi, V. Silani & R. Gouider

To cite this article: I. Kacem, I. Sghaier, S. Peverelli, Y. Abida, H. Ben Brahim, A. Ratti, A. Nasri, N. Ticozzi, V. Silani & R. Gouider (30 Oct 2023): Optineurin in patients with Amyotrophic Lateral Sclerosis associated to atypical Parkinsonism in Tunisian population, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, DOI: 10.1080/21678421.2023.2273961

To link to this article: https://doi.org/10.1080/21678421.2023.2273961

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2023; 0: 1–7

RESEARCH ARTICLE

Optineurin in patients with Amyotrophic Lateral Sclerosis associated to atypical Parkinsonism in Tunisian population

I. KACEM[1,2,3], I. SGHAIER[1,2,3], S. PEVERELLI[4], Y. ABIDA[1,2,3], H. BEN BRAHIM[1], A. RATTI[4,5], A. NASRI[1,2,3], N. TICOZZI[4,6], V. SILANI[4,6] & R. GOUIDER[1,2,3]

[1]Neurology Department, LR18SP03, Razi University Hospital, Tunis, Tunisia, [2]Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia, [3]Clinical Investigation Center (CIC) "Neurosciences and Mental Health", Razi Universitary Hospital, Tunis, Tunisia, [4]Department of Neurology and Laboratory of Neuroscience, Istituto Auxologico Italiano, IRCCS, Milan, Italy, [5]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy, and [6]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

Abstract

Amyotrophic Lateral Sclerosis (ALS) is a heterogeneous disorder and the phenotypic variability goes far beyond the used clinical stratification parameter. Evidence has emerged that ALS may coexist with distinct neurodegenerative diseases in single cases. We aim to study the clinical features of two familial cases of ALS carriers of two distinct variants harbored in the Optineurin (*OPTN*) gene. We included definite familial ALS followed up in the Department of Neurology of Razi University Hospital, Tunisia, and selected according to Byrne criteria. Preliminary screening for the four main ALS genes (*SOD1*, *C9ORF72*, *TARDBP*, *FUS*) was conducted. Given the negative results, we proceeded to NGS target-re-sequencing with a custom panel including genes associated with ALS-FTD, Alzheimer's, and Parkinson's diseases. Both families are carriers of two different *OPTN* variants and they present very different ALS clinical features. The first family comprises two siblings diagnosed with ALS and Corticobasal syndrome (ALS-CBS) at an early age of onset and carriers of *OPTN* p.E135X in the homozygous state. The proband for the second family was diagnosed with ALS at an early age of onset presenting as progressive muscular atrophy with rapid progression. Genetic analysis revealed the presence of the homozygous variant p.R520H.Our findings highlight the peculiarity of genetic Tunisian drift. Indeed, genes with a recessive mode of inheritance may explain part of ALS diversity in clinical features. Therefore, the screening of the *OPTN* gene is highly recommended among inbreeding populations such as the Tunisian one.

Keywords: *Amyotrophic Lateral Sclerosis, optineurin, atypical Parkinsonism*

Introduction

Amyotrophic lateral sclerosis (ALS) is a multi-systemic neurodegenerative disease characterized by a progressive degeneration of motor neurons in both brain and spinal cord (1). The constantly evolving effort in understanding ALS nature and etiology has gradually led to the present vision of this fatal disease as a multifactorial one, including genetic and environmental risk factors and affecting several cell pathways (2).

The functional convergence of all these diverse entities in determining ALS clinical features (age of onset, progression, survival, etc.) is still poorly understood (3). However, the common pathological hallmark of ALS is the presence of ubiquitinated and phosphorylated TDP-43 protein that aggregates into soluble inclusions in affected brain tissues (4).

Recent evidences highlight the oligogenic and polygenic basis of ALS that might explain its clinical features diversity (5,6). Along with these reports, several causative ALS genes emerged (7) which were associated, with the exception of *SOD1* and *FUS*, with the presence of TDP-43-positive neuronal cytoplasmic inclusions, pointing to the idea that these single genes could be an upstream cause for TDP-43 pathology in ALS (8).

Correspondence: R. Gouider Head of Neurology Department, LR18SP03, Razi University Hospital, La Manouba, 2010, Tunis, Tunisia. E-mail: riadh.gouider@gnet.tn

Supplemental data for this article can be accessed online at https://doi.org/10.1080/21678421.2023.2273961.

(Received 2 June 2023; revised 26 September 2023; accepted 10 October 2023)

ISSN 2167-8421 print/ISSN 2167-9223 online
DOI: 10.1080/21678421.2023.2273961

Stem Cell Research 66 (2023) 102998

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Multiple Cell Lines

Generation of five induced pluripotent stem cells lines from four members of the same family carrying a *C9orf72* repeat expansion and one wild-type member

Chiara Lattuada [a], Serena Santangelo [a,b], Silvia Peverelli [a], Philip McGoldrick [c], Ekaterina Rogaeva [c], Lorne Zinman [d], Georg Haase [e], Vincent Géli [f], Vincenzo Silani [a,g], Janice Robertson [c], Antonia Ratti [a,b,1], Patrizia Bossolasco [a,1,*]

[a] *Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy*
[b] *Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy*
[c] *Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Canada*
[d] *Sunnybrook Health Sciences Centre, Toronto, Canada*
[e] *MPATHY Laboratory, Institute of Systems Neuroscience, U1106 INSERM & Aix-Marseille University, Marseille, France*
[f] *Marseille Cancer Research Centre (CRCM), Inserm U1068, CNRS UMR7258, Institut Paoli-Calmettes, Aix-Marseille University, Marseille, France*
[g] *"Dino Ferrari" Center, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy*

A B S T R A C T

The most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) is the expansion of a G4C2 hexanucleotide repeat in the *C9orf72* gene. The size of the repeat expansion is highly variable and a cut-off of 30 repeats has been suggested as the lower pathological limit. Repeat size variability has been observed intergenerationally and intraindividually in tissues from different organs and within the same tissue, suggesting instability of the pathological repeat expansion. In order to study this genomic instability, we established iPSCs from five members of the same family of which four carried a *C9orf72* repeat expansion and one was wild-type.

Resource Table:

Unique stem cell lines identifier	IAIi005-A IAIi006-A IAIi007-A IAIi008-A IAIi009-A
Alternative name(s) of stem cell lines	AC52 (IAIi005-A)BC6 (IAIi006-A)CC5 (IAIi007-A)DC2 (IAIi008-A)EC1 (IAIi009-A)
Institution	IRCCS Istituto Auxologico Italiano, Milan, Italy
Contact information of distributor	Patrizia Bossolasco, p. bossolasco@auxologico.it
Type of cell lines	iPSC
Origin	Human
Additional origin info required	Ethnicity: Caucasian (IAIi005-A), Age:89, Sex: MaleEthnicity: Caucasian (IAIi006-A) , Age:65, Sex: FemaleEthnicity: Caucasian (IAIi007-A) , Age:57, Sex: FemaleEthnicity: Caucasian (IAIi008-A) , Age:51, Sex: FemaleEthnicity: Caucasian (IAIi009-A) , Age:65, Sex: Female
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus
Genetic Modification	No
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Amyotrophic lateral sclerosis (ALS)
Gene/locus	*C9orf72* gene/chromosome 9p21.2
Date archived/stock date	
Cell line repository/bank	https://hpscreg.eu/cell-line/IAIi005-A https://hpscreg.eu/cell-line/IAIi006-A https://hpscreg.eu/cell-line/IAIi007-A

(*continued on next column*)

(*continued*)

(*continued on next page*)

* Corresponding author.
E-mail address: p.bossolasco@auxologico.it (P. Bossolasco).
[1] Joint last authors.

https://doi.org/10.1016/j.scr.2022.102998
Received 26 September 2022; Received in revised form 21 November 2022; Accepted 4 December 2022
Available online 9 December 2022

Lombardo *et al. Trials* (2023) 24:792
https://doi.org/10.1186/s13063-023-07638-w

UPDATE

Open Access

A randomized double-blind clinical trial on safety and efficacy of tauroursodeoxycholic acid (TUDCA) as add-on treatment in patients affected by amyotrophic lateral sclerosis (ALS): the statistical analysis plan of TUDCA-ALS trial

Flavia L. Lombardo[1*†], Stefania Spila Alegiani[2†], Flavia Mayer[2], Marta Cipriani[3,4], Maria Lo Giudice[5], Albert Christian Ludolph[6,7], Christopher J. McDermott[8], Philippe Corcia[9,10,11,12], Philip Van Damme[13,14], Leonard H. Van den Berg[15], Orla Hardiman[16,17], Gabriele Nicolini[18], Nicola Vanacore[1], Brian Dickie[19], Alberto Albanese[5], Maria Puopolo[4] and TUDCA-ALS Study Group

Abstract

Background Amyotrophic lateral sclerosis (ALS) is a highly debilitating neurodegenerative condition. Despite recent advancements in understanding the molecular mechanisms underlying ALS, there have been no significant improvements in therapeutic options for ALS patients in recent years. Currently, there is no cure for ALS, and the only approved treatment in Europe is riluzole, which has been shown to slow the disease progression and prolong survival by approximately 3 months. Recently, tauroursodeoxycholic acid (TUDCA) has emerged as a promising and effective treatment for neurodegenerative diseases due to its neuroprotective activities.

Methods The ongoing TUDCA-ALS study is a double-blinded, parallel arms, placebo-controlled, randomized multicenter phase III trial with the aim to assess the efficacy and safety of TUDCA as add-on therapy to riluzole in patients with ALS. The primary outcome measure is the treatment response defined as a minimum of 20% improvement in the ALS Functional Rating Scale-Revised (ALSFRS-R) slope during the randomized treatment period (18 months) compared to the lead-in period (3 months). Randomization will be stratified by country. Primary analysis will be conducted based on the intention-to-treat principle through an unadjusted logistic regression model. Patient recruitment commenced on February 22, 2019, and was closed on December 23, 2021. The database will be locked in September 2023.

Discussion This paper provides a comprehensive description of the statistical analysis plan in order to ensure the reproducibility of the analysis and avoid selective reporting of outcomes and data-driven analysis. Sensitivity analyses have been included in the protocol to assess the impact of intercurrent events related to the coronavirus

†Flavia L. Lombardo and Stefania Spila Alegiani contributed equally as first authors.

*Correspondence:
Flavia L. Lombardo
flavia.lombardo@iss.it
Full list of author information is available at the end of the article

MI	Multiple imputation
MNAR	Missing not at random
MMP-9	Matrix metalloproteinase 9
MRC	Medical Research Council
SAP	Statistical analysis plan
TUDCA	Tauroursodeoxycholic acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-023-07638-w.

Additional file 1. Statistical Analysis Plan (SAP) Cheklist.

Acknowledgements
Members of TUDCA-ALS study group: Paolo Tornese, Antoniangela Cocco, IRCCS Humanitas Research Hospital, Rozzano (Italy); Michela Matteoli, Eliana Lauranzano, Maria Luisa Malosio, Chiara Adriana Elia, IRCCS Humanitas Research Hospital, Rozzano (Italy) and Institute of Neuroscience, National Research Council, Rozzano (Italy); Adriano Chiò, Umberto Manera, Cristina Moglia, Andrea Calvo, Paolina Salamone, Giuseppe Fuda "Rita Levi Montalcini", University of Turin (Italy); Carlo Colosimo, Cristina Spera, Prabha Cristina Ranchicchio, Azienda Ospedaliera Santa Maria, Terni (Italy). Giuseppe Stipa, Domenico Frondizi, Azienda Ospedaliera Santa Maria, Terni (Italy); Christian Lunetta, Valeria Sansone, Claudia Tarlarini, Francesca Gerardi, Centro Clinico NEMO Fondazione Serena ONLUS, Milan (Italy); Vincenzo Silani, Alberto Doretti, Eleonora Colombo, Gianluca Demirtzidis, IRCCS Istituto Auxologico Italiano, Milan (Italy) and "Dino Ferrari" Center, Universitá degli Studi di Milano, Milan (Italy); Gioacchino Tedeschi, Francesca Trojsi, Carla Passaniti, Università degli Studi della Campania "Luigi Vanvitelli," Naples (Italy); Stefania Ballestrero, Bruschettini S.r.l, Genova (Italy); Johannes Dorst,Ulrike Weiland, Andrea Fromm, Maximilian Wiesenfarth, Katharina Kandler, Simon Witzel, Markus Otto, Joachim Schuster, University of Ulm, Ulm (Germany); Thomas Meyer, André Maier, Dagmar Kettemann, Charité, Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität Zu Berlin and Berlin Institute of Health, Outpatient Center for ALS and Other Motor Neuron Diseases, Berlin (Germany); Susanne Petri, Lars Müschen, Camilla Wohnrade, Anastasia Sarikidi, Alma Osmanovic, Hannover Medical School, Hannover (Germany); Julian Grosskreutz, Annekathrin Rödiger, Robert Steinbach, Benjamin Ilse, Uta Smesny, University Hospital Jena, Jena (Germany); Robert Untucht, René Günther, Maximilian Vidovic, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden (Germany); Pamela Shaw, Alexis Collins, Helen Wollff, Theresa Walsh, Lee Tuddenham, Mbombe Kazoka, David White, Stacy Young, Benjamin Thompson, Daniel Madarshahian, University of Sheffield, Sheffield (UK); Suresh K. Chhetri, Lancashire Teaching Hospitals NHS Foundation Trust, Preston (UK); Amina Chaouch, Manchester Centre for Clinical Neuroscience, Salford Royal NHS Foundation Trust, Northern Care Alliance Salford (UK); Carolyn A. Young, Heike Arndt, The Walton Centre NHS Foundation Trust, Liverpool (UK); Coliver Hanemann, University of Plymouth, Plymouth (UK); Thomas Lambert, Royal Stoke University Hospital, Stoke on Trent (UK); Stephane Beltran, CHU Bretonneau, Tours (France); Philippe Couratier, Hôpital Dupuytren, CRMR SLA, Limoges (France); Florence Esselin, William Camu, Elisa De La Cruz, Université Montpellier, Montpellier (France); Gwendal Lemasson, Groupe Hospitalier Pelegrin, Bordeaux (France); Pegah Masrori, University Hospitals Leuven, KU Leuven, Leuven (Belgium); Sinead Maguire, Liz Fogarty, Toyosi Atoyebi, Niamh Ní Obáin, Trinity Biomedical Sciences Institute, Dublin (Ireland) and Beaumont Hospital, Dublin (Ireland).

Authors' contributions
FL, SSA, FM, and MP conceived, planned, and finalized the statistical analysis plan, with review by AA, ACL, CJM, PC, PVD, LHVdB, OH, GN, NV, and BD. AA is the chief investigator of the TUDCA-ALS study. MP is the senior statistician responsible. FL, SSA, FM, MC, and MP drafted the manuscript. AA, ACL, CJM, PC, PVD, LHVdB, OH, NV, and BD contributed to developing the research questions and study designs. FL and SSA contributed equally as co-first authors of the manuscript. FL, SSA, FM, MC, and MP read, amended, and approved the statistical analysis plan. All authors have read and approved the final manuscript.

Funding
This study was funded by the European Union's Horizon 2020 program SC1-PM-08–2017 "New therapies for rare diseases" (grant agreement 755094).

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
The TUDCA-ALS trial has been approved by all the involved national and local Ethical Committees (the full list is available in the published protocol). Informed consent was obtained from all study participants.

Consent for publication
Not applicable.

Competing interests
Author AL declares participation in Advisory Boards of Roche Pharma AG, Biogen, Alector, and Amylyx. Author CJM reports consultancy with Biogen, Amylyx, and Cytokinetics. Author PV declares participation in Advisory Board meetings for Biogen, UCB, argenx, Cytokinetics, Ferrer, Muna Therapeutics, Augustine Therapeutics, Alector, Alexion, QurAlis, VectorY, and Amylyx (paid to institution) and grant from CSL Behring (E. von Behring Chair for Neuromuscular and Neurodegenerative Disorders; paid to institution). Author OH declares participation in Advisory Boards for Accelsiors, Biogen Idec, Cytokinetics, NeuroSense Therapeutics, Neuropath Therapeutics, Novartis, Orion, Denali, and Wave Pharmaceuticals; role as principal investigator on the PRECISION ALS Project; Academic/Industry Collaboration funded by Science Foundation Ireland; Research collaboration with Biogen, Cytokinetics, and Ionis in delivering the IMPACT ALS survey and with Cytokinetics in delivering the REVEALS study of respiratory decline in ALS; editor-in-chief of ALS and the Frontotemporal Degeneration; editorial board member of the Journal of Neurology, Neurosurgery, and Psychiatry. Author GN declares that Bruschettini S.R.L is the pharmaceutical company providing the investigational medicinal product and industrial partner of the project, in which he is an employee as medical director. The remaining authors declare no competing interests.

Author details
[1]National Centre for Disease Prevention and Health Promotion, Italian National Institute of Health, Rome, Italy. [2]National Center for Drug Research and Evaluation, Italian National Institute of Health, Rome, Italy. [3]Department of Statistical Sciences, Sapienza University of Rome, Rome, Italy. [4]Department of Neuroscience, Italian National Institute of Health, Rome, Italy. [5]Neurology Department, IRCCS Humanitas Research Hospital, Rozzano, Italy. [6]Neurology Department, University of Ulm, Ulm, Germany. [7]German Centre of Neurodegenerative Diseases, Site Ulm, Ulm, Germany. [8]Department of Neuroscience, Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK. [9]Centre de Référence Maladie Rare (CRMR) SLA Et Les Autres Maladies du Neurone Moteur (FILSLAN), Tours, France. [10]CHU Bretonneau, Tours, France. [11]Federation des CRMR-SLA Tours-Limoges, LITORALS, Tours, France. [12]Faculté de Médecine, INSERM U1253, "iBrain," Université François-Rabelais de Tours, Tours, France. [13]Neurology Department, University Hospitals Leuven, Louvain, Belgium. [14]Neuroscience Department, KU Leuven, Louvain, Belgium. [15]Department of Neurology, UMC Utrecht Brain Center, University Medical Centre Utrecht, Utrecht, Netherlands. [16]Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Dublin, Ireland. [17]Clinical Research Centre, Beaumont Hospital, Dublin, Ireland. [18]Medical Affairs, Bruschettini S.R.L, Genoa, Italy. [19]Motor Neurone Disease Association, Northampton, UK.

Received: 23 July 2023 Accepted: 22 August 2023
Published online: 05 December 2023

References

1. Chio A, Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA, et al. Global epidemiology of amyotrophic lateral sclerosis: a systematic review

scientific reports

Check for updates

OPEN

The preferences of people with amyotrophic lateral sclerosis on riluzole treatment in Europe

Albert C. Ludolph[1], Harish Grandjean[2], Evy Reviers[3], Valentina De Micheli[4], Cosetta Bianchi[4], Leonardo Cardosi[5], Hermann Russ[6]✉ & Vincenzo Silani[7,8]

The Patient Preference Survey aims to understand unmet needs related to riluzole management in people with Amyotrophic Lateral Sclerosis (ALS) and to identify which characteristics of a new formulation could better match their preferences. The survey involved 117 people with ALS (PALS) treated with riluzole in four European countries. The dysphagic PALS were least satisfied with the riluzole tablet and oral suspension and with ease in self-administration; up to 68% of respondents postponed or missed the treatment due to swallowing difficulties and need of caregiver assistance. Overall, 51% of tablet and 53% of oral suspension users regularly crushed or mixed riluzole with beverages, respectively; PALS who always manipulated riluzole showed low satisfaction with the formulation and considered the risk of choking and pneumonia the most worrisome event. The survey evaluated the driving factors in choosing/switching the therapy: 67% of PALS declared a low risk of choking. The research finally evaluated which attributes of a new formulation would be preferred: the most relevant were ease of use (4.3/5), convenient/portable packaging (4.0/5) and oral-dissolving properties without tongue motility (3.9/5). The Patient Preference Survey suggests that patients have several unmet needs and preferences that could be addressed by a different formulation, e.g. using oral film technologies.

Amyotrophic lateral sclerosis (ALS) is a rare and progressive, neurodegenerative disease. It is characterized by a loss of upper and/or lower motor neurons with heterogeneous features and more than 30 genes identified as causative (the pathology is quite homogeneous indeed being TDP-43 + in 98% of cases both familial or sporadic)[1–4]. In Europe, the annual incidence and prevalence range from 2.1 to 3.8 and 4.1 to 8.4, per 100,000 persons, respectively[5,6]. The mean age at onset of symptoms is 51 to 66 years[3–5]. The course of ALS ends with respiratory failure and death, with a median survival rate of 2–4 years after onset[4].

After the first clinical symptoms (e.g. muscular weakness, twitches or cramps), degeneration of the thoracic and respiratory muscles' motor neurons leads to problems in daily activities. People with ALS (PALS) become increasingly dependent on caregiver support, including the administration of treatment[7–9]. This significantly affects their quality of life (QoL) and creates a substantial socioeconomic burden[10,11].

A cure for ALS is not available yet, and riluzole is the only approved Disease Modifying Treatment in Europe[12–14]. To date, riluzole is available in two formulations: tablets (50 mg) and oral suspension (5 mg/mL in 300 mL bottles). An independent meta-analysis of observational studies showed that riluzole leads to a broad spectrum of outcomes; in certain survival studies, the median survival increased to up to 19 months compared to placebo[15]. Thanks to long-term evidence, it is recommended that riluzole be administered as early as possible after diagnosis[16] and maintained long-term to slow the progression of the disease in PALS[15,17].

Adherence to riluzole treatment, whether with a tablet or liquid formulation, seems to be directly related to the progression of the disease and the onset of dysphagia. Therefore, the need for a riluzole formulation that may allow for better continuity of administration and thereby the best possible therapeutic effect on ALS disease progression, is an unresolved issue[18].

[1]Department of Neurology, German Center for Neurodegenerative Diseases (DZNE), University of Ulm, Ulm, Germany. [2]Charles River Associates International, Zürich, Switzerland. [3]European Organization for Professionals and Patients with ALS (EUpALS), Leuven, Belgium. [4]Business Integration Partners S.p.A, Milan, Italy. [5]Zambon Biotech SPA, Bresso, Milan, Italy. [6]Sirius Scientific Consulting AG, 8852 Altendorf, Switzerland. [7]Department of Neuroscience and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy. [8]Department of Pathophysiology and Transplantation, Dino Ferrari Center, Università degli Studi di Milano, Milan, Italy. ✉email: hermann.russ@hotmail.de

Neurological Sciences
https://doi.org/10.1007/s10072-023-06619-w

ORIGINAL ARTICLE

Psychometrics and diagnostics of the Italian version of the Beck Depression Inventory-II (BDI-II) in Parkinson's disease

Gianpaolo Maggi[1] · Alfonsina D'Iorio[1] · Edoardo Nicolò Aiello[2,3] · Barbara Poletti[2] · Nicola Ticozzi[2,4] · Vincenzo Silani[2,4] · Marianna Amboni[5,6] · Carmine Vitale[5,7] · Gabriella Santangelo[1]

Received: 3 August 2022 / Accepted: 9 January 2023

Abstract

Introduction Depression is one of the most disabling neuropsychiatric manifestations of Parkinson's disease (PD) and requires proper screening and diagnosis because it affects the overall prognosis and quality of life of patients. This study aimed to assess the psychometric and diagnostic properties of the Beck Depression Inventory-II (BDI-II) in an Italian PD cohort.

Materials and methods Fifty consecutive outpatients with PD underwent the Italian version of the BDI-II and other questionnaires to evaluate anxiety and apathetic symptoms. Patients' caregivers completed the depression/dysphoria domain of the Neuropsychiatric Inventory (NPI-D). We evaluated the internal consistency, convergent and divergent validity, and factorial structure of BDI-II. Sensitivity, specificity, positive and negative predictive values, and likelihood ratios were computed using ROC analyses, and an optimal cutoff was defined using the Youden index.

Results The BDI-II proved to be internally consistent (Cronbach's $\alpha = 0.840$) and substantially met the bi-factorial structure. Regarding construct validity, the BDI-II was substantially related to anxiety measures, but not to apathy. With the combination of the NPI-D and anxiety score used as the gold standard, the BDI-II overall showed good accuracy (AUC = 0.859) with adequate sensitivity (75%) and specificity (87%). The optimal cutoff point was defined at 14.50.

Conclusions We provide evidence of the psychometric and diagnostic properties of the Italian version of the BDI-II as a screening tool for depression in patients with PD. The BDI-II was found to be reliable and valid for the measurement of depression in patients with PD; therefore, it is available for use in clinical research and practice.

Keywords Beck Depression Inventory · Parkinson's disease · Depression · Anxiety · Diagnostics · Psychometrics

✉ Gabriella Santangelo
gabriella.santangelo@unicampania.it

Gianpaolo Maggi
Gianpaolo.maggi@unicampania.it

Alfonsina D'Iorio
alfonsina.diorio@unicampania.it

Edoardo Nicolò Aiello
e.aiello@auxologico.it

Barbara Poletti
b.poletti@auxologico.it

Nicola Ticozzi
n.ticozzi@auxologico.it

Vincenzo Silani
vincenzo.silani@unimi.it

Marianna Amboni
marianna.amboni@gmail.com

Carmine Vitale
cavit69@hotmail.com

[1] Department of Psychology, University of Campania "Luigi Vanvitelli", Caserta, Italy

[2] Department of Neurology and Laboratory of Neuroscience, IRCCS, Istituto Auxologico Italiano, Milano, Italy

[3] PhD Program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy

[4] Department of Pathophysiology and Transplantation, "Dino Ferrari Center", Università degli Studi di Milano, Milano, Italy

[5] Institute of Diagnosis and Health, IDC-Hermitage Capodimonte, Naples, Italy

[6] Department of Medicine, Surgery and Dentistry, University of Salerno, Salerno, Italy

[7] Department of Motor Sciences and Wellness, University "Parthenope", Naples, Italy

Published online: 18 January 2023

healthcare

MDPI

Article

Relationship between Reaction Times and Post-COVID-19 Symptoms Assessed by a Web-Based Visual Detection Task

Natale Vincenzo Maiorana [1], Edoardo Nicolò Aiello [2], Barbara Poletti [2], Fabrizio Carusi [1], Angelica De Sandi [3], Matteo Guidetti [1,4], Roberto Prandin [5], Sara Marceglia [5], Nicola Ticozzi [2,6], Vincenzo Silani [2,6], Alberto Priori [1,3] and Roberta Ferrucci [1,3,*]

[1] Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics, Department of Health Sciences, International Medical School, University of Milan, 20142 Milan, Italy
[2] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, 20149 Milan, Italy
[3] ASST Santi Paolo e Carlo, San Paolo University Hospital, 20142 Milano, Italy
[4] Department of Electronics, Information and Bioengineering, Politecnico di Milano, 20133 Milan, Italy
[5] Department of Engineering and Architecture, University of Trieste, 34127 Trieste, Italy
[6] Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, 20122 Milan, Italy
* Correspondence: roberta.ferrucci@unimi.it

Abstract: Long-COVID is a clinical condition in which patients affected by SARS-CoV-2 usually report a wide range of physical and cognitive symptoms from 3 to 6 months after the infection recovery. The aim of the current study was to assess the link between self-reported long-COVID symptoms and reaction times (RTs) in a self-administered Visual Detection Task (VDT) in order to identify the predictor symptoms of the slowing in reaction times to determine attention impairment. In total, 362 participants (age (mean ± S.D.: 38.56 ± 13.14); sex (female–male: 73.76–26.24%)) responded to a web-based self-report questionnaire consisting of four sections: demographics, disease-related characteristics, and medical history questions. The final section consisted of a 23 item 5-point Likert-scale questionnaire related to long-term COVID-19 symptoms. After completing the questionnaire, subjects performed a VDT on a tablet screen to assess reaction times (RTs). An exploratory factorial analysis (EFA) was performed on the 23 long-COVID symptom questions, identifying 4 factors (cognition, behavior, physical condition, presence of anosmia and/or ageusia). The most important predictors of RTs were cognition and physical factors. By dissecting the cognitive and physical factors, learning, visual impairment, and headache were the top predictors of subjects' performance in the VDT. Long-COVID subjects showed higher RTs in the VDT after a considerable time post-disease, suggesting the presence of an attention deficit disorder. Attention impairment due to COVID-19 can be due to the presence of headaches, visual impairments, and the presence of cognitive problems related to the difficulty in learning new activities. The link between the slowing of reaction times and physical and cognitive symptoms post-COVID-19 suggests that attention deficit disorder is caused by a complex interaction between physical and cognitive symptoms. In addition, the study provides evidence that RTs in a VDT represent a reliable measure to detect the presence of long-COVID neurological sequelae.

Keywords: COVID-19; SARS-CoV-2; neuropsychology; attention; post-COVID syndrome

Citation: Maiorana, N.V.; Aiello, E.N.; Poletti, B.; Carusi, F.; De Sandi, A.; Guidetti, M.; Prandin, R.; Marceglia, S.; Ticozzi, N.; Silani, V.; et al. Relationship between Reaction Times and Post-COVID-19 Symptoms Assessed by a Web-Based Visual Detection Task. *Healthcare* **2023**, *11*, 284. https://doi.org/10.3390/healthcare11030284

Academic Editor: Christian Napoli

Received: 9 December 2022
Revised: 9 January 2023
Accepted: 11 January 2023
Published: 17 January 2023

1. Introduction

SARS-CoV-2 is a human coronavirus that causes the respiratory infectious disease known as COVID-19 and, due to its high contagiousness, caused a global pandemic in the first months of the 2020s that is still ongoing [1,2]. In its worst form, SARS-CoV-2 infection causes severe lung damage and breathing disorders that require hospitalization in intensive care units (ICUs), representing a burden on healthcare systems [3]. A large

Journal of Neurology (2023) 270:5274–5287
https://doi.org/10.1007/s00415-023-11898-6

REVIEW

Role of expectations in clinical outcomes after deep brain stimulation in patients with Parkinson's disease: a systematic review

Francesca Mameli[1] · Eleonora Zirone[1] · Roberta Girlando[1] · Elena Scagliotti[1] · Giulia Rigamonti[1] · Edoardo Nicolò Aiello[3] · Barbara Poletti[3,4] · Roberta Ferrucci[2,4] · Nicola Ticozzi[3,5] · Vincenzo Silani[3,5] · Marco Locatelli[1] · Sergio Barbieri[1] · Fabiana Ruggiero[1]

Received: 23 May 2023 / Revised: 21 July 2023 / Accepted: 22 July 2023 / Published online: 30 July 2023

Abstract

Deep brain stimulation (DBS) is a well-established treatment that significantly improves the motor symptoms of patients with Parkinson's disease (PD); however, patients may experience post-operative psychological distress and social maladjustments. This phenomenon has been shown to be related to patients' pre-operative cognitive representations, such as expectations. In this systematic review, we discuss the findings on the role of the expectations of patients with PD regarding the clinical outcomes of DBS to identify areas of intervention to improve pre-operative patient education and promote successful post-operative psychosocial adjustment. PubMed was searched for relevant articles published up to 16 January 2023. Of the 84 identified records, 10 articles focusing on the treatment expectations of patients with PD undergoing DBS were included in this review. The selected studies were conducted among cohorts of patients with different DBS targets, among which the most common was the bilateral subthalamic nucleus. Overall, the data showed that patients' expectations contribute to treatment efficacy. Experiments investigating the placebo effect itself have shown clinical improvement after the induction of positive therapeutic expectations; conversely, unrealistic treatment expectations can affect patient satisfaction after surgery, clinical outcomes, and subjective well-being. This review highlights the need for routine clinical practice to better investigate and manage patients' pre-operative expectations, as well as multidisciplinary education to improve patient satisfaction and psychosocial adjustment after DBS.

Keywords Deep brain stimulation · Parkinson disease · Subthalamic nucleus · Patients' expectations · Placebo

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by a variety of motor and non-motor impairments primarily caused by the selective loss of dopaminergic neurones in the nigrostriatal pathway [1]. Bilateral deep brain stimulation (DBS) reduces motor symptoms and dopaminergic-related complications in patients with advanced PD [2–5]. While successful functional neurosurgery leading to the sudden alleviation of symptoms is expected to significantly improve patients' quality of life (QoL), growing evidence suggests that such positive effects are questionable [6–11]. This phenomenon, first characterised by Bladin as the '*Burden of Normality*', has been mostly investigated in patients with medically intractable epilepsy undergoing anterior temporal lobectomy [12, 13]; despite successful treatment and alleviation of seizures, some patients experienced psychosocial maladjustments (e.g.,

Francesca Mameli and Eleonora Zirone: contributed equally.

✉ Francesca Mameli
francesca.mameli@policlinico.mi.it

1 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via F. Sforza, 35, 20122 Milan, Italy

2 ASST Santi Paolo e Carlo, San Paolo University Hospital, Milan, Italy

3 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy

4 Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy

5 Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

frontiers | Frontiers in Aging Neuroscience

TYPE Original Research
PUBLISHED 01 February 2023
DOI 10.3389/fnagi.2023.1067954

OPEN ACCESS

EDITED BY
Michael Swash,
Queen Mary University of London,
United Kingdom

REVIEWED BY
Emanuele Buratti,
International Centre for Genetic Engineering and Biotechnology, Italy
Antonio Canosa,
University of Turin,
Italy

*CORRESPONDENCE
Nicola Ticozzi
✉ n.ticozzi@auxologico.it

†These authors share co-senior authorship

SPECIALTY SECTION
This article was submitted to Parkinson's Disease and Aging-related Movement Disorders, a section of the journal Frontiers in Aging Neuroscience

RECEIVED 12 October 2022
ACCEPTED 03 January 2023
PUBLISHED 01 February 2023

CITATION
Manini A, Casiraghi V, Brusati A, Maranzano A, Gentile F, Colombo E, Bonetti R, Peverelli S, Invernizzi S, Gentilini D, Messina S, Verde F, Poletti B, Fogh I, Morelli C, Silani V, Ratti A and Ticozzi N (2023) Association of the risk factor *UNC13A* with survival and upper motor neuron involvement in amyotrophic lateral sclerosis. *Front. Aging Neurosci.* 15:1067954. doi: 10.3389/fnagi.2023.1067954

Association of the risk factor *UNC13A* with survival and upper motor neuron involvement in amyotrophic lateral sclerosis

Arianna Manini[1,2], Valeria Casiraghi[1,3], Alberto Brusati[1,4], Alessio Maranzano[1,2], Francesco Gentile[1,2], Eleonora Colombo[1], Ruggero Bonetti[1,2], Silvia Peverelli[1], Sabrina Invernizzi[1], Davide Gentilini[4,5], Stefano Messina[1], Federico Verde[1,6], Barbara Poletti[1], Isabella Fogh[7], Claudia Morelli[1], Vincenzo Silani[1,6], Antonia Ratti[1,3†] and Nicola Ticozzi[1,6*†]

[1]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy, [2]Neurology Residency Program, Università degli Studi di Milano, Milan, Italy, [3]Department of Medical Biotechnology and Molecular Medicine, Università degli Studi di Milano, Milan, Italy, [4]Department of Brain and Behavioral Sciences, Università degli Studi di Pavia, Pavia, Italy, [5]Bioinformatics and Statistical Genomics Unit, IRCCS Istituto Auxologico Italiano, Milan, Italy, [6]Department of Pathophysiology and Transplantation, 'Dino Ferrari' Center, Università degli Studi di Milano, Milan, Italy, [7]Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College, London, United Kingdom

Background: The *UNC13A* gene is an established susceptibility locus for amyotrophic lateral sclerosis (ALS) and a determinant of shorter survival after disease onset, with up to 33.0 months difference in life expectancy for carriers of the rs12608932 risk genotype. However, its overall effect on other clinical features and ALS phenotypic variability is controversial.

Methods: Genotype data of the *UNC13A* rs12608932 SNP (A–major allele; C–minor allele) was obtained from a cohort of 972 ALS patients. Demographic and clinical variables were collected, including cognitive and behavioral profiles, evaluated through the Edinburgh Cognitive and Behavioral ALS Screen (ECAS) – Italian version and the Frontal Behavioral Inventory (FBI); upper and lower motor neuron involvement, assessed by the Penn Upper Motor Neuron Score (PUMNS) and the Lower Motor Neuron Score (LMNS)/Medical Research Council (MRC) scores, respectively; the ALS Functional Rating Scale Revised (ALSFRS-R) score at evaluation and progression rate; age and site of onset; survival. The comparison between the three rs12608932 genotypes (AA, AC, and CC) was performed using the additive, dominant, and recessive genetic models.

Results: The rs12608932 minor allele frequency was 0.31 in our ALS cohort, in comparison to 0.33–0.41 reported in other Caucasian ALS populations. Carriers of at least one minor C allele (AC+CC genotypes) had a shorter median survival than patients with the wild-type AA genotype (–11.7 months, p = 0.013), even after adjusting for age and site of onset, *C9orf72* mutational status and gender. Patients harboring at least one major A allele (AA+AC genotypes) and particularly those with the wild-type AA genotype showed a significantly higher PUMNS compared to CC carriers (p = 0.015 and p_{adj} = 0.037, respectively), thus indicating a more severe upper motor neuron involvement. Our analysis did not detect significant associations with all the other clinical parameters considered.

BRAIN
ORIGINAL ARTICLE

Regional spreading pattern is associated with clinical phenotype in amyotrophic lateral sclerosis

Alessio Maranzano,[1] Federico Verde,[1,2] Eleonora Colombo,[1] Barbara Poletti,[1] Alberto Doretti,[1] Ruggero Bonetti,[3] Delia Gagliardi,[2,4] Megi Meneri,[2,4] Luca Maderna,[1] Stefano Messina,[1] Stefania Corti,[2,4] Claudia Morelli,[1] Vincenzo Silani[1,2] and Nicola Ticozzi[1,2]

Increasing evidence shows that disease spreading in amyotrophic lateral sclerosis (ALS) follows a preferential pattern with more frequent involvement of contiguous regions from the site of symptom onset. The aim of our study was to assess if: (i) the burden of upper (UMN) and lower motor neuron (LMN) involvement influences directionality of disease spreading; (ii) specific patterns of disease progression are associated with motor and neuropsychological features of different ALS subtypes (classic, bulbar, primary lateral sclerosis, UMN-predominant, progressive muscular atrophy, flail arm, flail leg); and (iii) specific clinical features may help identify ALS subtypes, which remain localized to the site of onset for a prolonged time (regionally entrenching ALS).

A single-centre, retrospective cohort of 913 Italian ALS patients was evaluated to assess correlations between directionality of the disease process after symptom onset and motor/neuropsychological phenotype. All patients underwent an extensive evaluation including the following clinical scales: Penn Upper Motor Neuron Score (PUMNS), MRC Scale for Muscle Strength and the Edinburgh Cognitive and Behavioural ALS Screen (ECAS).

The most frequent initial spreading pattern was that towards adjacent horizontal regions (77.3%), which occurred preferentially in patients with lower MRC scores ($P = 0.038$), while vertical diffusion (21.1%) was associated with higher PUMNS ($P < 0.001$) and with reduced survival ($P < 0.001$). Non-contiguous disease spreading was associated with more severe UMN impairment ($P = 0.003$), while contiguous disease pattern with lower MRC scores. Furthermore, non-contiguous disease spreading was associated with more severe cognitive impairment in both executive and visuospatial ECAS domains. Individuals with regionally entrenching ALS were more frequently female (45.6% versus 36.9%; $P = 0.028$) and had higher frequencies of symmetric disease onset (40.3% versus 19.7%; $P < 0.001$) and bulbar phenotype (38.5% versus 16.4%; $P < 0.001$).

Our study suggests that motor phenotypes characterized by a predominant UMN involvement are associated with a vertical pattern of disease progression reflecting ipsilateral spreading within the motor cortex, while those with predominant LMN involvement display more frequently a horizontal spreading from one side of the spinal cord to the other. These observations raise the hypothesis that one of the mechanisms underlying disease spreading in ALS pathology is represented by diffusion of toxic factors in the neuron microenvironment. Finally, it is possible that in our cohort, regionally entrenching ALS forms are mainly observed in patients with atypical bulbar phenotypes, characterized by a slowly progressive course and relatively benign prognosis.

1 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, 20149, Italy
2 Department of Pathophysiology and Transplantation, 'Dino Ferrari' Center, Università degli Studi di Milano, Milan, 20122, Italy

Received September 20, 2022. Revised March 01, 2023. Accepted April 01, 2023. Advance access publication April 19, 2023

Cerebrovascular Diseases

Clinical Research in Stroke

Cerebrovasc Dis
DOI: 10.1159/000531160

Received: March 27, 2023
Accepted: May 12, 2023
Published online: June 1, 2023

The Single-Matrix Digit Cancellation Test, a Screener for Selective Attention Deficits: Standardization in an Italian Population Sample and Clinical Usability in Acute Stroke Patients

Fabrizio Pasotti[a] Edoardo Nicolò Aiello[b] Alessandra Bollani[c] Matteo Querzola[c] Sara Cozzi[a] Francesca Manfrin[a] Stefania Bruno[a] Barbara Poletti[b] Nicola Ticozzi[b, d] Vincenzo Silani[b, d] Gabriella Bottini[a, c]

[a]Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy; [b]IRCCS Istituto Auxologico Italiano, Department of Neurology and Laboratory of Neuroscience, Milan, Italy; [c]Cognitive Neuropsychology Centre, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; [d]Deparment of Pathophysiology and Transplanation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

Keywords
Selective attention · Stroke · Digit cancellation test · Cognitive screening · Neuropsychology

Abstract
Introduction: This study aimed at validating and providing Italian norms for the Single-Matrix Digit Cancellation Test (SMDCT), a cancellation task to screen for selective attention deficits, as well as providing clinical usability evidence for it in acute stroke patients. ***Methods:*** The SMDCT stimulus is a specular, 4-quadrant, horizontally oriented matrix, across which target distribution is homogeneous. Both accuracy (-A) and time (-T) outcomes were computed. *N* = 263 healthy participants (HPs) and *N* = 76 acute stroke patients were recruited. *N* = 108 HPs also underwent the Mini-Mental State Examination, Frontal Assessment Battery (FAB), and Trail-Making Test (TMT), while patients were further assessed by the Mental Performance in Acute Stroke (MEPS). Regression-based norms were derived (equivalent scores). Construct and factorial validity, as well as case-control discrimination, were tested. ***Results:*** The matrix was underpinned by a two-component structure reflecting left and right hits. The SMDCT-T and -A were associated with TMT and FAB scores, respectively. Education predicted the SMDCT-A/-T, whereas age predicted the SMDCT-T only. In patients, the SMDCT converged with the MEPS, also accurately discriminating them from HPs. An index of right-left difference differentiated right- from left-damaged patients. ***Conclusions:*** The SMDCT is a valid and normed screener for selective attention deficits, encompassing measures of both accuracy and time, whose adoption is encouraged in acute stroke patients. Relatedly, the horizontal disposition of its matrix does allow for the qualitative report of either leftward of rightward biases due to underlying visual or attentional-representational deficits in this population.

Fabrizio Pasotti and Edoardo Nicolò Aiello contributed equally to this work.

karger@karger.com
www.karger.com/ced

Correspondence to:
Fabrizio Pasotti, fabrizio.pasotti@unipv.it

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iafd20

Analysis of normal *C9orf72* repeat length as possible disease modifier in amyotrophic lateral sclerosis

Silvia Peverelli, Alberto Brusati, Valeria Casiraghi, Marta Nice Sorce, Sabrina Invernizzi, Serena Santangelo, Claudia Morelli, Federico Verde, Vincenzo Silani, Nicola Ticozzi & Antonia Ratti

To cite this article: Silvia Peverelli, Alberto Brusati, Valeria Casiraghi, Marta Nice Sorce, Sabrina Invernizzi, Serena Santangelo, Claudia Morelli, Federico Verde, Vincenzo Silani, Nicola Ticozzi & Antonia Ratti (01 Nov 2023): Analysis of normal *C9orf72* repeat length as possible disease modifier in amyotrophic lateral sclerosis, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, DOI: 10.1080/21678421.2023.2273965

To link to this article: https://doi.org/10.1080/21678421.2023.2273965

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2023; 0: 1–4

BRIEF REPORT

Analysis of normal *C9orf72* repeat length as possible disease modifier in amyotrophic lateral sclerosis

SILVIA PEVERELLI[1], ALBERTO BRUSATI[2], VALERIA CASIRAGHI[3], MARTA NICE SORCE[1], SABRINA INVERNIZZI[1], SERENA SANTANGELO[3], CLAUDIA MORELLI[1], FEDERICO VERDE[1,4], VINCENZO SILANI[1,4], NICOLA TICOZZI[1,4] & ANTONIA RATTI[1,3]

[1]*Department of Neurology—Laboratory of Neuroscience, IRCCS, Istituto Auxologico Italiano, Milan, Italy,* [2]*Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy,* [3]*Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy, and* [4]*"Dino Ferrari" Center, Dept. of Pathophysiology and Transplantation, University of Milan, Milan, Italy*

Abstract

The *C9orf72* hexanucleotide repeat (HR) expansion is the main genetic cause of amyotrophic lateral sclerosis (ALS), with expansion size from 30 to >4000 units. Normal *C9orf72* HR length is polymorphic (2–23 repeats) with alleles >8 units showing a low frequency in the general population. This study aimed to investigate if the normal *C9orf72* HR length influences *C9orf72* gene expression and acts as disease modifier in ALS patients negative for *C9orf72* mutation (ALS-C9Neg). We found that the distribution of HR alleles was similar in 325 ALS-C9Neg and 303 healthy controls. Gene expression analysis in blood revealed a significant increase of total *C9orf72* and V3 mRNA levels in ALS-C9Neg carrying two long alleles (L/L; ≥8 units) compared to patients homozygous for the 2-unit short allele (S/S). However, HR allele genotypes (L/L, S/L, S/S) correlated with no clinical parameters. Our data suggest that normal *C9orf72* HR length does not act as disease modifier in ALS-C9Neg despite increasing gene expression.

Keywords: *amyotrophic lateral sclerosis, C9orf72, gene expression, disease modifier*

Introduction

The intronic hexanucleotide repeat (HR) expansion of the *C9orf72* gene is the main genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (1). Conventionally, the *C9orf72* pathogenic threshold is >30 repeats, although mutated patients may carry alleles up to 4000 HRs (2). The *C9orf72*-associated pathomechanisms are the haploinsufficiency of *C9orf72* protein, due to reduced transcription of the main V2 isoform from exon 1b downstream of HR expansion, and the accumulation of repeat-containing RNAs transcribed from the upstream exon 1a and of dipeptide repeat proteins through repeat-associated non-AUG translation of V1/V3 transcripts (1).

Normal *C9orf72* HR alleles are polymorphic (2–23 units) with a trimodal distribution (2, 5, 8 units) (3). Alleles >8 repeats have a low frequency both in ALS cases and healthy controls (CTR), and they have been already investigated as risk factors in different neurodegenerative diseases (4). A previous functional assay showed that 9-, 17- and 24-unit alleles reduced luciferase gene transcription in a length-dependent manner compared to the 2-unit allele (5), but whether normal HR length also influences *C9orf72* gene expression in ALS patients' biosamples remains uninvestigated. Indeed, the purpose of this study was to assess whether normal HR length may influence *C9orf72* gene expression acting as disease modifier in ALS patients without *C9orf72* mutation (ALS-C9Neg).

Materials and methods

Our study included 325 ALS-C9Neg diagnosed according to the revised El Escorial criteria, and

Supplemental data for this article can be accessed online at https://doi.org/10.1080/21678421.2023.2273965.

Email address for correspondence: a.ratti@auxologico.it, antonia.ratti@unimi.it

(Received 11 August 2023; revised 1 October 2023; accepted 10 October 2023)

ISSN 2167-8421 print/ISSN 2167-9223 online
DOI: 10.1080/21678421.2023.2273965

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2023; 0: 1–4

SHORT REPORT

Single task-level, 2SD-based cutoffs for the Italian version of the Edinburgh Cognitive and Behavioral ALS screen (ECAS)

BARBARA POLETTI[1]*, EDOARDO NICOLÒ AIELLO[1]*, ANTONINO LA TONA[2], FEDERICA SOLCA[1], SILVIA TORRE[1], ELEONORA COLOMBO[1], ALESSIO MARANZANO[1], CLAUDIA MORELLI[1], ALBERTO DORETTI[1], FEDERICO VERDE[1,3], ALESSIA MONTI[4], AGOSTINO BRUGNERA[2], ANGELO COMPARE[2], ROBERTA FERRUCCI[5,6], SERGIO BARBIERI[6], FRANCESCA MAMELI[6], ALBERTO PRIORI[7,8], GABRIELLA PRAVETTONI[5,9], VINCENZO SILANI[1,3] & NICOLA TICOZZI[1,3]

[1]*Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milano, Italy,* [2]*Department of Human and Social Sciences, University of Bergamo, Bergamo, Italy,* [3]*Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milano, Italy,* [4]*Department of Neurorehabilitation Sciences, Casa di Cura Igea (CCI), Milano, Italy,* [5]*Department of Oncology and Hemato-Oncology, University of Milan, Milano, Italy,* [6]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy,* [7]*Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics, Department of Health Sciences, International Medical School, University of Milan, Milano, Italy,* [8]*ASST Santi Paolo e Carlo, San Paolo University Hospital, Milano, Italy, and* [9]*Applied Research Division for Cognitive and Psychological Science, IEO, European Institute of Oncology, IRCCS, Milano, Italy*

Abstract

The present study aimed at deriving, by means of a traditional "2 standard deviation-based" (2SD) approach, single task-level cutoffs for the Italian version of the Edinburgh Cognitive and Behavioral ALS Screen (ECAS). Cutoffs were derived – as *M*-2**SD* – from the sample of healthy participants (HPs) included within 2016 Poletti *et al.*'s normative study – $N = 248$; 104 males; age: 57.8 ± 10.6; education: 14.1 ± 4.6 – separately for the four, original demographic classes: 1) education <14 years and age ≤60 years; 2) education <14 years and age >60 years; 3) education ≥14 years and age ≤60 years; 4) education ≥14 years and age >60 years. The prevalence of deficits on each task was then estimated within a cohort of $N = 377$ amyotrophic lateral sclerosis (ALS) patients without dementia. The distribution of abnormal performance prevalences was overall consistent with the cognitive phenotype of ALS. In conclusion, the single task-level cutoffs herewith provided for the Italian version of the ECAS, which complement those already available within Poletti *et al.*'s normative framework, will help better profile Italian ALS patients' cognitive phenotype within both clinical and research settings.

Keywords: *Edinburgh Cognitive and Behavioral ALS Screen, amyotrophic lateral sclerosis, frontotemporal degeneration, cognitive screening, neuropsychology*

1. Background

The Edinburgh Cognitive and Behavioral ALS Screen (ECAS) is currently regarded as a gold-standard screener for frontotemporal-*spectrum* disorders in amyotrophic lateral sclerosis (ALS) patients, having gained major clinimetric and feasibility support worldwide [1].

Within the Italian scenario, the ECAS has been thoroughly assessed for its psychometrics [2], diagnostics [3] and feasibility [2,4,5], having been also normed at a single subscale level both *via* a traditional "2 standard deviation-based" (2SD) approach by Poletti *et al.* [2] and *via* a regression-based, non-parametric method by Siciliano *et al.* [6].

*These authors contributed equally.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/21678421.2023.2220746.

Correspondence: Barbara Poletti, IRCCS Istituto Auxologico Italiano, Department of Neurology and Laboratory of Neuroscience, Piazzale Brescia 20, 20149 Milano (MI), Italy. E-mail: b.poletti@auxologico.it

(Received 18 April 2023; revised 16 May 2023; accepted 28 May 2023)

ISSN 2167-8421 print/ISSN 2167-9223 online
DOI: 10.1080/21678421.2023.2220746

frontiers | Frontiers in Psychology

TYPE Brief Research Report
PUBLISHED 04 December 2023
DOI 10.3389/fpsyg.2023.1281976

OPEN ACCESS

EDITED BY
Sara Palermo,
University of Turin, Italy

REVIEWED BY
Fausto Viader,
Université de Caen Normandie, France
Graziella Orrù,
University of Pisa, Italy

*CORRESPONDENCE
Barbara Poletti
b.poletti@auxologico.it

†These authors have contributed equally to this work

RECEIVED 23 August 2023
ACCEPTED 31 October 2023
PUBLISHED 04 December 2023

CITATION
Poletti B, Aiello EN, Tagini S, Solca F, Torre S, Colombo E, Maranzano A, Bonetti R, Schevegher F, Morelli C, Doretti A, Verde F, Barbieri S, Mameli F, Priori A, Ferrucci R, Silani V, Cherubini P, Pravettoni G and Ticozzi N (2023) An exploratory study on counterfactual thinking in amyotrophic lateral sclerosis. *Front. Psychol.* 14:1281976. doi: 10.3389/fpsyg.2023.1281976

An exploratory study on counterfactual thinking in amyotrophic lateral sclerosis

Barbara Poletti [1,2*†], Edoardo Nicolò Aiello[1†], Sofia Tagini[3], Federica Solca[1], Silvia Torre[1], Eleonora Colombo[1], Alessio Maranzano[1], Ruggero Bonetti[4], Francesco Schevegher[4], Claudia Morelli[1], Alberto Doretti[1], Federico Verde[1,5], Sergio Barbieri[6], Francesca Mameli[6], Alberto Priori[7,8], Roberta Ferrucci[2,6], Vincenzo Silani[1,5], Paolo Cherubini[9,10], Gabriella Pravettoni[2,11†] and Nicola Ticozzi[1,5†]

[1]Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milano, Italy, [2]Department of Oncology and Hemato-Oncology, University of Milan, Milano, Italy, [3]"Rita Levi Montalcini" Department of Neurosciences, University of Turin, Torino, Italy, [4]Neurology Residency Program, Università degli Studi di Milano, Milano, Italy, [5]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milano, Italy, [6]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy, [7]Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics, Department of Health Sciences, International Medical School, University of Milan, Milano, Italy, [8]ASST Santi Paolo e Carlo, San Paolo University Hospital, Milano, Italy, [9]Department of Neural and Behavioral Sciences, University of Pavia, Pavia, Italy, [10]Milan Center for Neuroscience, University of Milano-Bicocca, Milano, Italy, [11]Applied Research Division for Cognitive and Psychological Science, IEO, European Institute of Oncology, IRCCS, Milano, Italy

Objectives: This study aimed at exploring (1) the motor and non-motor correlates of counterfactual thinking (CFT) abilities in non-demented amyotrophic lateral sclerosis (ALS) patients and (2) the ability of CFT measures to discriminate these patients from healthy controls (HCs) and patients with and without cognitive impairment.

Methods: $N = 110$ ALS patients and $N = 51$ HCs were administered two CFT tasks, whose sum, resulting in a CFT Index (CFTI), was addressed as the outcome. Patients further underwent an in-depth cognitive, behavioral, and motor-functional evaluation. Correlational analyses were run to explore the correlates of the CFTI in patients. Logistic regressions were performed to test whether the CFTI could discriminate patients from HCs.

Results: The CFTI was selectively associated ($p \leq 0.005$) with fluency and memory subscales of the Edinburgh Cognitive and Behavioral ALS Screen (ECAS), but not with other variables. CFTI scores discriminated patients from HCs ($p < 0.001$) with high accuracy (82%), but not patients with a normal vs. defective performance on the ECAS-Total.

Conclusion: CFT measures in non-demented ALS patients were associated with verbal fluency and memory functions, and they were also able to discriminate them from HCs.

KEYWORDS

counterfactual thinking, cognition, amyotrophic lateral sclerosis, frontotemporal degeneration, neuropsychology, dementia

brain sciences

MDPI

Article

The Effects of a New Integrated and Multidisciplinary Cognitive Rehabilitation Program Based on Mindfulness and Reminiscence Therapy in Patients with Parkinson's Disease and Mild Cognitive Impairment: A Pilot Study

Maria Rita Reitano [1], Matteo Guidetti [2,3], Natale Vincenzo Maiorana [2], Angelica De Sandi [1], Fabrizio Carusi [2], Chiara Rosci [1], Fabiana Ruggiero [4], Barbara Poletti [5], Nicola Ticozzi [5,6], Francesca Mameli [4], Sergio Barbieri [4], Vincenzo Silani [5,6], Alberto Priori [1,2] and Roberta Ferrucci [1,2,*]

1 ASST Santi Paolo e Carlo, San Paolo University Hospital, 20142 Milan, Italy
2 Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics, Department of Health Sciences, University of Milan, 20142 Milan, Italy
3 Department of Electronics, Information and Bioengineering, Politecnico di Milano, 20133 Milan, Italy
4 Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
5 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, 20149 Milan, Italy
6 Department of Pathophysiology and Transplantation, "Dino Ferrari Center", University of Milan, 20122 Milan, Italy
* Correspondence: roberta.ferrucci@unimi.it

Abstract: *Background*: Mindfulness trainings have shown promising results as treatment for behavioural symptoms in several pathologies. In addition, mindfulness protocols induced an improvement in memory and attention. Therefore, mindfulness could be an effective intervention for patients affected by Parkinson's disease (PD) and mild cognitive impairment (MCI), who are characterized by both behavioural and cognitive dysfunctions. *Methods*: We assessed differences in Montreal Cognitive Assessment (MoCA) scores and in Beck Depression Inventory II (BDI-II) scores in patients affected by PD and MCI enrolled in two different rehabilitation programs (an experimental vs. an usual structured program for cognitive rehabilitation). Participants in the experimental group (MILC-tr) underwent innovative rehabilitation program involving mindfulness and reminiscence activities. Assessments were performed before (T0) and at the end of the rehabilitation program (T1). *Results*: Friedman test showed a significant improvement between timepoints in MoCA global score ($x^2 = 4.000$, $p = 0.046$), MoCA memory sub-scale score ($x^2 = 4.571$, $p = 0.033$), and BDI-II cognitive and affective factors ($x^2 = 4.000$, $p = 0.046$) only for patients in MILC-tr group. Mann–Whitney test showed a significant difference between group comparing differences in Δ scores between T0 and T1 in the MoCA memory sub-scale score ($U = 190.50$, $p = 0.035$). *Conclusions*: Mindfulness-based rehabilitation programs could be effective in patients affected by PD and MCI.

Keywords: mindfulness; reminiscence and life review; verbal long-term memory; Parkinson's disease; mild cognitive impairment

Citation: Reitano, M.R.; Guidetti, M.; Maiorana, N.V.; De Sandi, A.; Carusi, F.; Rosci, C.; Ruggiero, F.; Poletti, B.; Ticozzi, N.; Mameli, F.; et al. The Effects of a New Integrated and Multidisciplinary Cognitive Rehabilitation Program Based on Mindfulness and Reminiscence Therapy in Patients with Parkinson's Disease and Mild Cognitive Impairment: A Pilot Study. *Brain Sci.* **2023**, *13*, 201. https://doi.org/10.3390/brainsci13020201

Academic Editor: Notger G. Müller

Received: 23 December 2022
Revised: 9 January 2023
Accepted: 23 January 2023
Published: 25 January 2023

1. Introduction

Mild cognitive impairment (MCI) is a condition characterized by a cognitive impairment greater than that due to normal aging, but not severe enough for a diagnosis of dementia [1,2] nor for a significant impact on the activities of daily living [3]. Although the true values are difficult to define [4], the literature suggests that prevalence of MCI in elderly people (>65 years old) would be 3–22% [2,5,6], depending on the demographics of the population studied. Although in the past MCI was considered simply a "precursor" to or a "paucisymptomatic" phase of Alzheimer's disease (AD) [7], not all cases of MCI

Neurological Sciences (2023) 44:3885–3894
https://doi.org/10.1007/s10072-023-06903-9

ORIGINAL ARTICLE

Telemedicine for cognitive impairment: a telephone survey of patients' experiences with neurological video consultation

Fabiana Ruggiero[1] · Eleonora Zirone[1] · Maria Takeko Molisso[1] · Tiziana Carandini[1] · Giorgio Fumagalli[1] · Anna Pietroboni[1] · Roberta Ferrucci[2,5] · Edoardo Nicolò Aiello[3] · Barbara Poletti[2,3] · Vincenzo Silani[3,4] · Giacomo Comi[1,4] · Elio Scarpini[1,4] · Sergio Barbieri[1] · Andrea Arighi[1] · Francesca Mameli[1]

Received: 27 January 2023 / Accepted: 5 June 2023 / Published online: 27 June 2023

Abstract

Objective This study aimed to evaluate the experience with telemedicine in patients with cognitive impairments and their caregivers.

Methods We conducted a survey-based study of patients who completed neurological consultation via video link between January and April 2022.

Results A total of 62 eligible neurological video consultations were conducted for the following categories of patients: Alzheimer's disease (33.87%), amnesic mild cognitive impairment (24.19%), frontotemporal dementia (17.74%), Lewy body dementia (4.84%), mixed dementia (3.23%), subjective memory disorders (12.90%), non-amnesic mild cognitive impairment (1.61%), and multiple system atrophy (1.61%).

The survey was successfully completed by 87.10% of the caregivers and directly by the patients in 12.90% of cases. Our data showed positive feedback regarding the telemedicine experience; both caregivers and patients reported that they found neurological video consultation useful (caregivers: 87.04%, 'very useful'; patients: 87.50%, 'very useful') and were satisfied overall (caregivers: 90.74%, 'very satisfied'; patients: 100%, 'very satisfied'). Finally, all caregivers (100%) agreed that neurological video consultation was a useful tool to reduce their burden (Visual Analogue Scale mean ± SD: 8.56 ± 0.69).

Conclusions Telemedicine is well received by patients and their caregivers. However, successful delivery incorporates support from staff and care partners to navigate technologies. The exclusion of older adults with cognitive impairment in developing telemedicine systems may further exacerbate access to care in this population. Adapting technologies to the needs of patients and their caregivers is critical for the advancement of accessible dementia care through telemedicine.

Keywords Cognitive impairment · Dementia · Caregiver · COVID-19 · Telemedicine · Neurological video consultation

Introduction

The coronavirus disease (COVID-19) pandemic has rendered older adults more vulnerable to not receiving the healthcare needed. Furthermore, it has placed those living with dementia at an even increased risk of developing other mental health symptoms due to social isolation and loneliness [1]. Indeed, as the virus spreads, it has become necessary to introduce social distancing measures, such as quarantine within urban areas, prohibition of travel to and from certain countries, and suspension of a large range of clinical activities. Elective face-to-face consultations had to be rescheduled, and the need for health care during the pandemic required telehealth solutions. National initiatives have been launched to review and update previous restrictions

✉ Francesca Mameli
francesca.mameli@policlinico.mi.it

1 Department of Neuroscience and Mental Health, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

2 Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

3 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy

4 Department of Pathophysiology and Transplantation (DEPT), Dino Ferrari Centre, University of Milan, Neuroscience Section, Milan, Italy

5 ASST Santi Paolo e Carlo, Milan, Italy

Stem Cell Research 66 (2023) 103008

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Single Cell Line

Generation of an iPSC line from a patient with spastic paraplegia type 10 carrying a novel mutation in *KIF5A* gene

Serena Santangelo [a,b], Patrizia Bossolasco [b], Stefania Magri [c], Claudia Colombrita [b], Sabrina Invernizzi [b], Cinzia Gellera [c], Lorenzo Nanetti [c], Daniela Di Bella [c], Vincenzo Silani [b,d], Franco Taroni [c], Antonia Ratti [a,b,*]

[a] Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy
[b] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy
[c] Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[d] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

A B S T R A C T

We generated an iPSC line from a patient with spastic paraplegia type 10 (SPG10) carrying the novel missense variant c.50G > A (p.R17Q) in the *N*-terminal motor domain of the kinesin family member 5A (*KIF5A*) gene.

This patient-derived *in vitro* cell model will help to investigate the role of different *KIF5A* mutations in inducing neurodegeneration in spastic paraplegia and in other *KIF5A*-related disorders, including Charcot-Marie-Tooth type 2 (CMT2) and amyotrophic lateral sclerosis (ALS).

Resource table

Unique stem cell line identifier	IAIi010-A
Alternative name(s) of stem cell line	KIF5A_C3
Institution	IRCCS Istituto Auxologico Italiano, Milan, Italy
Contact information of distributor	Antonia Ratti, antonia.ratti@unimi.it
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Ethnicity: Caucasian Age: 79 Sex: Female
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune iPS 2.0 Sendai Reprogramming Kit
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Autosomal dominant Spastic Paraplegia type 10 (SGP10)
Gene/locus	KIF5A, chromosome 12q13.13 NM_004984.3: c.50G > A (p.R17Q)
Date archived/stock date	October 2022
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/IAIi010-A
Ethical approval	Ethical committee Regione Lombardia, sezione Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy, Approval n.64

(*continued on next column*)

Resource table (*continued*)

1. Resource utility

Allelic mutations in *KIF5A* gene are associated to different neurodegenerative disorders, such as spastic paraplegia type 10 (SPG10), axonal Charcot-Marie-Tooth type 2 (CMT2), and amyotrophic lateral sclerosis (ALS) as well as to neonatal intractable myoclonus (NEIMY) with distinct mutational hotspots.

We generated an iPSC line from a SPG10 individual carrying the novel missense mutation p.R17Q (c.50G > A) in KIF5A protein motor domain.

This iPSC line represents a new *in vitro* disease model to elucidate, upon differentiation into motoneurons, the pathomechanisms associated with *KIF5A* mutations.

* Corresponding author.
E-mail address: antonia.ratti@unimi.it (A. Ratti).

https://doi.org/10.1016/j.scr.2022.103008
Received 18 November 2022; Accepted 19 December 2022
Available online 21 December 2022

BRAIN
ORIGINAL ARTICLE

Genetic variability in sporadic amyotrophic lateral sclerosis

Sien Hilde Van Daele,[1,2,3,4] Matthieu Moisse,[1,2] Joke J. F. A. van Vugt,[5] Ramona A. J. Zwamborn,[5] Rick van der Spek,[5] Wouter van Rheenen,[5] Kristel Van Eijk,[5] Kevin Kenna,[5] Philippe Corcia,[6,7] Patrick Vourc'h,[7] Philippe Couratier,[8] Orla Hardiman,[9] Russell McLaughin,[10] Marc Gotkine,[11] Vivian Drory,[12] Nicola Ticozzi,[13,14] Vincenzo Silani,[13,14] Antonia Ratti,[13,15] Mamede de Carvalho,[16] Jesús S. Mora Pardina,[17] Monica Povedano,[18] Peter M. Andersen,[19] Markus Weber,[20] Nazli A. Başak,[21] Chris Shaw,[22] Pamela J. Shaw,[23] Karen E. Morrison,[24] John E. Landers,[25] Jonathan D. Glass,[26] Michael A. van Es,[5] Leonard H. van den Berg,[5] Ammar Al-Chalabi,[22] Jan Veldink[5] and Philip Van Damme[1,2,3], on behalf of Project MinE ALS Sequencing Consortium

With the advent of gene therapies for amyotrophic lateral sclerosis (ALS), there is a surge in gene testing for this disease. Although there is ample experience with gene testing for *C9orf72*, *SOD1*, *FUS* and *TARDBP* in familial ALS, large studies exploring genetic variation in all ALS-associated genes in sporadic ALS (sALS) are still scarce. Gene testing in a diagnostic setting is challenging, given the complex genetic architecture of sALS, for which there are genetic variants with large and small effect sizes. Guidelines for the interpretation of genetic variants in gene panels and for counselling of patients are lacking.

We aimed to provide a thorough characterization of genetic variability in ALS genes by applying the American College of Medical Genetics and Genomics (ACMG) criteria on whole genome sequencing data from a large cohort of 6013 sporadic ALS patients and 2411 matched controls from Project MinE.

We studied genetic variation in 90 ALS-associated genes and applied customized ACMG-criteria to identify pathogenic and likely pathogenic variants. Variants of unknown significance were collected as well. In addition, we determined the length of repeat expansions in *C9orf72*, *ATXN1*, *ATXN2* and *NIPA1* using the ExpansionHunter tool.

We found *C9orf72* repeat expansions in 5.21% of sALS patients. In 50 ALS-associated genes, we did not identify any pathogenic or likely pathogenic variants. In 5.89%, a pathogenic or likely pathogenic variant was found, most commonly in *SOD1*, *TARDBP*, *FUS*, *NEK1*, *OPTN* or *TBK1*. Significantly more cases carried at least one pathogenic or likely pathogenic variant compared to controls (odds ratio 1.75; *P*-value 1.64×10^{-5}). Isolated risk factors in *ATXN1*, *ATXN2*, *NIPA1* and/or *UNC13A* were detected in 17.33% of cases. In 71.83%, we did not find any genetic clues. A combination of variants was found in 2.88%.

This study provides an inventory of pathogenic and likely pathogenic genetic variation in a large cohort of sALS patients. Overall, we identified pathogenic and likely pathogenic variants in 11.13% of ALS patients in 38 known ALS genes. In line with the oligogenic hypothesis, we found significantly more combinations of variants in cases compared to controls. Many variants of unknown significance may contribute to ALS risk, but diagnostic algorithms to reliably identify and weigh them are lacking. This work can serve as a resource for counselling and for the assembly of gene panels for ALS. Further characterization of the genetic architecture of sALS is necessary given the growing interest in gene testing in ALS.

Received November 16, 2022. Revised March 10, 2023. Accepted March 23, 2023. Advance access publication April 12, 2023

1 Department of Neurosciences, Experimental Neurology, KU Leuven—University of Leuven, and Leuven Institute for Neuroscience and Disease (LIND), 3000 Leuven, Belgium
2 VIB, Center for Brain & Disease Research, Laboratory of Neurobiology, 3000 Leuven, Belgium
3 Department of Neurology, University Hospitals Leuven, 3000 Leuven, Belgium
4 Department of Human genetics, University Hospitals Leuven, 3000 Leuven, Belgium
5 Department of Neurology, UMC Utrecht Brain Center, Utrecht University, 3584 CX Utrecht, The Netherlands
6 Centre SLA, CHRU de Tours, 37044 Tours, France
7 UMR 1253, iBrain, Université de Tours, Inserm, 37032 Tours, France
8 Centre SLA, CHU Limoges, 87042 Limoges, France
9 Academic Unit of Neurology, Trinity College Dublin, Trinity Biomedical Sciences Institute, Dublin D02 PN40, Republic of Ireland
10 Complex Trait Genomics Laboratory, Smurfit Institute of Genetics, Trinity College Dublin, Dublin D02 PN40, Republic of Ireland
11 The Agnes Ginges Center for Human Neurogenetics, Hadassah Medical Organization and Faculty of Medicine, Hebrew University of Jerusalem, 91120 Jerusalem, Israel
12 Department of Neurology, Tel-Aviv Sourasky Medical Centre, 64239 Tel Aviv, Israel
13 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, 20149 Milano, Italy
14 Department of Pathophysiology and Transplantation, 'Dino Ferrari' Center, Università degli Studi di Milano, 20122 Milan, Italy
15 Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, 20133 Milano, Italy
16 Instituto de Fisiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisbon, Portugal
17 ALS Unit, Hospital University San Rafael, 28016 Madrid, Spain
18 Servei de Neurologia, HUB-IDIBELL, 08908 Barcelona, Spain
19 Department of Clinical Science, Neurosciences, Umeå University, 901 87 Umeå, Sweden
20 Neuromuscular Diseases Unit/ALS Clinic, Kantonsspital St. Gallen, 9007 St. Gallen, Switzerland
21 Koç University, School of Medicine, KUTTAM-NDAL, 34010 Istanbul, Turkey
22 Maurice Wohl Clinical Neuroscience Institute, King's College London, Department of Basic and Clinical Neuroscience, London SE5 9RT, UK
23 Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield S10 2HQ, UK
24 School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast BT9 7BL, UK
25 Department of Neurology, University of Massachusetts Medical School, Worcester, MA 01655, USA
26 Department Neurology, Emory University School of Medicine, Atlanta, GA 30322, USA

Correspondence to: Philip Van Damme
Laboratorium voor Neurobiologie, VIB-KU Leuven
ON IV Herestraat 49—bus 602
3000 Leuven, Belgium
E-mail: philip.vandamme@uzleuven.be

Keywords: complex genetic disease; oligogenic inheritance; motor neuron disease

Introduction

Amyotrophic lateral sclerosis (ALS) is a motor neuron disorder characterized by upper and lower motor neuron degeneration, which leads to progressive muscle weakness and wasting.[1] Up to 50% of patients develop extramotor symptoms, such as cognitive or behavioural dysfunction, as seen in frontotemporal dementia (FTD).[1] The disease is relentlessly progressive and most people die between 2 and 5 years after disease onset, as effective treatments are lacking.[1,2] ALS has a strong genetic component. In 5–10%, there is a familial history of ALS (fALS). Highly penetrant causal variants are found in ~70% of fALS patients, most commonly in *C9orf72*, *SOD1*, *TARDPB* and *FUS*, which are responsible for about 40%, 20%, 4% and 3% of familial cases in Western populations, respectively.[3] The remaining 90–95% of patients present with apparently sporadic ALS (sALS),[4] but mutations in the same genes are found at lower frequencies.[5] Twin studies suggest a heritability in sALS patients of around 60%.[6] Both rare variants with a variable effect size, common variants with small effect size and combinations of such variants are thought to confer genetic risk in sALS patients, but convincing data showing this are still lacking.[7,8] Nevertheless, much of the genetic architecture of ALS remains unknown. Over the past few years, many efforts have been made to unravel the missing heritability. Genetic research has linked a considerable number of genes and variants to ALS through various techniques.[7,9] However, strong evidence for association is variable, and some findings have failed to be replicated in subsequent studies.[10] Furthermore, the clinical significance of individual variants is often unclear (e.g. monogenetic with high penetrance, modifier, risk factor or in linkage with causal variant), especially in sALS patients. As the risk of ALS is age-dependent, the life-time risk is in the order of 1/400 for males and 1/550 for females and since many of the reported disease-associated variants have been associated with incomplete penetrance (in fALS pedigrees), such variants will invariably also be found in control populations.[11] One of the difficulties in the interpretation of variants in complex genetic diseases like ALS is how to weigh the pathogenicity of variants, since

Activation of long non-coding RNA NEAT1 leads to survival advantage of multiple myeloma cells by supporting a positive regulatory loop with DNA repair proteins

Elisa Taiana,[1,2] Cecilia Bandini,[3,4] Vanessa Katia Favasuli,[1,2] Domenica Ronchetti,[1,2] Ilaria Silvestris,[1,2] Noemi Puccio,[1,2] Katia Todoerti,[1] Silvia Erratico,[5,6] Domenica Giannandrea,[7] Niccolò Bolli,[1,2] Nicola Amodio,[8] Alessia Ciarrocchi,[9] Raffaella Chiaramonte,[7] Yvan Torrente,[6] Roberto Piva[3,4] and Antonino Neri[1,2°]

[1]Hematology, Fondazione Cà Granda IRCCS Policlinico, Milan; [2]Department of Oncology and Hemato-oncology, University of Milan, Milan; [3]Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin; [4]Città Della Salute e della Scienza Hospital, Turin; [5]Novystem Spa, Milan; [6]Stem Cell Laboratory, Department of Pathophysiology and Transplantation, University of Milan, Centro Dino Ferrari, Unit of Neurology, Fondazione Cà Granda IRCCS Policlinico, Milan; [7]Department of Health Sciences, University of Milan, Milan; [8]Department of Experimental and Clinical Medicine, Magna Graecia University of Catanzaro, Catanzaro and [9]Laboratory of Translational Research, Azienda Unità Sanitaria Locale-IRCCS Reggio Emilia, Reggio Emilia, Italy

°Current address: Scientific Directorate, Azienda USL-IRCCS Reggio Emilia, Italy

Correspondence: E. Taiana
elisa.taiana@unimi.it
A. Neri
antonino.neri@unimi.it

Received: March 31, 2022.
Accepted: September 1, 2022.
Prepublished: September 8, 2022.

https://doi.org/10.3324/haematol.2022.281167

Abstract

Long non-coding RNA NEAT1 is the core structural component of the nuclear paraspeckle (PS) organelles and it has been found to be deregulated in multiple myeloma (MM) patients. Experimental evidence indicated that NEAT1 silencing negatively impacts proliferation and viability of MM cells, both *in vitro* and *in vivo*, suggesting a role in DNA damage repair (DDR). In order to elucidate the biological and molecular relevance of NEAT1 upregulation in MM disease we exploited the CRISPR/Cas9 synergistic activation mediator genome editing system to engineer the AMO-1 MM cell line and generate two clones that para-physiologically transactivate NEAT1 at different levels. NEAT1 overexpression is associated with oncogenic and prosurvival advantages in MM cells exposed to nutrient starvation or a hypoxic microenvironment, which are stressful conditions often associated with more aggressive disease phases. Furthermore, we highlighted the NEAT1 involvement in virtually all DDR processes through, at least, two different mechanisms. On one side NEAT1 positively regulates the post-translational stabilization of essential PS proteins, which are involved in almost all DDR systems, thus increasing their availability within cells. On the other hand, NEAT1 plays a crucial role as a major regulator of a molecular axis that includes ATM and the catalytic subunit of DNA-PK kinase proteins, and their direct targets pRPA32 and pCHK2. Overall, we provided novel important insightsthe role of NEAT1 in supporting MM cells adaptation to stressful conditions by improving the maintenance of DNA integrity. Taken together, our results suggest that NEAT1, and probably PS organelles, could represent a potential therapeutic target for MM treatment.

Introduction

Multiple myeloma (MM) is a malignant proliferation of bone marrow plasma cells (PC) characterized by a different clinical course and a highly heterogeneous genetic background with both structural chromosomal alterations and specific gene mutations.[1,2]

Over the past decade, a causal relationship between the regulation of long non-coding RNA (lncRNA) and the pathogenesis of human cancers, including MM, has emerged from different functional studies.[3-6] lncRNA participate in several biological processes, such as transcriptional gene regulation, genomic integrity maintenance, cell differentiation and development.[7]

We have identified the nuclear paraspeckle assembly transcript 1 (NEAT1) as one of the abundantly expressed lncRNA in malignant PC compared to its normal counterpart,[6,8,9] consistently with its high expression levels in many solid

Article

SOURCE DATA | TRANSPARENT PROCESS | OPEN ACCESS

EMBO Molecular Medicine

Combinatorial activation of the WNT-dependent fibrogenic program by distinct complement subunits in dystrophic muscle

Francesca Florio[1,2,3], Sara Vencato[1,2], Filomena T Papa[1,2], Michela Libergoli[1,2], Eyemen Kheir[1,2], Imen Ghzaiel[1,2], Thomas A Rando[4], Yvan Torrente[3,5] & Stefano Biressi[1,2,*]

Abstract

Fibrosis is associated with compromised muscle functionality in Duchenne muscular dystrophy (DMD). We report observations with tissues from dystrophic patients and mice supporting a model to explain fibrosis in DMD, which relies on the crosstalk between the complement and the WNT signaling pathways and the functional interactions of two cellular types. Fibro-adipogenic progenitors and macrophages, which populate the inflamed dystrophic muscles, act as a combinatorial source of WNT activity by secreting distinct subunits of the C1 complement complex. The resulting aberrant activation of the WNT signaling in responsive cells, such as fibro-adipogenic progenitors, contributes to fibrosis. Indeed, pharmacological inhibition of the C1r/s subunits in a murine model of DMD mitigated the activation of the WNT signaling pathway, reduced the fibrogenic characteristics of the fibro-adipogenic progenitors, and ameliorated the dystrophic phenotype. These studies shed new light on the molecular and cellular mechanisms responsible for fibrosis in muscular dystrophy and open to new therapeutic strategies.

Keywords complement C1 complex; Duchenne muscular dystrophy; fibro-adipogenic progenitors; fibrosis; skeletal muscle regeneration
Subject Category Musculoskeletal System
DOI 10.15252/emmm.202317405 | Received 10 January 2023 | Revised 10 October 2023 | Accepted 11 October 2023 | Published online 6 November 2023
EMBO Mol Med (2023) 15: e17405

Introduction

Duchenne muscular dystrophy (DMD) is one of the most severe and frequent forms of dystrophy (Emery, 2002; Mercuri *et al*, 2019). DMD patients show progressive dysfunction of skeletal and cardiac muscles (Emery, 2002; Mercuri *et al*, 2019). Although multidisciplinary care and glucocorticoid treatment are associated with reduced disease progression and improved patient survival, no definitive cure is currently available for DMD, and patients die by their third decade of life (Birnkrant *et al*, 2018; McDonald *et al*, 2018). DMD occurs due to mutations in the X-chromosome dystrophin gene (O'Brien & Kunkel, 2001). The absence of functional dystrophin causes repetitive cycles of degeneration/regeneration of the muscle fibers. Portions of the dystrophic muscles (regeneration foci) continuously attempt to regenerate and are characterized by the infiltration of inflammatory cells (Ciciliot & Schiaffino, 2010). This chronic condition of inflammation and degeneration determines impairment of muscle repair potential, and fibrotic extracellular matrix progressively substitutes contractile fibers, determining a severe deficit of muscular function (Ciciliot & Schiaffino, 2010).

Upon damage, muscle regeneration is ensured by muscle satellite cells (MuSCs), a muscle-specific stem cell population required to restore tissue functionality (Scharner & Zammit, 2011). MuSCs' activity is influenced by intrinsic and extrinsic factors (Sousa-Victor *et al*, 2022). Particularly, MuSCs function is supported by a heterogeneous pool of cells occupying the interstitial space between fibers or associated with the vasculature (Wosczyna & Rando, 2018). Among them, mesenchymal cells, called fibro-adipogenic progenitors (FAPs), characterized by the expression of PDGF receptor-α (PDGFRα) and Sca1 critically influence muscle regeneration and homeostasis (Joe *et al*, 2010; Uezumi *et al*, 2010; Wosczyna *et al*, 2019). Moreover, a large body of evidence identified various subpopulations of immune cells as crucial mediators of effective muscle repair (Shen *et al*, 2008; Burzyn *et al*, 2013; Heredia *et al*, 2013; Lemos *et al*, 2015; Liu *et al*, 2017). Importantly, in diseased muscle, macrophages' fate is disturbed, and the communication between MuSCs and different subpopulations of inflammatory and interstitial cells is compromised (Tidball & Villalta, 2010; Mozzetta *et al*, 2013). These alterations are believed to contribute to the defective regeneration and promotion of fibrosis (Desguerre *et al*, 2009; Serrano & Munoz-Canoves, 2010).

1 Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy
2 Dulbecco Telethon Institute at University of Trento, Trento, Italy
3 Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
4 Broad Stem Cell Research Center, University of California Los Angeles, Los Angeles, CA, USA
5 Stem Cell Laboratory, Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy
*Corresponding author. Tel: ++39 0461285290; E-mail: stefano.biressi@unitn.it

DIS3 depletion in multiple myeloma causes extensive perturbation in cell cycle progression and centrosome amplification

Vanessa K. Favasuli,[1,2*] Domenica Ronchetti,[1*] Ilaria Silvestris,[1] Noemi Puccio,[3,4] Giuseppina Fabbiano,[5] Valentina Traini,[5] Katia Todoerti,[6] Silvia Erratico,[7,8] Alessia Ciarrocchi,[3] Valentina Fragliasso,[3] Domenica Giannandrea,[9] Francesca Tumiatti,[10] Raffaella Chiaramonte,[9] Yvan Torrente,[7] Palma Finelli,[10,11] Eugenio Morelli,[2] Nikhil C. Munshi,[2] Niccolò Bolli,[1,5] Antonino Neri[12] and Elisa Taiana[5]

[1]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy; [2]Department of Medical Oncology, Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, Boston, MA, USA; [3]Laboratory of Translational Research, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy; [4]Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy; [5]Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [6]Department of Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; [7]Stem Cell Laboratory, Department of Pathophysiology and Transplantation, University of Milan, Centro Dino Ferrari, Unit of Neurology, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy; [8]Novystem Spa, Milan, Italy; [9]Department of Health Sciences, University of Milan, Milan, Italy; [10]Medical Genetics Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [11]Department of Medical Biotechnology and Translational Medicine, University of Milan, Segrate, Italy and [12]Scientific Directorate, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

VKF and DR contributed equally as first authors.

Correspondence: A Neri
antonino.neri@ausl.re.it

Received: April 4, 2023.
Accepted: July 5, 2023.
Early view: July 13, 2023.

https://doi.org/10.3324/haematol.2023.283274

Abstract

DIS3 gene mutations occur in approximately 10% of patients with multiple myeloma (MM); furthermore, *DIS3* expression can be affected by monosomy 13 and del(13q), found in roughly 40% of MM cases. Despite the high incidence of *DIS3* mutations and deletions, the biological significance of *DIS3* and its contribution to MM pathogenesis remain poorly understood. In this study we investigated the functional role of *DIS3* in MM, by exploiting a loss-of-function approach in human MM cell lines. We found that *DIS3* knockdown inhibits proliferation in MM cell lines and largely affects cell cycle progression of MM plasma cells, ultimately inducing a significant increase in the percentage of cells in the G0/G1 phase and a decrease in the S and G2/M phases. *DIS3* plays an important role not only in the control of the MM plasma cell cycle, but also in the centrosome duplication cycle, which are strictly co-regulated in physiological conditions in the G1 phase. Indeed, *DIS3* silencing leads to the formation of supernumerary centrosomes accompanied by the assembly of multipolar spindles during mitosis. In MM, centrosome amplification is present in about a third of patients and may represent a mechanism leading to genomic instability. These findings strongly prompt further studies investigating the relevance of *DIS3* in the centrosome duplication process. Indeed, a combination of *DIS3* defects and deficient spindle-assembly checkpoint can allow cells to progress through the cell cycle without proper chromosome segregation, generating aneuploid cells which ultimately lead to the development of MM.

Introduction

Multiple myeloma (MM) is a hematologic malignancy that is still incurable despite the recent introduction of a large array of innovative therapies.[1] MM is characterized by the abnormal proliferation of plasma cells (PC) in the bone marrow and has different clinical courses and a highly heterogeneous genetic background with both structural chromosomal alterations and specific gene mutations affecting the expression and the activity of both putative oncogenes and tumor suppressor genes.[2]

Among the frequently mutated genes in MM, *DIS3* has been reported to be mutated in roughly 10% of patients and to have a significant impact on clinical outcome.[3-6] Despite the

Article

SOURCE DATA | TRANSPARENT PROCESS | OPEN ACCESS

EMBO Molecular Medicine

Microbiota dysbiosis influences immune system and muscle pathophysiology of dystrophin-deficient mice

Andrea Farini[1], Luana Tripodi[2], Chiara Villa[2], Francesco Strati[3], Amanda Facoetti[4,5], Guido Baselli[6,†], Jacopo Troisi[7,8], Annamaria Landolfi[7,8], Caterina Lonati[9], Davide Molinaro[1,2], Michelle Wintzinger[10,11], Stefano Gatti[9], Barbara Cassani[5,12], Flavio Caprioli[13], Federica Facciotti[13], Mattia Quattrocelli[10,11] & Yvan Torrente[1,2,*]

Abstract

Duchenne muscular dystrophy (DMD) is a progressive severe muscle-wasting disease caused by mutations in *DMD*, encoding dystrophin, that leads to loss of muscle function with cardiac/respiratory failure and premature death. Since dystrophic muscles are sensed by infiltrating inflammatory cells and gut microbial communities can cause immune dysregulation and metabolic syndrome, we sought to investigate whether intestinal bacteria support the muscle immune response in mdx dystrophic murine model. We highlighted a strong correlation between DMD disease features and the relative abundance of *Prevotella*. Furthermore, the absence of gut microbes through the generation of mdx germ-free animal model, as well as modulation of the microbial community structure by antibiotic treatment, influenced muscle immunity and fibrosis. Intestinal colonization of mdx mice with eubiotic microbiota was sufficient to reduce inflammation and improve muscle pathology and function. This work identifies a potential role for the gut microbiota in the pathogenesis of DMD.

Keywords Duchenne muscular dystrophy; gut microbiota; immunity; skeletal muscle metabolism; T-lymphocytes

Subject Categories Digestive System; Microbiology, Virology & Host Pathogen Interaction; Musculoskeletal System

DOI 10.15252/emmm.202216244 | Received 29 April 2022 | Revised 24 November 2022 | Accepted 1 December 2022 | Published online 19 December 2022

EMBO Mol Med (2023) 15: e16244

See also: **A Jayaraman & S Pettersson** (March 2023)

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked disease caused by mutations in the *DMD* gene and loss of the dystrophin protein, leading to myofiber membrane fragility and necrosis with weakness and contractures. Affected DMD boys typically die in their second or third decade of life due to either respiratory failure or cardiomyopathy (Emery, 2002). Although the primary defects rely on skeletal muscle structure, a multitude of secondary defects exist involving deregulated metabolic and inflammatory pathways. Immune cell infiltration into skeletal muscle is, indeed, a typical feature of DMD pathophysiology and is strongly associated with disease severity (Farini *et al*, 2009). In the dystrophic dystrophin-deficient mdx murine model, we recently found the presence of activated T lymphocytes and the overexpression of immunoproteasome (IP), an enzymatic complex that cleaves peptides to produce epitopes for antigen presentation to T lymphocytes. We have demonstrated that IP inhibition improved dystrophic muscle functions by reducing the

1 Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
2 Stem Cell Laboratory, Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, Milan, Italy
3 Mucosal Immunology Lab, Department of Experimental Oncology, IEO-European Institute of Oncology, Milan, Italy
4 Humanitas University, Milan, Italy
5 Humanitas Clinical and Research Center IRCCS, Milan, Italy
6 Translational Medicine – Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
7 Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, University of Salerno, Baronissi, Italy
8 Theoreo Srl, Spinoff Company of the University of Salerno, Montecorvino Pugliano, Italy
9 Center for Surgical Research, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy
10 Molecular Cardiovascular Biology Division, Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
11 Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA
12 Department of Medical Biotechnologies and Translational Medicine, Università Degli Studi di Milano, Milan, Italy
13 Unit of Gastroenterology and Endoscopy, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda, Ospedale Policlinico di Milano, Milan, Italy
*Corresponding author. Tel: +39 0255033874; E-mail: yvan.torrente@unimi.it
†Present address: SciLifeLab, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solna, Sweden

BRAIN
REVIEW ARTICLE

Multifaceted nanoparticles: emerging mechanisms and therapies in neurodegenerative diseases

Miriam Mistretta,[1] Andrea Farini,[2] Yvan Torrente[1,2] and Chiara Villa[1]

Neurodegenerative diseases are a major global health burden particularly with the increasing ageing population. Hereditary predisposition and environmental risk factors contribute to the heterogeneity of existing pathological phenotypes. Traditional clinical interventions focused on the use of small drugs have often led to failures due to the difficulties in crossing the blood–brain barrier and reaching the brain. In this regard, nanosystems can specifically deliver drugs and improve their bioavailability, overcoming some of the major challenges in neurodegenerative disease treatment.

This review focuses on the use of nanosystems as an encouraging therapeutic approach targeting molecular pathways involved in localized and systematic neurodegenerative diseases. Among the latter, Friedreich's ataxia is an untreatable complex multisystemic disorder and the most widespread type of ataxia; it represents a test case to validate the clinical potential of therapeutic strategies based on nanoparticles with pleiotropic effects.

1 Stem Cell Laboratory, Dino Ferrari Center, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy
2 Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy

Correspondence to: Chiara Villa
Stem Cell Laboratory, Dino Ferrari Center
Department of Pathophysiology and Transplantation
University of Milan, via Francesco Sforza, 20122 Milan, Italy
E-mail: chiara.villa2@unimi.it

Keywords: neurodegeneration; neuroinflammation; ROS; autophagy; gold quantum clusters

Introduction

Neurodegenerative diseases (NDs) are a heterogeneous group of CNS disorders characterized by chronic and selective neuronal cell death, decreased strength, coordination and mobility, respiratory distress and cognitive deficit.[1] Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) are the major NDs.[1,2] Although genetic and hereditary predisposition seem to play an important role, especially combined with environmental risk factors,[3] NDs differ in pathophysiology and symptomatology. On the contrary, protein misfolding, aggregation and accumulation of proteins, neuroinflammation, mitochondrial dysfunctions, oxidative stress, dysregulated autophagy and apoptosis[4] are some of the most important shared biological processes. Most of these processes are also characteristic of Friedreich's ataxia (FRDA), a multisystemic autosomal recessive degenerative disorder affecting central and peripheral nervous system, heart, skeletal muscle and endocrine pancreas.[5,6] With onset before 25 years of age, FRDA affects 1 in 30 000–50 000 people, with prominent neurological manifestations including limb ataxia, spinocerebellar ataxia, dysarthria, muscle weakness of lower limbs, loss of tone and

Received September 03, 2022. Revised December 20, 2022. Accepted January 08, 2023. Advance access publication February 2, 2023