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Common and rare variant association analyses in Amyotrophic Lateral Sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology

Wouter van Rheenen, Rick A.A. van der Spek, Mark K Bakker, Joke J.F.A. van Vugt ...+209 more authors

Institutions: Utrecht University, University of Groningen, University of Exeter, Katholieke Universiteit Leuven ...+78 more institutions

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Wouter van Rheenen (w.vanrheenen-2@umcutrecht.nl)
UMC Utrecht https://orcid.org/0000-0002-5860-1533

Rick van der Spek
Brain Center Rudolf Magnus

Mark Bakker
University Medical Center Utrecht https://orcid.org/0000-0002-7887-9014

Leonard van den Berg
UMC Utrecht

Jan Veldink
Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht
https://orcid.org/0000-0001-5572-9657

Joke van Vugt
University Medical Center Utrecht

Paul Hop
Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht

Ramona Zwamborn
Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht

Niek de Klein
University of Groningen https://orcid.org/0000-0003-4640-9904

Harm-Jan Westra
University Medical Center Groningen https://orcid.org/0000-0001-7038-567X

Olivier Bakker
University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, the Netherlands

Patrick Deelen
University Medical Center Groningen https://orcid.org/0000-0002-5654-3966

Gemma Shireby
University of Exeter Medical School, College of Medicine and Health, University of Exeter, Exeter, UK.

Eilis Hannon
University of Exeter https://orcid.org/0000-0001-6840-072X

Matthieu Moisse
KU Leuven – University of Leuven, Department of Neurosciences, Experimental Neurology, and Leuven Brain Institute (LBI), Leuven, Belgium.
Denis Baird
Translational Biology, Biogen, Boston, Massachusetts, USA.
Restuadi Restuadi
Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia.
Egor Dolzhenko
Illumina Inc
Annelot Dekker
University Medical Center Utrecht
Klara Gawor
Department of Neurology, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht Univer
https://orcid.org/0000-0001-7571-5420
Henk-Jan Westeneng
University Medical Center Utrecht Brain Center, Utrecht University
Gijs Tazelaar
Department of Neurology, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht Univer
Kristel van Eijk
Brain Center Rudolf Magnus, Department of Psychiatry, UMC Utrecht
Maarten Kooyman
https://orcid.org/0000-0002-9023-5617
Ross Byrne
Trinity College Dublin https://orcid.org/0000-0002-4082-6072
Mark Doherty
Complex Trait Genomics Laboratory, Smurfit Institute of Genetics, Trinity College Dublin, Dublin D02 PN40, Ireland.
Mark Heverin
Complex Trait Genomics Laboratory, Smurfit Institute of Genetics, Trinity College Dublin, Dublin D02 PN40, Ireland.
Ahmad Al Khleifat
Maurice Wohl Clinical Neuroscience Institute https://orcid.org/0000-0002-7406-9831
Alfredo Iacoangeli
King's College London
Aleksey Shatunov
King's College London, Maurice Wohl Clinical Neuroscience Institute
Nicola Ticozzi
IRCCS Istituto Auxologico Italiano https://orcid.org/0000-0001-5963-7426
Johnathan Cooper-Knock

University of Sheffield

Bradley Smith

Institute of Psychiatry, Kings College London

Marta Gromicho

Instituto de Fisiologia, Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal. https://orcid.org/0000-0003-2111-4579

Siddharthan Chandran

University of Edinburgh

Suvankar Pal

University of Edinburgh

Karen Morrison

School of Medicine, Dentistry, and Biomedical Sciences, Queen's University Belfast, Belfast, UK.

Pamela Shaw

University of Sheffield

John Hardy

University College London

Richard Orrell

Institute of Neurology, UCL

Michael Sendtner

University Hospital Wuerrzburg

Thomas Meyer

Charité Universitätsmedizin Berlin https://orcid.org/0000-0002-2736-7350

Nazli Basak

Bogazici University,

Anneke van der Kooi

Amsterdam Medical Center

Antonia Ratti

University of Milan

Isabella Fogh

King's College London, Institute of Psychiatry

Cinzia Gellera

Fondazione IRCCS Istituto Neurologico Carlo Besta

Guiseppe Lauria Pinter

3rd Neurology Unit, Motor Neuron Diseases Center, Fondazione IRCCS Istituto Neurologico "Carlo Besta", MIlan, Italy.

Stefania Corti

Università degli Studi di Milano

Cristina Cereda

Fondazione Istituto Neurologico Nazionale Casimiro Mondino

Daisy Sproviero
Genomic and Post-Genomic Center, IRCCS Mondino Foundation, Pavia, Italy.
Sandra D'Alfonso
University of Eastern Piedmont
Gianni Soraru
University of Padova, Italy
Gabriele Siciliano
University of Pisa
Massimiliano Filosto
Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.
Alessandro Padovani
Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.
Adriano Chio
University of Torino
Andrea Calvo
University of Torino https://orcid.org/0000-0002-5122-7243
Cristina Moglia
'Rita Levi Montalcini' Department of Neuroscience, University of Turin
Maura Brunetti
"Rita Levi Montalcini" Department of Neuroscience, ALS Centre, University of Torino, Turin, Italy.
Antonio Canosa
University of Turin
Maurizio Grassano
"Rita Levi Montalcini" Department of Neuroscience, ALS Centre, University of Torino, Turin, Italy.
Ettore Beghi
IRCCS Istituto di Ricerche Farmacologiche Mario Negri
Elisabetta Pupillo
IRCCS Istituto di Ricerche Farmacologiche Mario Negri
Giancarlo Logroscino
Neurosciences and Sense Organs of the "Aldo Moro" University of Bari
Beatrice Nefussy
Department of Neurology, Tel-Aviv Sourasky Medical Centre, Tel-Aviv, Israel.
Alma Osmanovic
Department of Neurology, Hannover Medical School, Hannover, Germany.
Angelica Nordin
Department of Clinical Sciences, Neurosciences, Umeå University, SE-901 85 Umeå, Sweden.
Yossef Lerner
Faculty of Medicine, Hebrew University of Jerusalem, Israel.
Michal Zabari

Faculty of Medicine, Hebrew University of Jerusalem, Israel.

Marc Gotkine

Hadassah Medical Center

Robert Baloh

Center for Neural Science and Medicine, Cedars-Sinai Medical Center, Los Angeles, California, 90048, USA

Shaugn Bell

Center for Neural Science and Medicine, Cedars-Sinai Medical Center, Los Angeles, California, 90048, USA

Patrick Vourc'h

Université François Rabelais

Philippe Corcia

CHU de Tours, Université François Rabelais

Philippe Couratier

Centre de compétence SLA-fédération Tours-Limoges

Stephanie Millecamps

Inserm U1127, CNRS UMR7225, Sorbonne Universités, UPMC Univ Paris 6 UMRS1127

Vincent Meininger

Hôpital des Peupliers, Ramsay Générale de Santé, 75013 Paris, France.

Francois Salachas

APHP, Département de Neurologie, Hôpital Pitié-Salpêtrière, Centre référent SLA

Jesus Mora Pardina

Hospital Universitario San Rafael

Abdelilah Assialioui

L'Hospitalet de Llobregat

Ricardo Rojas-García

Hospital de la Santa Creu i Sant Pau de Barcelona https://orcid.org/0000-0003-1411-5573

Patrick Dion

McGill University

Jay Ross

McGill University https://orcid.org/0000-0002-8183-2524

Albert Ludolph

German Center for Neurodegenerative Diseases

Jochen Weishaupt

Division of Neurodegeneration, Department of Neurology, University Medicine Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

David Brenner

Division of Neurodegeneration, Department of Neurology, University Medicine Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

Axel Freischmidt
Department of Neurology, Ulm University, Ulm, Germany.
Gilbert Bensimon
Département de Pharmacologie Clinique, Hôpital de la Pitié-Salpêtrière, UPMC Pharmacologie, AP-HP, Paris, France.
Alexis Brice
INSERM U679
Alexandra Durr
INSERM
Christine Payan
AP-HP
Safa Saker-Delye
Genethon
Nicholas Wood
University College London https://orcid.org/0000-0002-9500-3348
Simon Topp
King's College London https://orcid.org/0000-0002-5200-3284
Rosa Rademakers
Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, Florida, USA.
Lukas Tittmann
Christian-Albrechts University Kiel and Biobank popgen
Wolfgang Lieb
Institute of Epidemiology and Biobank popgen, University of Kiel
Andre Franke
Christian-Albrechts-University of Kiel https://orcid.org/0000-0003-1530-5811
Stephan Ripke
Massachusetts General Hospital
Alice Braun
Department of Psychiatry and Psychotherapy, Charité - Universitätsmedizin, Berlin 10117, Germany.
Julia Kraft
Department of Psychiatry and Psychotherapy, Charité - Universitätsmedizin, Berlin 10117, Germany.
https://orcid.org/0000-0001-7306-1179
David Whiteman
QIMR Berghofer Medical Research Institute https://orcid.org/0000-0003-2563-9559
Catherine Olsen
QIMR Berghofer Medical Research Institute https://orcid.org/0000-0003-4483-1888
André Uitterlinden
Erasmus MC https://orcid.org/0000-0002-7276-3387
Albert Hofman

Erasmus MC University Medical Centre Rotterdam

Marcella Rietschel

University of Mannheim https://orcid.org/0000-0002-5236-6149

Sven Cichon

University of Bonn

Markus Nöthen

University of Bonn

Philippe Amouyel

Institut Pasteur de Lille https://orcid.org/0000-0001-9088-234X

Bryan Traynor

National Institute on Aging

Andrew Singleton

National Institute on Aging

Miguel Mitne Neto

Fleury Group

Ruben Cauchi

Center for Molecular Medicine and Biobanking & Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Malta.

Roel Ophoff

University of California Los Angeles, University Medical Center Utrecht

Martina Wiedau-Pazos

University of California Los Angeles

Catherine Lomen-Hoerth

University of California

Vivianna Van Deerlin

University of Pennsylvania https://orcid.org/0000-0002-7400-9097

Julian Grosskreutz

University Hospital Jena

Annekathrin Rödiger

Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany.

Alexander Jörk

Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany.

Tabea Barthel

Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany.

Erik Theele

Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany.

Berjamin Ilse

Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany.

Beatrice Stubendorff

University Hospital Jena

Otto Witte

Hans Berger Department of Neurology, Jena University Hospital

Robert Steinbach

Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany.

Christian Hübner

Jena University Hospital

Caroline Graff

Karolinska Institutet

Lev Brylev

Department of Neurology, Bujanov Moscow Clinical Hospital, Moscow, Russia.

Vera Fominykh

Department of Neurology, Bujanov Moscow Clinical Hospital, Moscow, Russia.

Vera Demeshonok

ALS-care center, "GAOORDI", Medical Clinic of the St. Petersburg, St. Petersburg, Russia.

Anastasia Ataulina

Department of Neurology, Bujanov Moscow Clinical Hospital, Moscow, Russia.

Boris Rogelj

Jožef Stefan Institute https://orcid.org/0000-0003-3898-1943

Blaž Koritnik

Ljubljana ALS Centre, Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, SI-1000 Ljubljana https://orcid.org/0000-0002-5083-8261

Janez Zidar

University Clinical Center Ljubljana

Metka Ravnik-Glavač

University of Ljubljana

Damjan Glavač

Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

Zorica Stević

Clinic of Neurology, Clinical Center of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia

Vivian Drory

Department of Neurology, Tel-Aviv Sourasky Medical Centre, Tel-Aviv, Israel.

Mónica Povedano

L'Hospitalet de Llobregat

Ian Blair

Macquarie University

Matthew Kiernan

University of Syndey

Beben Benyamin

Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia.

Robert Henderson

Department of Neurology, Royal Brisbane and Women's Hospital, Brisbane, QLD 4029, Australia.

Sarah Furlong

Centre for Motor Neuron Disease Research, Macquarie University, NSW 2109, Australia.

Susan Mathers

Calvary Health Care Bethlehem, Parkdale, VIC 3195, Australia.

Pamela McCombe

Centre for Clinical Research, The University of Queensland, Brisbane, QLD 4019, Australia.

Merrilee Needham

Fiona Stanley Hospital, Perth, WA 6150, Australia.

Shyuan Ngo

The Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD 4072, Australia.

Garth Nicholson

Macquarie University

Roger Pamphlett

The University of Sydney https://orcid.org/0000-0003-3326-7273

Dominic Rowe

Centre for Motor Neuron Disease Research, Macquarie University, New South Wales 2109, Australia
https://orcid.org/0000-0003-0912-2146

Frederik Steyn

School of Biomedical Sciences, The University of Queensland, Brisbane, QLD 4072, Australia.

Kelly Williams

Macquarie University https://orcid.org/0000-0001-6319-9473

Karen Mather

Centre for Healthy Brain Ageing, Psychiatry, University of New South Wales (UNSW)
https://orcid.org/0000-0003-4143-8941

Perminder Sachdev

https://orcid.org/0000-0002-9595-3220

Anjali Henders

University of Queensland

Leanne Wallace

University of Queensland

Mamede de Carvalho

Instituto de Medicina Molecular and Institute of Physiology, Faculty of Medicine, University of Lisbon, Portugal

Susana Pinto
Instituto de Fisiologia, Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.
Susanne Petri
Otto-von-Guericke University Magdeburg
Markus Weber
Kantonspital St. Gallen
Guy Rouleau
Department of Human Genetics, McGill University, Montréal, QC, Canada; Montreal Neurological Institute and Hospital, McGill University, Montréal, QC https://orcid.org/0000-0001-8403-1418
Vincenzo Silani
IRCCS Istituto Auxologico Italiano-University of Milan Medical School https://orcid.org/0000-0002-7698-3854
Charles Curtis
King's College London
Gerome Breen
King's College London https://orcid.org/0000-0003-2053-1792
Jonathan Glass
Emory University https://orcid.org/0000-0002-3295-4971
Robert Brown
University of Massachusetts Medical School
John Landers
University of Massachusetts Medical School
Christopher Shaw
Maurice Wohl Clinical Neuroscience Institute, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London
Peter Andersen
Umeå University https://orcid.org/0000-0003-0094-5429
Ewout Groen
University Medical Center Utrecht https://orcid.org/0000-0002-2330-9444
Michael van Es
University Medical Center Utrecht https://orcid.org/0000-0002-7709-5883
Jeroen Pasterkamp
University Medical Center Utrecht https://orcid.org/0000-0003-1631-6440
Dongsheng Fan
Peking University Third Hospital
Fleur Garton
Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia.
Allan McRae

University of Queensland

George Davey Smith

University of Bristol https://orcid.org/0000-0002-1407-8314

Tom Gaunt

University of Bristol https://orcid.org/0000-0003-0924-3247

Michael Eberle

Illumina Inc. https://orcid.org/0000-0001-8965-1253

Jonathan Mill

University of Exeter https://orcid.org/0000-0003-1115-3224

Russell McLaughlin

Trinity College Dublin https://orcid.org/0000-0003-3915-2135

Orla Hardiman

Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin D02 PN40, Ireland.

Kevin Kenna

University Medical Center Utrecht

Naomi Wray

University of Queensland https://orcid.org/0000-0001-7421-3357

Ellen Tsai

Biogen https://orcid.org/0000-0002-5625-1189

Heiko Runz

Translational Genome Sciences, Biogen, 225 Binney Street, Cambridge, MA 02142, USA

Lude Franke

University Medical Center Groningen https://orcid.org/0000-0002-5159-8802

Ammar Al-Chalabi

King's College London https://orcid.org/0000-0002-4924-7712

Philip Van Damme

Universitaire Ziekenhuizen Leuven https://orcid.org/0000-0002-4010-2357

Nayana Gaur

Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany.

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Authors

Wouter van Rheenen[1,#,@], Rick A.A. van der Spek[1,#], Mark K. Bakker[1,#], Joke J.F.A. van Vugt[1], Paul J. Hop[1], Ramona A.J. Zwamborn[1], Niek de Klein[2], Harm-Jan Westra[2], Olivier B. Bakker[2], Patrick Deelen[2,3], Gemma Shireby[4], Eilis Hannon[4], Matthieu Moisse[5,6,7], Denis Baird[8,9], Restuadi Restuadi[10], Egor Dolzhenko[11], Annelot M. Dekker[1], Klara Gawor[1], Henk-Jan Westeneng[1], Gijs H.P. Tazelaar[1], Kristel R. van Eijk[1], Maarten Kooyman[1], Ross P. Byrne[12], Mark Doherty[12], Mark Heverin[13], Ahmad Al Khleifat[14], Alfredo Iacoangeli[14,15,16], Aleksey Shatunov[14], Nicola Ticozzi[17,18], Johnathan Cooper-Knock[19], Bradley N. Smith[14], Marta Gromicho[20], Siddharthan Chandran[21,22], Suvankar Pal[21,22], Karen E. Morrison[23], Pamela J. Shaw[19], John Hardy[24], Richard W. Orrell[25], Michael Sendtner[26], Thomas Meyer[27], Nazli Başak[28], Anneke J. van der Kooi[29], Antonia Ratti[17,30], Isabella Fogh[14], Cinzia Gellera[31], Giuseppe Lauria Pinter[32,33], Stefania Corti[34,18], Cristina Cereda[35], Daisy Sproviero[35], Sandra D'Alfonso[36], Gianni Sorarù[37], Gabriele Siciliano[38], Massimiliano Filosto[39], Alessandro Padovani[39], Adriano Chiò[40,41], Andrea Calvo[40,41], Cristina Moglia[40,41], Maura Brunetti[40], Antonio Canosa[40,41], Maurizio Grassano[40], Ettore Beghi[42], Elisabetta Pupillo[42], Giancarlo Logroscino[43], Beatrice Nefussy[44], Alma Osmanovic[45], Angelica Nordin[46], Yossef Lerner[47,48], Michal Zabari[47,48], Marc Gotkine[47,48], Robert H. Baloh[49,50], Shaughn Bell[49,50], Patrick Vourc'h[51,52], Philippe Corcia[53,52], Philippe Couratier[54,55], Stéphanie Millecamps[56], Vincent Meininger[57], François Salachas[58,56], Jesus S. Mora Pardina[59], Abdelilah Assialioui[60], Ricardo Rojas-García[61], Patrick Dion[62], Jay P. Ross[62,63], Albert C. Ludolph[64], Jochen H. Weishaupt[65], David Brenner[65], Axel Freischmidt[64,66], Gilbert Bensimon[67], Alexis Brice[68,69,70], Alexandra Dürr[71], Christine A.M. Payan[67], Safa Saker-Delye[72], Nicholas Wood[73], Simon Topp[14], Rosa Rademakers[74], Lukas Tittmann[75], Wolfgang Lieb[75], Andre Franke[76], Stephan Ripke[77,78,79], Alice Braun[79], Julia Kraft[79], David C. Whiteman[80], Catherine M. Olsen[80], Andre G. Uitterlinden[81,82], Albert Hofman[82], Marcella Rietschel[83], Sven Cichon[84,85,86,87], Markus M. Nöthen[84,85], Philippe Amouyel[88], SLALOM Consortium*, PARALS Consortium*, SLAGEN Consortium*, SLAP Consortium*, Bryan Traynor[89,90], Adrew B. Singleton[91], Miguel Mitne Neto[92], Ruben J. Cauchi[93], Roel A. Ophoff[94,95,96], Martina Wiedau-Pazos[97], Catherine Lomen-Hoerth[98], Vivianna M. van Deerlin[99], Julian Grosskreutz[100], Annekathrin Rödiger[100], Nayana Gaur[100], Alexander Jörk[100], Tabea Barthel[100], Erik Theele[100], Benjamin Ilse[100], Beatrice Stubendorff[100], Otto W. Witte[100], Robert Steinbach[100], Christian A. Hübner[101], Caroline Graff[102], Lev Brylev[103,104,105], Vera Fominykh[103,105], Vera Demeshonok[106], Anastasia Ataulina[103], Boris Rogelj[107,108,109], Blaž Koritnik[110], Janez Zidar[110], Metka Ravnik-Glavač[111], Damjan Glavač[112], Zorica Stević[113], Vivian Drory[44], Monica Povedano[60], Ian P. Blair[114], Matthew C. Kiernan[115], Beben Benyamin[10,116], Robert D. Henderson[117,118,119], Sarah Furlong[114], Susan Mathers[120], Pamela A. McCombe[117,121], Merrilee Needham[119,122,123], Shyuan T. Ngo[117,124,118], Garth A. Nicholson[114], Roger Pamphlett[125], Dominic B. Rowe[114], Frederik J. Steyn[126,121], Kelly L. Williams[114], Karen A. Mather[127,128], Perminder S. Sachdev[127,129], Anjali K. Henders[10], Leanne Wallace[10], Mamede de Carvalho[20], Susana Pinto[20], Susanne Petri[45], Markus Weber[130], Guy A. Rouleau[62], Vincenzo Silani[17,18], Charles Curtis[131], Gerome Breen[132,133], Jonathan Glass[134], Robert H. Brown[135], John E. Landers[135], Christopher E. Shaw[14], Peter M. Andersen[46], Ewout J.N. Groen[1], Michael A. van Es[1], R. Jeroen Pasterkamp[136], Dongsheng Fan[137], Fleur C. Garton[10], Allan F. McRae[10], George Davey Smith[9,138], Tom R. Gaunt[9,138], Michael A. Eberle[11], Jonathan Mill[4], Russell L. McLaughlin[12], Orla Hardiman[13], Kevin P. Kenna[136,1], Naomi R.

Wray[10,117], Ellen Tsai[8], Heiko Runz[8], Lude Franke[2], Ammar Al-Chalabi[14,139], Philip Van Damme[5,6,7], Leonard H. van den Berg[1,+] & Jan H. Veldink[1,+,@]

Affiliations

1: Department of Neurology, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands.

2: Department of Genetics, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands.

3: Department of Genetics, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands.

4: University of Exeter Medical School, College of Medicine and Health, University of Exeter, Exeter, UK.

5: KU Leuven – University of Leuven, Department of Neurosciences, Experimental Neurology, and Leuven Brain Institute (LBI), Leuven, Belgium.

6: VIB, Center for Brain & Disease Research, Laboratory of Neurobiology, Leuven, Belgium.

7: University Hospitals Leuven, Department of Neurology, Leuven, Belgium.

8: Translational Biology, Biogen, Boston, Massachusetts, USA.

9: MRC Integrative Epidemiology Unit (IEU), Population Health Sciences, University of Bristol, Bristol, UK.

10: Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia.

11: Illumina, San Diego, California, USA.

12: Complex Trait Genomics Laboratory, Smurfit Institute of Genetics, Trinity College Dublin, Dublin D02 PN40, Ireland.

13: Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin D02 PN40, Ireland.

14: Maurice Wohl Clinical Neuroscience Institute, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK.

15: Department of Biostatistics and Health Informatics, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.

16: National Institute for Health Research Biomedical Research Centre and Dementia Unit, South London and Maudsley NHS Foundation Trust and King's College London, London, UK.

17: Department of Neurology-Stroke Unit and Laboratory of Neuroscience, Istituto Auxologico Italiano IRCCS, Milan, Italy.

18: Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy.

19: Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, UK.

20: Instituto de Fisiologia, Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.

21: Euan MacDonald Centre for Motor Neurone Disease Research, Edinburgh, UK.

22: Centre for Neuroregeneration and Medical Research Council Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK.

23: School of Medicine, Dentistry, and Biomedical Sciences, Queen's University Belfast, Belfast, UK.

24: Department of Molecular Neuroscience, Institute of Neurology, University College London, London, UK.

25: Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, London, UK.

26: Institute of Clinical Neurobiology, University Hospital Würzburg, Würzburg, Germany.

27: Charité University Hospital, Humboldt-University, Berlin, Germany.

28: Neurodegeneration Research Laboratory, Bogazici University, Istanbul, Turkey.

29: Department of Neurology, Academic Medical Center, Amsterdam, The Netherlands.

30: Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy.

31: Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy.

32: 3rd Neurology Unit, Motor Neuron Diseases Center, Fondazione IRCCS Istituto Neurologico "Carlo Besta", MIlan, Italy.

33: 'L. Sacco' Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan, Italy.

34: Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

35: Genomic and Post-Genomic Center, IRCCS Mondino Foundation, Pavia, Italy.

36: Department of Health Sciences, University of Eastern Piedmont, Novara, Italy.

37: Department of Neurosciences, University of Padova, Padova, Italy.

38: Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.

39: Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.

40: "Rita Levi Montalcini" Department of Neuroscience, ALS Centre, University of Torino, Turin, Italy.

41: Neurologia 1, Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Turin, Italy.

42: Laboratory of Neurological Diseases, Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.

43: Department of Clinical Research in Neurology, University of Bari at "Pia Fondazione Card G. Panico" Hospital, Bari, Italy.

44: Department of Neurology, Tel-Aviv Sourasky Medical Centre, Tel-Aviv, Israel.

45: Department of Neurology, Hannover Medical School, Hannover, Germany.

46: Department of Clinical Sciences, Neurosciences, Umeå University, SE-901 85 Umeå, Sweden.

47: Faculty of Medicine, Hebrew University of Jerusalem, Israel.

48: The Agnes Ginges Center for Human Neurogenetics, Dept. of Neurology, Hadassah Medical Center, Jerusalem, Israel.

49: Center for Neural Science and Medicine, Cedars-Sinai Medical Center, Los Angeles, California, 90048, USA

50: Department of Neurology, Neuromuscular Division, Cedars-Sinai Medical Center, Los Angeles, California, 90048, USA.

51: Service de Biochimie et Biologie moléculaire, CHU de Tours, Tours, France.

52: UMR 1253, Université de Tours, Inserm, 37044 Tours, France.

53: Centre de référence sur la SLA, CHU de Tours, Tours, France.

54: Centre de référence sur la SLA, CHRU de Limoges, Limoges, France.

55: UMR 1094, Université de Limoges, Inserm, 87025 Limoges, France.

56: ICM, Institut du Cerveau, Inserm, CNRS, Sorbonne Université, Hôpital Pitié-Salpêtrière, Paris, France.

57: Hôpital des Peupliers, Ramsay Générale de Santé, 75013 Paris, France.

58: Département de Neurologie, Centre de référence SLA Ile de France, Hôpital de la Pitié Salpêtrière, AP-HP, Paris, France.

59: ALS Unit, Hospital San Rafael, Madrid, Spain.

60: Functional Unit of Amyotrophic Lateral Sclerosis (UFELA), Service of Neurology, Bellvitge University Hospital, L'Hospitalet de Llobregat, Barcelona, Spain.

61: MND Clinic, Neurology Department, Hospital de la Santa Creu i Sant Pau de Barcelona, Universitat Autonoma de Barcelona, Barcelona, Spain.

62: Montreal Neurological Institute and Hospital, McGill University, Montréal H3A 2B4, Canada.

63: Department of Human Genetics, McGill University, Montreal, QC H3A 0C7, Canada.

64: Department of Neurology, Ulm University, Ulm, Germany.

65: Division of Neurodegeneration, Department of Neurology, University Medicine Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

66: German Center for Neurodegenerative Diseases (DZNE) Ulm, Ulm, Germany.

67: Département de Pharmacologie Clinique, Hôpital de la Pitié-Salpêtrière, UPMC Pharmacologie, AP-HP, Paris, France.

68: INSERM U289, Hôpital Salpêtrière, AP-HP, Paris, France.

69: Département de Génétique, Cytogénétique et Embryologie, Hôpital Salpêtrière, AP-HP, Paris, France.

70: Fédération de Neurologie, Hôpital Salpêtrière, AP-HP, Paris, France.

71: Department of Medical Genetics, L'Institut du Cerveau et de la Moelle Épinière, Hôpital Salpêtrière, Paris, France.

72: Genethon, CNRS UMR, 8587 Evry, France.

73: Department of Neurogenetics, UCL Institute of Neurology, Queen Square, London, UK.

74: Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, Florida, USA.

75: Popgen Biobank and Institute of Epidemiology, Christian Albrechts-University Kiel, Kiel, Germany.

76: Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany.

77: Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts, USA.

78: Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

79: Department of Psychiatry and Psychotherapy, Charité - Universitätsmedizin, Berlin 10117, Germany.

80: Cancer Control Group, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia.

81: Department of Internal Medicine, Genetics Laboratory, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands.

82: Department of Epidemiology, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands.

83: Central Institute of Mental Health, Mannheim, Germany; Medical Faculty Mannheim, University of Heidelberg, Heidelberg, Germany.

84: Institute of Human Genetics, University of Bonn, Bonn, Germany.

85: Department of Genomics, Life and Brain Center, Bonn, Germany.

86: Division of Medical Genetics, University Hospital Basel and Department of Biomedicine, University of Basel, Basel, Switzerland.

87: Institute of Neuroscience and Medicine INM-1, Research Center Juelich, Juelich, Germany.

88: Univ. Lille, Inserm, Centre Hosp. Univ. Lille, Institut Pasteur de Lille, UMR1167 - RID-AGE LabEx DISTALZ - Risk factors and molecular determinants of aging-related diseases, F-59000 Lille, France.

89: Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, NIH, Porter Neuroscience Research Center, Bethesda, Maryland, USA

90: Department of Neurology, Johns Hopkins University, Baltimore, Maryland, USA.

91: Molecular Genetics Section, Laboratory of Neurogenetics, National Institute on Aging, NIH, Porter Neuroscience Research Center, Bethesda, Maryland, USA.

92: Universidade de São Paulo, São Paulo, Brazil.

93: Centre for Molecular Medicine and Biobanking & Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Malta.

94: University Medical Center Utrecht, Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, The Netherlands

95: Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, California, USA.

96: Center for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, California, USA.

97: Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, California, USA.

98: Department of Neurology, University of California, San Francisco, California, USA.

99: Center for Neurodegenerative Disease Research, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA.

100: Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany.

101: Institute of Human Genetics, Jena University Hospital, Jena, Germany.

102: Department of Geriatric Medicine, Karolinska University Hospital-Huddinge, Stockholm, Sweden.

103: Department of Neurology, Bujanov Moscow Clinical Hospital, Moscow, Russia.

104: Moscow Research and Clinical Center for Neuropsychiatry of the Healthcare Department, Moscow, Russia.

105: Department of Functional Biochemistry of the Nervous System, Institute of Higher Nervous Activity and Neurophysiology Russian Academy of Sciences, Moscow, Russia.

106: ALS-care center, "GAOORDI", Medical Clinic of the St. Petersburg, St. Petersburg, Russia.

107: Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia.

108: Biomedical Research Institute BRIS, Ljubljana, Slovenia.

109: Faculty of Chemistry and Chemical Technology, Universtiy of Ljubljana, Ljubljana, Slovenia.

110: Ljubljana ALS Centre, Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia.

111: Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

112: Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

113: Clinic of Neurology, Clinical Center of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia

114: Centre for Motor Neuron Disease Research, Faculty of Medicine, Health and Human Sciences, Macquarie University, NSW 2109, Australia.

115: Brain and Mind Centre, The University of Sydney, Sydney, New South Wales, Australia.

116: Australian Centre for Precision Health & Allied Health and Human Performance, University of South Australia, Adelaide, SA 5001 Australia.

117: Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia.

118: Centre for Clinical Research, The University of Queensland, Brisbane, Queensland, Australia.

119: Fiona Stanley Hospital, Perth, WA 6150, Australia.

120: Calvary Health Care Bethlehem, Parkdale, VIC 3195, Australia.

121: Department of Neurology, Royal Brisbane and Women's Hospital, Brisbane, QLD 4029, Australia.

122: Notre Dame University, Fremantle, WA 6160, Australia.

123: Institute for Immunology and Infectious Diseases, Murdoch University, Perth, WA 6150, Australia.

124: The Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Queensland, Australia.

125: Discipline of Pathology and Department of Neuropathology, Brain and Mind Centre, The University of Sydney, Sydney, NSW 2050, Australia.

126: The School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, Brisbane, QLD 4074, Australia.

127: Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Sydney, NSW 2031, Australia.

128: Neuroscience Research Australia Institute, Randwick, NSW 2031, Australia.

129: Neuropsychiatric Institute, The Prince of Wales Hospital, UNSW, Randwick, NSW 2031, Australia.

130: Neuromuscular Diseases Unit/ALS Clinic, Kantonsspital St. Gallen, 9007, St. Gallen, Switzerland.

131: MRC Social, Genetic and Developmental Psychiatry Centre, King's College London, London, UK.

132: IoPPN Genomics & Biomarker Core, Translational Genetics Group, MRC Social, Genetic and Developmental Psychiatry Centre, King's College London, London, UK.

133: NIHR Biomedical Research Centre for Mental Health, Maudsley Hospital and Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK.

134: Department Neurology, Emory University School of Medicine, Atlanta, Georgia, USA.

135: Department of Neurology, University of Massachusetts Medical School, Worcester, Massachusetts, USA.

136: Department of Translational Neuroscience, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands.

137: Department of Neurology, Peking University, Third Hospital, No. 49, North Garden Road, Haidian District, Beijing, 100191, China.

138: Population Health Science, Bristol Medical School, Bristol, Bristol BS8 1TH, UK.

139: King's College Hospital, Denmark Hill, SE5 9RS London, UK.

[#] Shared first authors

[+] Shared last authors

[@] Corresponding authors

* A list of authors and their affiliations appears at the end of the paper.

Correspondence

Wouter van Rheenen: w.vanrheenen-2@umcutrecht.nl

Jan H. Veldink: j.h.veldink@umcutrecht.nl

Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with a life-time risk of 1 in 350 people and an unmet need for disease-modifying therapies. We conducted a cross-ancestry GWAS in ALS including 29,612 ALS patients and 122,656 controls which identified 15 risk loci in ALS. When combined with 8,953 whole-genome sequenced individuals (6,538 ALS patients, 2,415 controls) and the largest cortex-derived eQTL dataset (MetaBrain), analyses revealed locus-specific genetic architectures in which we prioritized genes either through rare variants, repeat expansions or regulatory effects. ALS associated risk loci were shared with multiple traits within the neurodegenerative spectrum, but with distinct enrichment patterns across brain regions and cell-types. Of the environmental and life-style risk factors obtained from literature, Mendelian randomization analyses indicated a causal role for high cholesterol levels. All ALS associated signals combined reveal a role for perturbations in vesicle mediated transport and autophagy, and provide evidence for cell-autonomous disease initiation in glutamatergic neurons.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting 1 in 350 individuals. Due to degeneration of both upper and lower motor neurons patients suffer from progressive paralysis, ultimately leading to respiratory failure within three to five years after disease onset[1]. In ~10% of ALS patients there is a clear family history for ALS suggesting a strong genetic predisposition and currently in more than half of these cases a pathogenic mutation can be found[2]. On the other hand, apparently sporadic ALS is considered a complex trait where heritability is estimated at 40-50%.[3,4] To date, partially overlapping GWASs have identified up to six genome-wide significant loci, explaining a small proportion of the genetic susceptibility to ALS[5–10]. Some of these loci found in GWAS harbor rare variants with large effects also present in familial cases (e.g. *C9orf72* and *TBK1*) [11–13]. For other loci, the role of rare variants remains unknown.

While ALS is referred to as a motor neuron disease, cognitive and behavioral changes are observed in up to 50% of the patients, sometimes leading to frontotemporal dementia (FTD). The overlap with FTD is clearly illustrated by the pathogenic hexanucleotide repeat expansion in *C9orf72* which causes familial ALS and/or FTD[11,12] and the genome-wide genetic correlation between ALS and FTD[14]. Further expanding the ALS/FTD spectrum, a genetic correlation with progressive supranuclear palsy has been described[15]. Shared pathogenic mechanisms between ALS and other neurodegenerative diseases, including common diseases such as Alzheimer's and Parkinson's disease, can further reveal ALS pathophysiology and inform new therapeutic strategies.

Here, we combine new and existing individual level-genotype data in the largest GWAS of ALS to date. We present a comprehensive screen for pathogenic rare variants and short tandem repeat (STR) expansions as well as regulatory effects observed in brain cortex-derived RNA-seq and methylation datasets to prioritize causal genes within ALS risk loci. Furthermore, we reveal similarities and

differences between ALS and other neurodegenerative diseases as well as the biological processes in disease-relevant tissues and cell-types that affect ALS risk.

Results

Cross-ancestry meta-analysis reveals 15 risk loci for ALS. To generate the largest genome-wide association study in ALS to date, we merged individual level genotype data from 117 cohorts into 6 strata matched by genotyping platform. A total of 27,205 ALS patients and 110,881 control subjects of European ancestries passed quality control (Online Methods, Supplementary Table 1-2). Through meta-analysis of these six strata, we obtained association statistics for 10,461,755 variants down to a minor allele-frequency (MAF) of 0.1% in the Haplotype Reference Consortium resource[16]. We observed moderate inflation of the test statistics (λ_{GC} = 1.12, λ_{1000} = 1.003) and linkage-disequilibrium score regression yielded an intercept of 1.029 (SE = 0.0073), indicating that the majority of inflation is due to the polygenic signal in ALS. The European ancestries analysis identified 12 loci reaching genome-wide significance ($P < 5.0 \times 10^{-8}$, Supplementary Figure 1). Of these, 8 were present in GWAS of ALS in Asian ancestries[8,10] and all showed a consistent direction of effects ($P_{binom} = 3.9 \times 10^{-3}$). The genetic overlap between ALS risk in European and Asian ancestries resulted in a trans-ancestry genetic correlation of 0.57 (SE = 0.28) for genetic effect and 0.58 (SE = 0.30) for genetic impact, which were not statistically significant different from unity (P = 0.13 and 0.16, respectively). We therefore performed a cross-ancestry meta-analysis which revealed three additional loci, totaling 15 genome-wide significant risk loci for ALS risk (Figure 1, Table 1, Supplementary Figures 2-16, Supplementary Tables 4-18). Conditional and joint analysis did not identify secondary signals within these loci.

Of these findings, 8 loci have been reported in previous genome-wide association studies (*C9orf72, UNC13A, SCFD1, MOBP/RPSA, KIF5A, CFAP410, GPX3/TNIP1,* and *TBK1*)[7–9]. The rs80265967 variant corresponds to the p.D90A mutation in *SOD1* previously identified in a Finnish ALS cohort enriched for familial ALS[6]. Interestingly, we observed for the first time, a genome-wide significant common variant

association signal within the *NEK1* locus, where *NEK1* was previously shown to harbor rare variants associated with ALS [17]. The recently reported association at the *ACSL5-ZDHHC6* locus[10,18] did not reach the threshold for genome-wide significance (rs58854276, $P_{EUR} = 5.4 \times 10^{-5}$, $P_{ASN} = 4.9 \times 10^{-7}$, $P_{comb} = 6.5 \times 10^{-8}$, Supplementary Figure 17, Supplementary Table 19), despite that our analysis includes all data from the original discovery studies.

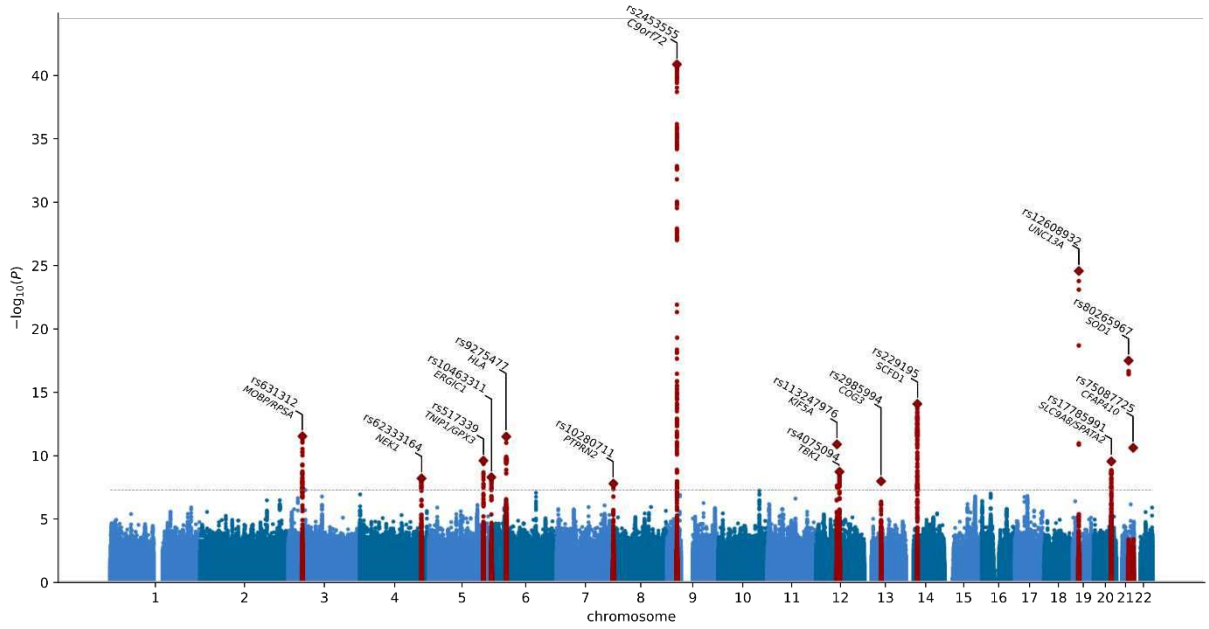

Figure 1. Manhattan plot of cross-ancestry meta-analysis. Horizontal dotted line reflects threshold for calling SNPs genome-wide significant ($P = 5 \times 10^{-8}$). Gene labels reflect those prioritized by gene prioritization analysis.

							European ancestries		Asian ancestries		Cross-ancestry	
Chr	**Basepair**	**ID**	**Prioritized gene**	**A1**	**A2**	**Freq**	**Effect (SE)**	**P**	**Effect (SE)**	**P**	**Effect (SE)**	**P**
9	27563868	rs2453555	*C9orf72*	A	G	0.248	0.174 (0.013)	1.0×10^{-43}	0.017 (0.066)	0.80	0.168 (0.012)	1.5×10^{-41}
19	17752689	rs12608932	*UNC13A*	C	A	0.347	0.125 (0.012)	8.8×10^{-25}	0.074 (0.038)	0.053	0.120 (0.012)	3.0×10^{-25}
21	33039603	rs80265967	*SOD1*	C	A	0.006	1.078 (0.124)	3.5×10^{-18}	-	-	1.078 (0.124)	3.5×10^{-18}
14	31045596	rs229195	*SCFD1*	A	G	0.337	0.091 (0.012)	9.2×10^{-15}	-	-	0.091 (0.012)	9.2×10^{-15}
3	39508968	rs631312	*MOBP/RPSA*	G	A	0.291	0.079 (0.012)	5.2×10^{-11}	0.084 (0.036)	0.020	0.080 (0.011)	3.3×10^{-12}
6	32672641	rs9275477	*HLA*	C	A	0.096	-0.143 (0.021)	5.5×10^{-12}	-0.110 (0.111)	0.32	-0.142 (0.02)	3.5×10^{-12}
12	57975700	rs113247976	*KIF5A*	T	A	0.016	0.332 (0.049)	1.4×10^{-11}	-	-	0.332 (0.049)	1.4×10^{-11}
21	45753117	rs75087725	*CFAP410*	A	C	0.012	0.418 (0.063)	2.7×10^{-11}	-	-	0.418 (0.063)	2.7×10^{-11}
5	150410835	rs10463311	*GPX3/TNIP1*	C	T	0.253	0.079 (0.013)	3.5×10^{-10}	0.042 (0.036)	0.24	0.075 (0.012)	2.7×10^{-10}
20	48438761	rs17785991	*SLC9A8/SPATA2*	A	T	0.353	0.074 (0.012)	3.5×10^{-10}	0.045 (0.076)	0.55	0.073 (0.012)	3.2×10^{-10}
12	64877053	rs4075094	*TBK1*	A	T	0.112	-0.098 (0.018)	1.7×10^{-8}	-0.216 (0.090)	0.017	-0.103 (0.017)	2.1×10^{-9}
5	172354731	rs517339	*ERGIC1*	C	T	0.397	-0.065 (0.011)	8.5×10^{-9}	-0.067 (0.074)	0.37	-0.065 (0.011)	5.6×10^{-9}
4	170583157	rs62333164	*NEK1*	G	A	0.335	0.063 (0.012)	7.0×10^{-8}	0.203 (0.070)	3.8×10^{-3}	0.067 (0.012)	6.9×10^{-9}
13	46113984	rs2985994	*COG3*	C	T	0.259	0.066 (0.013)	1.9×10^{-7}	0.100 (0.041)	0.014	0.069 (0.012)	1.2×10^{-8}
7	157481780	rs10280711	*PTPRN2*	G	C	0.124	0.076 (0.017)	5.8×10^{-6}	0.132 (0.037)	2.9×10^{-4}	0.086 (0.015)	1.8×10^{-8}

Table 1. Genome-wide significant loci. Details of the top associated SNPs within each genome-wide significant locus. Chr = chromosome, Basepair = position in reference genome GRCh37, A1 = effect allele, A2 = non-effect allele, Freq = frequency of the effect allele in European ancestries GWAS, SE = standard error of effect estimate.

Rare variant association analyses in ALS. To assess a general pattern of underlying architectures that link associated SNPs to causal genes, we first tested for annotation specific enrichment using stratified linkage disequilibrium score regression (LDSC). This revealed that 5' UTR regions as well as coding regions in the genome and those annotated as conserved were most enriched for ALS-associated SNPs (Supplementary figure 18). Subsequently we investigated how rare, coding variants contributed to ALS risk generating a whole-genome sequencing dataset of ALS patients (N = 6,538) and controls (N = 2,415). The exome-wide association analysis included transcript-level rare-variant burden testing for different models of allele-frequency thresholds and variant annotations (Online methods). This identified *NEK1* as the strongest associated gene (minimal $P = 4.9 \times 10^{-8}$ for disruptive and damaging variants at minor allele frequency < 0.005), which was the only gene to pass the exome-wide significance thresholds ($0.05/17{,}994 = 2.8 \times 10^{-6}$ and $0.05/58{,}058 = 8.6 \times 10^{-7}$ for number of genes and protein-coding transcripts, respectively, Supplementary figures 19-32). This association is independent from the previously reported increased rare variant burden in familial ALS patients[17] that were not included in this study.

Gene prioritization shows locus-specific underlying architectures. To assess whether rare variant associations could drive the common variant signals at the 15 genome-wide significant loci, we combined the common and rare variants analyses to prioritize genes within these loci. The SNP effects on gene expression were assessed through summary-based Mendelian Randomization (SMR) in blood (eQTLGen[19]) and a new brain cortex-derived expression quantitative trait locus (eQTL) dataset (MetaBrain[20]). Similarly, we analyzed methylation-QTL (mQTL) through SMR in blood and brain-derived mQTL datasets[21–23]. Finally, we leveraged the genome-wide signature of ALS associated gene features in a new gene prioritization method to calculate a polygenic priority score (PoPS)[24]. Through these multi-layered gene prioritization strategies we classified each locus into one of four classes of most likely underlying genetic architecture to prioritize the causal gene (Supplementary figures 33-47).

First, in three GWAS loci the strongest associated SNP was a low-frequency coding variant which was nominated as the causal variant. This is the case for rs80265967 (*SOD1*, p.D90A, Supplementary Figure 46) and rs113247976 (*KIF5A* p.P986L, Supplementary Figure 40) which are coding variants in known ALS risk genes. This is also the most likely causal mechanism for rs75087725 (*CFAP410*, formerly *C21orf2*, p.V58L, Supplementary Figure 46) as the GWAS variant is a missense variant, no evidence for other mechanisms including repeat expansions, eQTL or mQTL effects is observed within this locus, and *CFAP410* itself is known to directly interact with *NEK1*, another ALS gene[13,25]. These three loci illustrate the power of large-scale GWAS combined with modern imputation panels to directly identify low-frequency causal variants that confer disease risk.

Second, SNPs can tag a highly pathogenic repeat expansion, as is seen for rs2453555 (*C9orf72*) and the known GGGGCC hexanucleotide repeat in this locus. Conditional analysis revealed no residual signal after conditioning on the repeat expansion which is in LD with the top-SNP (r^2 = 0.14, |D'| = 0.99, MAF_{SNP} = 0.25, MAF_{STR} = 0.047). Besides the repeat expansion, both eQTL and mQTL analyses point to *C9orf72* (Supplementary Figure 39). The HEIDI outlier test, however, rejected the null hypothesis that gene expression or methylation mediated the causal effect of the associated SNP ($P_{HEIDI\_eQTL} = 3.7 \times 10^{-}$

[23] and $P_{HEIDI\_mQTL} = 4.1 \times 10^{-7}$). This is in line with the pathogenic repeat expansion as the causal variant in this locus as and that eQTL and mQTL effects do not mediate a causal effects. We found no similar pathogenic repeat expansions that fully explain the SNP association signal in the other genome-wide significant loci.

Third, in two loci (rs62333164 in *NEK1* and rs4075094 in *TBK1*) common and rare variants converge to the same gene, which are known ALS risk genes[13,17]. For both loci, the rare variant burden association is conditionally independent from the top SNP which was included in the GWAS (Supplementary figures 34 and 41). Here, the eQTL and mQTL analyses indicated that the risk-increasing effects of the common variants are mediated through both eQTL and mQTL effects on *NEK1* and *TBK1*. Furthermore, a polymorphic STR downstream of *NEK1* was associated with increased ALS risk (motif = TTTA, threshold = 10 repeat units, expanded allele-frequency = 0.51, $P = 5.2 \times 10^{-5}$, FDR = 4.7×10^{-4}, Supplementary figure 48). This polymorphic repeat is in LD with the top associated SNP within this locus ($r^2 = 0.24$, $|D'| = 0.70$). Within the whole-genome sequencing data, there was no statistically significant association for the top SNP to reliably determine its independent contribution to ALS risk.

Lastly, the fourth group contains remaining loci where there is no direct link to a causal gene through coding variants or repeat expansions. Here, we investigated regulatory effects of the associated SNPs on target genes acting as either eQTL or mQTL. Single genes were prioritized by SMR using both mQTL and eQTL for rs2985994 (*COG3* Supplementary Figure 42), rs229243 (*SCFD1,* Supplementary Figure 43), and rs517339 (*ERGIC1*, Supplementary Figure 36). In other loci, both methods prioritized multiple genes, such as rs631312 (*MOBP* and *RPSA,* Supplementary Figure 33) and rs10463311 (*GPX3* and *TNIP1*, Supplementary Figure 35). Besides the prioritized genes, each of these loci harbor multiple genes that are not prioritized by any method and are therefore less likely to contribute to ALS risk.

Locus-specific sharing of risk loci between ALS and neurodegenerative diseases. To investigate the pleiotropic properties of ALS-associated loci and shared genetic basis of neurodegeneration, we tested for shared effects among neurodegenerative diseases. We included GWAS from clinically-diagnosed

Alzheimer's disease (AD)[26], Parkinson's disease (PD)[27], frontotemporal dementia (FTD)[28], progressive supranuclear palsy (PSP)[15] and corticobasal degeneration (CBD)[29] to estimate genetic correlations. Bivariate LDSC confirmed a statistically significant genetic correlation between ALS and PSP (r_g = 0.44, SE = 0.11, P = 1.0 × 10^{-4}) as previously reported, and also found a significant genetic correlation between ALS and AD (r_g = 0.31, SE = 0.12, P = 9.6 × 10^{-3}) as well as between ALS and PD (r_g = 0.16, SE = 0.061, P = 0.011, Figure 2a). The point estimate for the genetic correlation between ALS and FTD was high (r_g = 0.59, SE = 0.41, P = 0.15), but not statistically significant due to the limited size of the FTD GWAS. Thus, power to detect a genetic correlation between ALS and FTD using LDSC was limited (Supplementary Figure 49).

Patterns of sharing disease-associated genetic variants appeared to be locus specific (Figure 2b, Supplementary Table 20). To assess whether two traits shared a common signal indicating shared causal variants, we performed colocalization analyses for all loci meeting P < 5 × 10^{-5} in any of the GWAS on neurodegenerative diseases (N = 161 loci). This revealed a shared signal in the *MOBP/RPSA* between ALS, PSP and CBD, as well as a shared signal in the *UNC13A* locus between ALS and FTD (posterior probability: PP_{H4} > 95%, Supplementary Figure 50). For the *HLA* locus, there was evidence for a shared causal variant between ALS and PD (PP_{H4} = 88%) but no conclusive evidence for ALS and AD (PP_{H4} = 51% for a shared causal variant and PP_{H3} = 49% for independent signals in both traits).

Furthermore, the colocalization analyses identified two additional shared loci that were not genome-wide significant in the ALS GWAS: between ALS and PD at the *GAK* locus (rs34311866, PP_{H4} = 99%) and between ALS and AD at the *BRZAP-AS1* locus (rs2632516, PP_{H4} = 90%). Of note, the association at *BZRAP-AS1* was not genome-wide significant in the GWAS of clinically diagnosed AD (P = 3.7 × 10^{-7}) either, but was identified in the larger AD-by-proxy GWAS[30]. For FTD subtypes, *C9orf72* showed a co-localization signal for a shared causal variant between ALS and the motor neuron disease subtype of FTD (mndFTD, PP_{H4} = 93%, Supplementary figure 50 and 51).

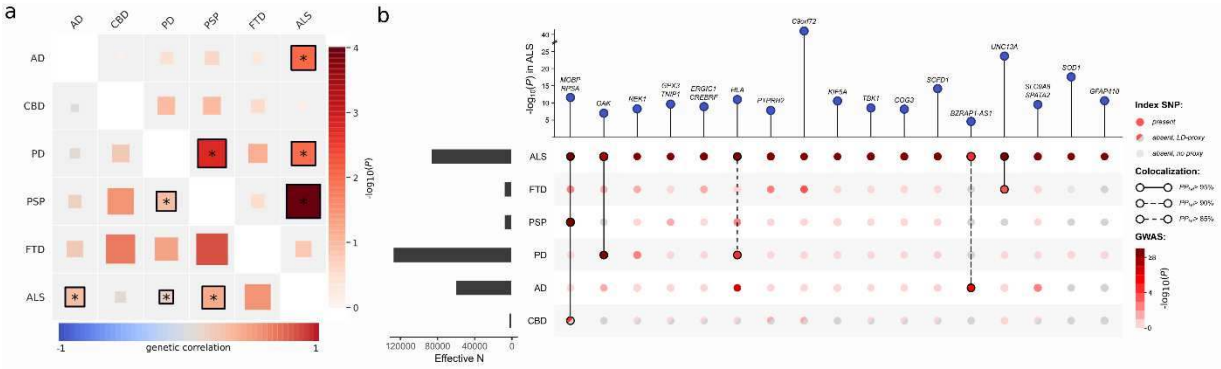

Figure 2. Shared genetic risk among ALS and neurodegenerative diseases. (a) Genetic correlation analysis. Genetic correlation was estimated with LD-score regression between each pair of neurodegenerative diseases being ALS, Alzheimer's disease (AD), corticobasal degeneration (CBD), Parkinson's disease (PD), progressive supranuclear palsy (PSP), and frontotemporal dementia (FTD). Lower left triangle shows correlation estimate and upper right triangle shows -$\log_{10}$(P-value). Correlations marked with an asterisk were statistically significant P < 0.05. **(b)** SNP associations of ALS lead SNPs or LD-proxies in neurodegenerative diseases. Effective sample size is shown on the left. Posterior probabilities of the same causal SNP affecting two diseases were estimated through colocalization analysis and highlighted as connections.

Enrichment of glutamatergic neurons indicate cell-autonomous processes in ALS susceptibility. To find tissues and cell-types which gene expression profiles are enriched for genes within ALS risk loci, we first combined gene-based association statistics calculated using MAGMA[31] with gene expression patterns from GTEx (v8) in a gene-set enrichment analysis using FUMA[32]. We observed a significant enrichment in genes expressed in brain tissues, specifically the cerebellum, basal ganglia (caudate nucleus, accumbens, and putamen), and cortex, but not peripheral nervous tissue or muscle. Whereas this pattern roughly resembles the enrichments observed in PD, it is strikingly different from that observed in AD where blood, lung and spleen were mostly enriched (Figure 3a). We subsequently queried single-cell RNA sequencing datasets of human-derived brain samples to further specify brain-specific enriched cell-types using the cell-type analysis module in FUMA[33]. This showed significant enrichment for neurons but not microglia or astrocytes (Figure 3b). Further subtyping of these neurons illustrated that genes expressed in glutamatergic neurons were mostly enriched for genes within the ALS-associated risk loci. Again, this contrasted AD which showed specific enrichment of microglia. In single-cell RNA sequencing data obtained from brain tissues in mice, a similar pattern was observed showing neuron-specific enrichment in ALS and PD, but microglia in AD (Supplementary Figure 52).

Together, this indicates that susceptibility to neurodegeneration in ALS is mainly driven by neuron-specific pathology and not by immune-related tissues and microglia.

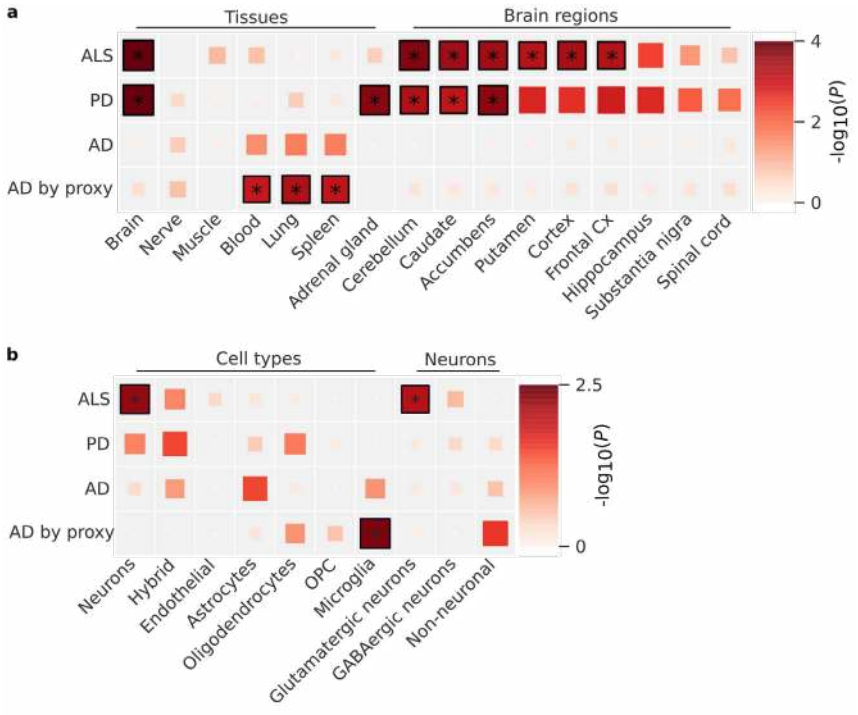

Figure 3. Tissue and cell-type enrichment analysis. (a) Enrichment of tissues and brain regions included in the GTEx v8 illustrates a brain-specific enrichment pattern in ALS, similar to Parkinson's disease but contrasting Alzheimer's disease. **(b)** Cell-type enrichment analyses indicate neuron-specific enrichment for glutamatergic neurons. No enrichment was found for microglia or other non-neuronal cell-types, contrasting the pattern observed in Alzheimer's disease. Statistically significant enrichments after correction for multiple testing with a false discovery rate (FDR) < 0.05 are marked with an asterisk. ALS = amyotrophic lateral sclerosis, PD = Parkinson's disease, AD = Alzheimer's disease, Cx = cortex, OPC = oligodendrocyte progenitor cells.

Brain-specific co-expression networks improve detection of ALS-relevant pathways. To assess which processes were mostly enriched in ALS, we performed enrichment analyses that combined gene-based association statistics with gene co-expression patterns obtained from either multi-tissue transcriptome datasets[34] or RNA-seq data from brain cortex samples (MetaBrain[20]). To validate this approach, we first tested for enrichment of Human Phenotype Ontology (HPO) terms that are linked to well-established disease genes in the Online Mendelian Inheritance in Man (OMIM) and Orphanet catalogues. Using the multi-tissue co-expression matrix, we found no enriched HPO terms after Bonferroni correction for multiple testing. Using the brain-specific co-expression matrix however, we found a strong enrichment of HPO terms that are related to ALS or neurodegenerative diseases in

general, including *Cerebral cortical atrophy* ($P = 1.8 \times 10^{-8}$), *Abnormal nervous system electrophysiology* ($P = 4.1 \times 10^{-7}$) and *Distal amyotrophy* ($P = 8.6 \times 10^{-7}$, full-list in Supplementary table 21). In general, HPO terms in the neurological branch (*Abnormality of the nervous system*) showed an increase in enrichment statistics in ALS when using the brain-specific co-expression matrix compared to the multi-tissue dataset (Supplementary Figure 53), which illustrates the benefit of the brain-specific co-expression matrix for ALS-specific enrichment analyses. Subsequently, we tested for enriched biological processes using Reactome and Gene Ontology terms. Again, using the multi-tissue expression profiles, we found no Reactome annotations to be enriched. Leveraging the brain-specific co-expression networks we identified Vesicle Mediated Transport ("*Membrane Trafficking*" $P = 4.2 \times 10^{-6}$, "*Intra-golgi and retrograde Golgi-to-ER trafficking*" $P = 1.4 \times 10^{-5}$) and Autophagy ("*Macroautophagy*" $P = 3.2 \times 10^{-5}$) as enriched processes after Bonferroni correction for multiple testing (Supplementary Table 22). The subsequently identified enriched Gene Ontology terms all related to vesicle mediated transport or autophagy (Supplementary Table 23 and 24).

Cholesterol levels are causally related to ALS. From previous observational case-control studies and our accompanying blood-based methylome-wide study[35], numerous non-genetic risk factors have been implicated in ALS. Here we studied a selection of those putative risk factors through causal inference in a Mendelian randomization (MR) framework[36]. We selected 22 risk factors for which robust genetic predictors were available including BMI, smoking, alcohol consumption, physical activity, cholesterol-related traits, cardiovascular diseases and inflammatory markers (Supplementary Table 25). These analyses provided the strongest evidence for cholesterol levels to be causally related to ALS risk ($P_{WeightedMedian} = 3.2 \times 10^{-4}$, Figure 4a, full results in Supplementary Table 26). These results were robust to removal of outliers through Radial MR analysis[37] and we observed no evidence for reverse causality (Supplementary Table 27 and 28). Importantly, ascertainment bias can lead to the selection of higher educated control subjects[38], compared to ALS patients that are mostly ascertained through the clinic. In line with control subjects being higher educated, MR analyses indicate a negative effect for years of schooling on ALS risk ($P_{IVW} = 2.0 \times 10^{-4}$, Figure 4b). As a result, years of schooling can

act as a confounder for the observed risk increasing effect of higher total cholesterol through ascertainment bias. To correct for this potential confounding, we applied multivariate MR analyses including both years of schooling and total cholesterol. The results for total cholesterol were robust in the multivariate analyses, suggesting a causal role for total cholesterol levels on ALS susceptibility (Supplementary Table 29).

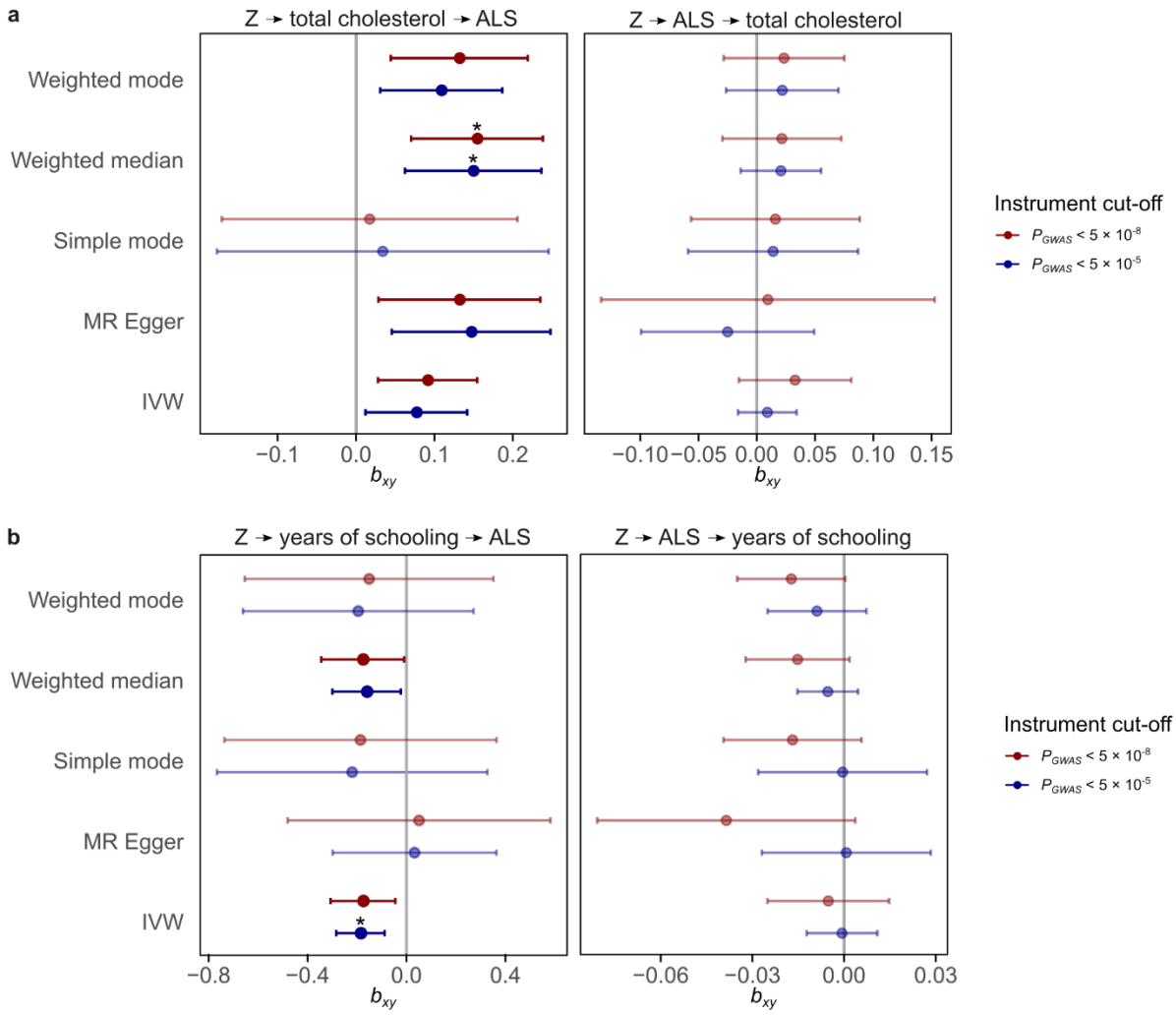

Figure 4. Causal inference of total cholesterol and years of schooling in ALS. **(a)** Mendelian randomization results for ALS and total cholesterol. Results for the five different Mendelian Randomization methods for two different P-value cut-offs for SNP instrument selection. All methods show a consistent positive effect for an increased risk of ALS with higher total cholesterol levels. There is no evidence for reverse causality. **(b)** Mendelian randomization results for ALS and years of schooling. Error-bars reflex 95% confidence intervals. Statistically significant effects that pass Bonferroni correction for multiple testing for all tested traits and MR methods are marked with an asterisk. Z = genetic instrument, MR = Mendelian Randomization, IVW = inverse-variance weighted, b_{xy} = estimated causal effect for one standard deviation increase in genetically predicted exposure.

Discussion

In summary, in the largest GWAS on ALS to date including 29,612 ALS patients and 122,656 control subjects, we have identified 15 risk loci contributing to ALS risk. Through in-depth analysis of these loci incorporating rare-variant burden analyses and repeat expansion screens in whole-genome sequencing data, blood and brain-specific eQTL and mQTL analysis we have prioritized genes in 14 of the loci. Across the spectrum of neurodegenerative diseases we identified a genetic correlation between ALS and AD, PD and PSP with locus-specific patterns of shared genetic risk across all neurodegenerative diseases. Colocalization analysis identified two additional loci, *GAK* and *BZRAP1-AS1*, with a high posterior probability of shared causal variants between ALS/PD, and ALS/AD respectively. We found glutamatergic neurons as the most enriched cell type in the brain and brain-specific co-expression network enrichment analyses indicated a role for vesicle-mediated transport and autophagy in ALS. Finally, causal inference of previously described risk factors provides evidence for high total cholesterol levels as a causal risk factor for ALS.

The cross-ancestry comparison illustrated similarities in the genetic risk factors for ALS in European and East Asian ancestries, providing an argument for cross-ancestry studies and to further expand ALS GWAS in non-European populations. Important to note is that 3 loci including those that harbor low-frequency variants (*KIF5A*, *SOD1,* and *CFAP410*) were not included in the East Asian GWAS due to their low minor allele frequency. Therefore, the shared genetic risk might not extend to rare genetic variation, for which population-specific frequencies have been observed even within Europe.

The multi-layered gene prioritization analyses highlighted four different classes of genome-wide significant loci in ALS. First, the sample size of this GWAS combined with accurate imputation of low-frequency variants directly identified rare coding variants that increase ALS risk. These include the known p.D90A mutation in *SOD1* (MAF = 0.006) as well as rare variants in *KIF5A* (MAF = 0.016) and *CFAP410* (MAF = 0.012) for which, after their identification through GWAS, experimental work

confirms their direct role in ALS pathophysiology[9,25,39]. Second, we confirmed that the pathogenic *C9orf72* repeat expansion is tagged by genome-wide significant GWAS SNPs, and that no residual signal is left by conditioning the SNP on the repeat expansion. Although more repeat expansions are known to affect ALS risk, we found no similar loci where the SNPs tag a highly pathogenic repeat expansion. This suggests that highly pathogenic repeat expansions on a stable haplotype are merely the exception rather than the rule in ALS. Third, common and rare variant association signals can converge on the same gene as is observed for *NEK1* and *TKB1*, consistent with observations for other traits and diseases[40–42]. We show that these signals are conditionally independent and that the common variants act on the same gene through regulatory effects as eQTL or mQTL. In the fourth class, we find evidence for regulatory effects of ALS associated SNPs that act as eQTL or mQTL. These locus-specific architectures illustrate the complexity of ALS associated GWAS loci where not one solution fits all, but instead warrants a multi-layered approach to prioritize genes.

In addition, we find locus-specific patterns of shared effects across neurodegenerative diseases. The *MOBP* locus has previously been identified in PSP and ALS and here we show that indeed both diseases, as well as CBD, are likely to share the same causal variant in this locus. The same is true for *UNC13A* and *C9orf72* with FTD and the motor neuron disease subtype of FTD, respectively. The colocalization analysis with PD identified a shared causal variant in the *GAK* locus, which was not found in the ALS GWAS alone. Furthermore the *BZRAP1-AS1* locus harbors SNPs associated with ALS and AD risk. Although this locus was not significant in either of the GWAS, larger GWAS including AD-by-proxy cases confirmed this as a risk locus for AD. This illustrates the power of cross-disorder analyses to leverage the shared genetic risk of neurodegenerative diseases.

We aimed to clarify the role of neuron-specific pathology in ALS susceptibility as opposed to non-cell autonomous pathology through detailed cell-type enrichment analyses. Previous experiments have illustrated multiple lines of evidence for non-cell autonomous pathology in microglia, astrocytes and oligodendrocytes which ultimately leads to neurodegeneration in ALS[43–45]. These experiments have

shown that non-cell autonomous processes, such as neuro-inflammation, mainly act as modifiers of disease in *SOD1* models of ALS[44,45]. Here, we show that genes within loci associated with ALS susceptibility are specifically expressed in (glutamatergic) neurons. This provides evidence for neuron-specific pathology as a driver of ALS susceptibility, which is in stark contrast to the signal of inflammation associated tissues and cell-types in Alzheimer's disease[30]. It also shows that disease susceptibility and disease modification can be distinct processes, while both can be targets for potential new treatments in ALS.

The subsequent functional enrichment analyses identified membrane trafficking, Golgi to Endoplasmatic Reticulum (ER) trafficking and autophagy to be enriched for genes within ALS associated loci. These terms and their related Gene Ontology (GO) terms of biological processes are all related to autophagy and degradation of (misfolded) proteins. This corroborates the central hypothesis of impaired protein degradation leading to aberrant protein aggregation in neurons which is the pathological hallmark of ALS. Our results suggest that this is a central mechanism in ALS even in the absence of rare known mutations in genes directly involved in these biological processes such as *TARDBP, FUS, UBQLN2* and *OPTN*[46].

Based on observational studies and MR analyses, conflicting evidence exists for lipid levels including cholesterol as a risk factor for ALS[47–49]. Potential selection bias, reverse causality and the subtype of cholesterol studied challenge the interpretation of these results. Here, we provided support for a causal relationship between high total cholesterol levels and ALS independent of educational attainment and ruling out reverse orientation of the MR effect. The total cholesterol effects were consistent across the different MR methods tested, indicating that this finding is robust to violation of the no horizontal pleiotropy assumption. This is in line with our accompanying study showing methylation changes associated with increased cholesterol levels in ALS[35]. We do not find a clear pattern for either LDL or HDL cholesterol subtypes in relation to ALS risk. Whereas cholesterol levels are closely related to cardiovascular risk, the association between cardiovascular risk and ALS risk

547 remains controversial with conflicting reports.[3,47,50]. Interestingly, recent work has shown that lipid
548 metabolism and autophagy are closely related which brings results of our pathway analyses and
549 Mendelian randomization together[51]. Both *in vitro* and *in vivo* experiments have shown that autophagy
550 regulates lipid homeostasis through lipolysis and that impaired autophagy increases triglyceride and
551 cholesterol levels. Conversely, high lipid levels were shown to impair autophagy[51]. Further studies on
552 the effect of high cholesterol levels and protein degradation through autophagy illustrate that high
553 cholesterol levels decrease fusogenic ability of autophagic vesicles through decreased SNARE
554 function[52,53] and lead to increased protein aggregation due to impaired autophagy in mouse models
555 for Alzheimer's disease[54]. Therefore, the risk increasing effect of cholesterol on ALS might be mediated
556 through impaired autophagy.

557 In conclusion, our genome-wide association study identifies 15 risk loci in ALS, and illustrates locus-
558 specific interplay between common and rare genetic variation that helps prioritize genes for future
559 follow-up studies. We show a causal role for cholesterol which can be linked to impaired autophagy as
560 common denominators of neuron-specific pathology that drive ALS susceptibility and serve as
561 potential targets for therapeutic strategies.

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Methods

GWAS

Data description

We obtained individual genotype level data for all individuals in the previously published GWAS in ALS in European ancestries[7,9] and publicly available control datasets including 120,971 controls genotyped on Illumina platforms. Additionally 6,374 cases and 22,526 controls were genotyped on the IlluminaOmniExpress and Illumina GSA array. Details for each cohort are provided in Supplementary Table 1. For ALS cases, both cases with and without a family-history for ALS and/or dementia were included. Cases were not pre-screened for specific ALS related mutations. Given the late onset and relatively low life-time risk of ALS, controls were not screened for (subclinical) signs of ALS. A detailed description of the newly genotyped cases and controls is provided in the Supplementary Information. All participants gave written informed consent and the relevant local institutional review boards approved this study (Supplementary Information). Cases and controls formed cohorts when they were processed in the same lab and were genotyped in the same batch, resulting in 117 independent cohorts.

GWAS quality control and imputation

For each cohort, SNPs were first annotated according to dbSNP150 and mapped to the hg19 reference genome. All multi-allelic and palindromic (A/T or C/G) SNPs were excluded. Subsequently, basic quality control was first performed by cohort, excluding extremely low-quality SNPs and genotyped individuals as well as excluding extreme population outliers. Low quality SNPs and genotyped individuals were excluded using PLINK 1.9 (--geno 0.1 and --mind 0.1)[55]. Population structure was assessed by projecting HapMap3 principal components (PCs) using EIGENSOFT[56] 6.1.4. Extreme outliers from the European

ancestries population were removed (> 25 SD on PC1-4). Finally, cohorts were merged into strata based on genotyping platforms to preserve the maximum number of SNPs (Supplementary Table 2). Four out of 6 strata were formed by only a single platform. The remaining two strata included multiple platforms with 420,952 and 299,625 overlapping SNPs across platforms in these strata.

After excluding major SNP and sample outliers in cohort QC and merging cohorts into strata, stringent SNP QC was performed per stratum. The following filter criteria were applied: MAF > 0.01, SNP genotyping rate > 0.98, Deviation from Hardy-Weinberg disequilibrium in controls $P > 1 \times 10^{-5}$, and haplotype-biased missingness $P > 1 \times 10^{-8}$ (PLINK --maf 0.01, --geno 0.02, --hwe 1e-5 midp include-nonctrl, --test-mishap). Then, more stringent QC thresholds were applied to exclude individuals: individual missingness > 0.02, inbreeding coefficient |F| > 0.2, mismatches between genetic and reported gender, and missing phenotypes (PLINK --mind 0.02, --het, --check-sex). Subsequently, SNPs with a differential missingness (--test-missing midp) $P < 1 \times 10^{-4}$ were excluded. Duplicate individuals were removed (PI_HAT > 0.8). Finally, outliers from the European ancestries reference population (projected on HapMap 3: > 10 SD from CEU on PC1-4 and projected on 1000 Genomes: > 4 SD from CEU on PC1-4) and outliers within the stratum itself (> 4SD from stratum mean on PC1-4) were removed (Supplementary Figures 54-59).

After removing outliers, principal components were recalculated for each stratum. To assess the result of quality control prior to imputation, genomic inflation factors per stratum were calculated using SAIGE[57] to run a logistic mixed model regressing SNP genotype on ALS case-control status. SAIGE internally calculates an equivalent of a genetic relationship matrix to correct for relatedness and population structure. Additionally, PC1-20 and genotyping platform were included as covariates.

The number of individuals and SNPs passing quality control for each stratum prior to imputation is described in Supplementary Table 2.

Post Imputation quality control

Strata were then imputed using the HRC reference panel (r.1.1 2016) on the Michigan Imputation Server[16]. Data was phased using Eagle 2.3. After imputation, one individual of each pair of related samples across strata (PI_HAT > 0.125) was removed whereas related pairs within a stratum were retained since the genetic relationship matrix corrects for relatedness. Post-imputation variant-level quality control included removing all monomorphic SNPs and multi-allelic SNPs from each stratum. SNPs with MAF < 0.1% in the HRC imputation panel were excluded. Subsequently, INFO scores were calculated for each stratum based on dosage information using SNPTEST[58] v2.5.4-beta3. Within each stratum, SNPs with an INFO-score < 0.6 and those deviating from Hardy-Weinberg equilibrium at $P < 1 \times 10^{-5}$ in control subjects were removed. Effective sample size was calculated for each stratum:

$$N_{effective} = \frac{4 \cdot N_{cases} \cdot N_{controls}}{N_{cases} + N_{controls}}$$

The difference in sample size and number of SNPs for each stratum prior to imputation, resulted in a different set of SNPs passing post-imputation quality control for each stratum. Therefore, only SNPs that were successfully imputed in an effective sample meeting > 50% of the maximum effective sample size were included.

The number of individuals and SNPs passing quality control for each stratum after imputation is described in Supplementary Table 2.

Association testing and meta-analysis

After quality control, a null logistic mixed model was fitted using SAIGE[57] 0.29.1 for each stratum with PC1-20 as covariates. The model was fit on a set of high-quality (INFO >0.95), pruned with PLINK 1.9, (--indep-pairwise 50 25 0.1) SNPs in a leave-one-chromosome-out scheme. Subsequently, a SNP-wise logistic mixed model including the saddle point approximation test was performed using genotype

742 dosages with SAIGE. Association statistics for all strata were combined in an inverse variance-weighted
743 fixed effects meta-analysis using METAL[59].

744 Genomic inflation factors were calculated per stratum and for the full meta-analysis. To assess any
745 residual confounding due to population stratification and artificial structure in the data we calculated
746 the LD Score regression (LDSC)[60] intercept using SNP LD-scores calculated in the HapMap3 CEU
747 population.

748 Cross-ancestry analyses.

749 GWAS summary statistics from two Asian ancestry studies were obtained[8,10]. These summary statistics
750 were meta-analyzed with all European ancestry in strata as described above. To assess genetic
751 correlation for ALS in the European and Asian ancestries, we used Popcorn[61] version 0.9.9. We used
752 population specific LD scores for genetic impact and genetic effect provided with the Popcorn
753 software. The regression model (--use_regression) was used to estimate genetic correlation. We
754 calculated both the correlation of genetic effects (correlation of allelic effect sizes) and genetic impact
755 (correlation of allelic effect size adjusted for difference in allele frequencies).

756 Conditional SNP analysis

757 Conditional and joint SNP analysis (COJO, GCTA v1.91.1b)[62,63] was performed to identify potential
758 secondary GWAS signals within a single locus. SNPs with association $P \leq 5 \times 10^{-8}$ were considered.
759 European ancestry controls from the health and retirement study (HRS, cohort 65, Supplementary
760 Table 1), included in stratum 4 of this study, were used as LD reference panel.

Gene prioritization.

Whole-genome sequencing

Sample selection, sequencing and data preparation.

ALS cases and controls from Project MinE[64] were recruited for whole genome sequencing. The participating cohorts are described in the Supplementary Note. A full description of Project MinE, the sequencing and quality control pipeline were described previously[65]. In summary, the first batch of 2,250 cases and control samples were sequenced on the Illumina HiSeq 2000 platform. All remaining 7,350 cases and controls were sequenced on the Illumina HiSeq X platform. All samples were sequenced to ~35X coverage with 100bp reads and ~25X coverage with 150bp reads for the HiSeq 2000 and HiSeq X respectively. Both sequencing sets used PCR-free library preparation. Samples were also genotyped on the Illumina 2.5M array. Sequencing data was then aligned to GRCh37 using the iSAAC Aligner, and variants called using the iSAAC variant caller; both the aligner and caller are standard to Illumina's aligning and calling pipeline.

Quality control

For variant-level quality control, we set sites with a genotype quality (GQ) < 10 to missing and SNVs and indels with quality (QUAL) scores < 20 and < 30, respectively, were removed. We subsequently performed sample-level quality control. An overview of the number of samples that have been excluded at each of the following QC steps, stratified by country of origin, is included in Supplementary Table 3.

We estimated kinship coefficients (i.e., relatedness) using the KING method, as implemented in the SNPRelate package in R. In some instances, cohorts were intentionally enriched for related samples. We identified all pairs of related individuals (kinship > 0.0625).

We calculated the transition-transversion ratio in each sample using SnpSift 4.3p. In WGS data, the expected transition-transversion ratio is ~2.0. Samples with a Ti/Tv ratio ± 6 SD from the full distribution of samples were removed.

Per sample, we calculated the total number of SNVs and total number of singletons. We removed samples with a total number of SNVs or Singletons > 6 SD from the mean. The transition in sequencing platforms from HiSeq 2000 to HiSeq X (which occurred in parallel with a change in the calling pipeline, to improve indel detection) caused an increase in observed indels per sample. Samples were thus filtered by platform (HiSeq 2000 or HiSeq X) and removed samples with number of indels ± 6 SD from the mean of their respective group.

We calculated average sample depth and again observed noticeable differences between those samples sequenced on the HiSeq 2000 and the HiSeq X, where average depth of coverage was somewhat higher (35X, on average) for samples sequenced on HiSeq 2000 compared to the samples sequenced on the HiSeqX (25X, on average). We removed no samples at this step.

Using the genetically inferred sex based on the number of X and Y chromosome, we tested to see if the inferred genetic sex was concordant with the sex as annotated in the available phenotype information. We excluded samples with mismatching information and samples for which phenotypic information is missing at this time.

We performed the remaining sample QC on high-quality variants: We removed all multi-allelic SNVs, Plink 1.9 (--geno), variants with a missingness > 2% were excluded. We calculated Hardy-Weinberg equilibrium (HWE) in controls only, PLINK 1.9 (--hwe midp), and removed all variants with HWE $P < 1 \times 10^{-5}$. We calculated differential missingness, PLINK 1.9 (--test-mishap) between cases and controls and removed variants with $P < 1 \times 10^{-8}$. Samples with a missingness > 2%, in SNV and indels, were excluded. Final steps of sample QC was performed on a set of variants with a MAF > 10%, SNP missingness < 0.1%, variants residing outside four complex regions (the major histocompatibility complex (MHC) on chromosome 6; the lactase locus (LCT), on chromosome 2; and inversions on chromosomes 8 and 17);

and we excluded the A/T and C/G variants. We used the SNVs to calculate observed and expected autosomal homozygous genotype counts for each sample PLINK 1.9 (--het); samples with |F| > 0.1 were excluded. We excluded duplicate samples; PLINK 1.9 (--genome) with a PIHAT > 0.8, keeping the maximum number of non-duplicated individuals.

Principal component analysis (PCA) implemented in EIGENSOFT was used to visualize potential structure in the data, induced by population stratification or other variables. Projections onto HapMap3 and the 1KG phase3 v5 populations indicated that the samples were primarily of European ancestry, though some were of African or East Asian ancestries, while other samples appeared to be admixed. Outliers from the European population (HapMap3: > 10 SD on PC1-4, 1KG: > 4 SD on PC1-4).

All samples were sent in batches to Illumina for sequencing. To prevent spurious association due to batch specific artifacts, we regressed all variants on a dummy coded variable indicating batch using PLINK 1.9 (--logistic). All variants with an association $P < 1 \times 10^{-10}$ in at least 1 batch were excluded.

Genic burden association analyses

To aggregate rare variants in a genic burden test framework we used a variety of variant filters to allow for different genetic architectures of ALS associated variants per gene as we and others have used previously[65,66]. In summary, variants were annotated according to allele-frequency threshold (MAF < 0.01 or MAF < 0.005) and predicted variant impact ("missense", "damaging", "disruptive"). "Disruptive" variants were those variants classified as frame-shift, splice-site, exon loss, stop gained, start loss and transcription ablation. "Damaging" variants were missense variants predicted to be damaging by seven prediction algorithms (SIFT[67], Polyphen-2[68], LRT[69], MutationTaster2[70], Mutations Assessor[71], and PROVEAN[72]). "Missense" variants are those missense variants that did not meet the "damaging" criteria. All combinations of allele frequency threshold and variant annotations were used to test the genic burden on a transcript level in a Firth logistic regression framework where burden was defined as the number of variants per individual. Sex and the first 20 principal components were

included as covariates. All ENSEMBL protein coding transcripts for which at least five individuals had a non-zero burden were included in the analysis.

Conditional genic burden analysis.

We selected for each gene the protein coding transcripts that were strongest associated with ALS across all different combinations of MAF and variant impact thresholds that exhibited the strongest association with ALS. For these transcripts and variants, we applied Firth logistic regression on individuals overlapping the GWAS and WGS dataset (5,158 cases and 2,167 controls). To assess whether the rare variant burden association and the signal from GWAS were conditionally independent we subsequently included the genotype of the top-associated SNP within that locus as covariate.

Short tandem repeat screen

For all individuals that were sequenced on the HiSeqX dataset (5,392 cases, 1,795 controls) we screened all loci harboring SNPs associated with ALS meeting genome-wide significance for expansions of known and new short tandem repeats (STRs) using ExpansionHunter[73] and ExpansionHunter Denovo[74].

First we used ExpansionHunter (v4.0) to screen for expansions of known STRs located within 1 MB of the top ALS-associated SNP. For this we used the STR catalogue of the ExpansionHunter software which is based on STRs identified from indels in 18 high quality genomes and the gangSTR STR catalogue based on STR annotations in the reference genome[75]. From these catalogues, we excluded all homopolymers. Repeat length was subsequently regressed on case-control status using Firth logistic regression including the first 20 principal components as covariates, recoding the STR size to a biallelic variant using a sliding window over all observed repeat lengths. To correct for multiple testing across all possible thresholds, we applied Benjamini Hochberg correction per STR.

To screen for extremely long STR expansions (similar to the *C9orf72* repeat expansion) at loci that not included in the predefined STR catalogues, we applied ExpansionHunter-Denovo[74]. This method aims to only find STR expansions that exceed the sequencing read-length (> 150 bp) by identifying reads (mapped, mismapped and unmapped) that contain STR motifs, using their mate pairs for *de novo* mapping to the reference genome.

For all STRs we calculated linkage disequilibrium statistics (r^2 and |D'|) between recoded repeat genotypes at the optimal threshold and the top associated GWAS SNP. Subsequently, we conditioned the SNP association on the repeat genotype in a Firth logistic regression.

Summary-based Mendelian randomization

We used multi-SNP SMR[76,77] to infer the effect of gene expression variation on ALS using eQTLs (the association of a SNP with expression of a gene) on ALS risk. MetaBrain is a harmonized set of 8,727 RNA-seq samples from 7 regions of the central nervous system from 15 datasets, and we selected eQTLs derived from the cortex region of the brain in samples of European ancestry (MetaBrain Cortex-EUR eQTLs) as our instrument variable[20]. The European-only ALS summary statistics were used as the outcome. To supplement this analysis, we also used eQTLs in blood from the eQTLGen consortium, as this is the largest eQTL resource available. European-ancestry samples in the Health and Retirement study (HRS, cohort 65 of this GWAS) were used as LD reference panel. SNP with MAF ≥ 1% in HRS were included. Further SMR settings were left as default, meaning probes with at least one eQTL with $P \leq 5 \times 10^{-8}$ were included.

We subsequently performed SMR using DNA methylation QTL (mQTL) data and European-only ALS summary statistics. Human prefrontal cortex and whole blood DNA mQTLs were generated as part of ongoing analyses by the Complex Disease Epigenomics Group at the University of Exeter (www.epigenomicslab.com) using the Illumina EPIC HumanMethylation array that quantifies DNAm at >850,000 sites across the genome[21]. The prefrontal cortex mQTL dataset was generated using DNA

879 methylation and SNP data from 522 individuals from the Brains for Dementia Research cohort[22] and
880 included 4,623,966 cis mQTLs (distance between QTL SNP and DNAm site ≤ 500 kb) between 1,744,102
881 SNPs and 43,337 DNA methylation sites. The whole blood mQTL dataset was generated using DNAm
882 and SNP data from 2,082 individuals[78] and included 30,432,023 cis mQTLs between 4,030,902 SNPs
883 and 167,854 DNA methylation sites. mQTLs reaching the significance threshold $P \leq 1 \times 10^{-10}$ were taken
884 forward for SMR analysis as described by Hannon and colleagues[78]. To map CpG sites to their putative
885 target genes we used the expression quantitative trait methylation (eQTM) results from a paired
886 methylation and gene expression (RNA-seq) study in blood[79]. For CpG sites where no eQTM were
887 present in this dataset, we used positional mapping based on the basal regulatory domains and
888 extended regulatory domains as defined in the Genomic Regions Enrichment of Annotations Tool
889 (GREAT)[80] which is applied in the `cpg_to_gene` function in the CpGtools toolkit[81].

890 ## Polygenic Priority Score (PoPS)

891 We used the polygenic priority score (PoPS[24] v0.1) to rank genes according to the gene features that
892 were enriched in ALS. For this we applied MAGMA in the European ancestries GWAS since it depends
893 on an LD reference panel (1000 Genomes Project, EUR population) to obtain gene-wise association
894 statistics. We used the default 57,543 gene features that were based on expression data, protein-
895 protein interaction networks and pathway membership. Genes were ranked based on the Polygenic
896 Priority Score.

897 Cross-trait analyses in neurodegenerative diseases.

898 Datasets and data preparation

899 GWAS summary statistics for clinically-diagnosed Alzheimer's disease (AD)[26], Parkinson's disease
900 (PD)[27], frontotemporal dementia (FTD)[28], corticobasal degeneration (CBD)[29], and progressive
901 supranuclear palsy (PSP)[15] in European ancestry individuals were obtained. For Alzheimer's disease we

used the clinically diagnosis as case definition to avoid spurious genetic correlations that could have been introduced through the by-proxy design[30] where by-proxy cases are defined as having a parent with Alzheimer's disease. Although this is a powerful design for gene discovery and the genetic correlation with clinically diagnosed Alzheimer's disease is high[82], mislabeling by-proxy cases when parents suffer from other types of dementia (e.g. Lewy-body dementia, Parkinson's dementia, FTD, or vascular dementia) can lead to spurious genetic correlations with ALS and other neurodegenerative diseases. For FTD, we primarily used the results of the cross-subtype meta-analysis which includes behavioral variant FTD (bvFTD), semantic dementia (sdFTD), progressive non-fluent aphasia (pnfaFTD) and motor neuron disease FTD (mndFTD). For CBD, allele coding were missing and effect alleles were inferred by matching allele frequencies to those observed in the Haplotype Reference Consortium. SNPs with minor allele frequency > 0.4 were excluded. Since downstream methods rely on LD-scores or population-specific LD patterns, the European ancestry summary statistics from the present study were used for ALS. For sample size parameters, effective sample size was calculated as described previously.

Genetic correlation

We first assessed residual confounding through estimating the LD Score regression[60] intercept using LDSC (v.1.0.0): ALS = 1.03 (SE 0.0073), AD = 1.03 (SE 0.013), PD = 0.98 (SE 0.0065), PSP = 1.05 (SE 0.0076), CBD = 0.98 (SE 0.0073), FTD = 1.00 (SE 0.0071), showing limited inflation of test statistics due to confounding across these studies. Genome-wide genetic correlation between neurodegenerative traits was calculated using LDSC (v1.0.0). Pre-computed LD-scores of European individuals in the 1000 Genomes project for high-quality HapMap3 SNPs were used (eur_w_ld_chr). A free intercept was modelled to allow for potential sample overlap.

Colocalization

For each locus (top-SNP +/- 100KB) harboring SNPs with an association with any of the neurodegenerative diseases at $P < 1 \times 10^{-5}$ we performed colocalization analysis using the `coloc` package in R.[83] We set the prior probabilities to $\pi_1 = 1 \times 10^{-4}$, $\pi_2 = 1 \times 10^{-4}$, $\pi_{12} = 1 \times 10^{-5}$ for a causal variant in trait 1, trait 2 and a shared causal variant between trait 1 and 2 respectively. Using the same parameters, we performed colocalization analysis for ALS and each of the FTD subtypes (bvFTD, sdFTD, pnfaFTD, mndFTD).

Enrichment analyses

LD-score regression annotation-specific enrichment analysis

We used LDSC (v1.0.0) to calculate SNP-based heritability, the LDSC intercept and SNP-based heritability enrichment for partitions of the genome. In all LDSC analyses, summary statistics excluding the HLA region of only European ancestry samples were included. LD scores and partitioned LD scores provided by LDSC were used for genome-wide and genic region-based heritability analyses. The option --overlap-annot was used in the partitioned heritability analysis to allow for overlapping SNP between MAF bins. SNPs with a MAF > 5% were included.

Tissue and cell-type enrichment analysis

Tissue and cell-type enrichment analyses were performed using the GWAS summary statistics of the European ancestries meta-analysis and FUMA[32] software v1.3.6a. FUMA performs a genic aggregation analysis of GWAS association signals to calculate gene-wise association signals using MAGMA v1.6 and subsequent tests whether tissues and cell-types are enriched for expression of these genes. For tissue enrichment analysis we used the GTEx v8 reference set. For cell-type enrichment analyses[33] we used human-derived single-cell RNA sequencing data on major brain cell-types (GSE67835 without fetal

946 samples[84]), the Allen Brain Atlas Cell-type[85] for the human-derived major neuronal subtypes and the
947 DropViz[86] dataset for mouse-derived brain cell-types across all brain regions.

948 Pathway enrichment analysis

949 We used the Downstreamer software[20] to identify enriched biological pathways and processes. First,
950 gene-based association statistics are obtained through the PASCAL method[87] which aggregates SNP
951 association statistics including SNPs up to 10kb up- and downstream of a gene, accounting for linkage
952 disequilibrium using the non-Finish European individuals from the 1000 Genomes Project phase 3 (ref.
953 [88]) as a reference. In the Downstreamer method, putative core genes are defined as those that are
954 coexpressed with disease-associated genes and can therefore be implicated in disease. Co-expression
955 networks are based on either a large, multi-tissue transcriptome dataset including 56,435 genes and
956 31,499 individuals, or brain-specific RNA-sequencing data obtained in the MetaBrain resource. The
957 gene-based association statistics, co-expression matrix and gene Z-scores per pathway or HPO term
958 are then combined in a generalized least squares regression model to obtain enrichment statistics.[20]
959 Enrichment analyses were performed for Reactome, Gene Ontology and Human Phenotype Ontology
960 (HPO) terms using the multi-tissue or brain-specific transcriptome datasets to calculate the co-
961 expression matrix.

962 The distribution of enrichment Z-score statistics were compared between the analyses using the multi-
963 tissue or the brain-specific co-expression matrices. Using the 'pyhpo' module in Python, all HPO terms
964 were assigned to their parent term(s) in the "*Phenotypic abnormality*" (HP:0000118) branch which
965 includes phenotypic abnormalities grouped per organ system.

966 Mendelian Randomization

967 Causal inference through MR analysis was performed for 22 exposures for which large-scale GWAS are
968 available and for which there is prior evidence for an association with ALS. These include 7 behavioral

969 related traits: body mass index (anthropometric)[89], years of schooling (educational attainment)[90],
970 alcoholic drinks per week, age of smoking initiation and cigarettes per day from Liu et al.[91], days per
971 week moderate physical activity and days per week vigorous activity from UK Biobank[92]; 4 blood
972 pressure traits: coronary artery disease[93], stroke[94], diastolic blood pressure and systolic blood
973 pressure[95]; 7 immune system traits from Vuckovic et al.[96] (basophil, eosinophil, lymphocyte, monocyte,
974 neutrophil and while blood cells) and C-reactive protein[97]; and 4 lipid traits from Willer et al.[98] (HDL
975 cholesterol, LDL cholesterol, total cholesterol and triglycerides). A full description of the included
976 studies is provided in Supplementary Table 25. From these GWASs, SNPs to serve as instruments for
977 MR analyses were selected at two different p-value cut-offs ($P < 5 \times 10^{-8}$ and $P < 5 \times 10^{-5}$) and then LD
978 clumped to obtain independent SNPs. SNP effect estimates on ALS risk were obtained from the
979 European ancestries only GWAS and if needed an LD-proxy was selected ($r^2 > 0.8$).

980 After harmonizing effect-alleles and excluding palindromic SNPs, we performed a series of quality
981 control steps to avoid biased estimates of causal effects, checking for each exposure the (i) instrument
982 coverage (> 85% overlapping SNPs, Supplementary Table 30), (ii) instrument strength (F-statistic[36,99,100]
983 > 10, Supplementary Table 31), (iii) distribution and significance of the Wald ratios (visual inspection
984 of volcano plots, Supplementary Table 32) and (iv) heterogeneity across the instrument-exposure
985 effects (Q-statistic at $P < 0.05$ indicating heterogeneity, Supplementary Table 33).

986 We applied 5 different MR methods: Inverse variance weighted (IVW) using the random effects model,
987 MR-Egger, simple mode, weighted median and weighted mode methods. When only a single SNP was
988 available the Wald ratio (WR) test was conducted. MR analysis was conducted in R using the `mr()`
989 function in the `TwoSampleMR` package[101].

990 Subsequently, Radial MR analysis was conducted to determine if Wald ratio outliers needed to be
991 removed from the IVW or MR-Egger MR estimates[37]. In addition, we conducted a Q-test to identify
992 outlier SNPs ($P < 0.05$). These outliers were then removed from the original MR analyses (across all 5
993 MR methods). The Radial MR analysis was conducted using the RadialMR R package

(https://github.com/WSpiller/RadialMR). In order to determine that the MR effects were orientated in the correct direction (from exposure to ALS) we conducted both reverse MR[102] and Steiger filtering[103] on our top MR findings.

Finally, we explored whether the MR effects of our total and LDL cholesterol and systolic blood pressure exposures may be confounded by the effect we observed for years of schooling by conducting multivariate MR analysis[104]. Conditional F and Q statistics were calculated using the `MVMR` package[105] in R.

Data availability

All summary statistics will be made publicly available in centralized repositories upon publication.

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Consortium members

SLALOM Consortium

Ettore Beghi[42], Elisabetta Pupillo[42], Giancarlo Comi[140], Nilo Riva[140], Christian Lunetta[141], Francesca Gerardi[141], Maria Sofia Cotelli[142], Fabrizio Rinaldi[142], Luca Chiveri[143], Maria Cristina Guaita[144], Patrizia Perrone[144], Mauro Ceroni[145], Luca Diamanti[145], Carlo Ferrarese[146], Lucio Tremolizzo[146], Maria Luisa Delodovici[147] & Giorgio Bono[147]

Affiliations

42: Laboratory of Neurological Diseases, Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.

140: IRCCS San Raffaele Hospital, Milan, Italy.

141: NEMO Clinical Center, Serena Onlus Foundation, Niguarda Ca' Granda Hospital, Milan, Italy.

142: Civil Hospital of Brescia, Brescia, Italy.

143: Ospedale Valduce, Como, Italy.

144: A.O. Ospedale Civile di Legnano, Legnano, Italy.

145: IRCCS Istituto Neurologico Nazionale "C.Mondino", Pavia, Italy.

146: A.O. "San Gerardo" di Monza and University of Milano-Bicocca, Italy

147: A.O. "Ospedale di Circolo Fondazione Macchi" di Varese, Varese, Italy.

PARALS Consortium

Adriano Chiò[40,41], Andrea Calvo[40,41], Cristina Moglia[40,41], Antonio Canosa[40,41,148], Umberto Manera[40], Rosario Vasta[40], Alessandro Bombaci[40], Maurizio Grassano[40], Maura Brunetti[40], Federico Casale[40], Giuseppe Fuda[40], Paolina Salamone[40], Barbara Iazzolino[40], Laura Peotta[40], Paolo Cugnasco[40], Giovanni De Marco[41], Maria Claudia Torrieri[40], Francesca Palumbo[40], Salvatore Gallone[41], Marco Barberis[149], Luca Sbaiz[149], Salvatore Gentile[150], Alessandro Mauro[40,151], Letizia Mazzini[152,153], Fabiola De Marchi[152,153], Lucia Corrado[154,153], Sandra D'Alfonso[154,153], Antonio Bertolotto[155], Maurizio Gionco[156], Daniela Leotta[157], Enrico Odddenino[157], Daniele Imperiale[158], Roberto Cavallo[159], Pietro Pignatta[160], Marco De Mattei[161], Claudio Geda[162], Diego Maria Papurello[163], Graziano Gusmaroli[164], Cristoforo Comi[165,166], Carmelo Labate[167], Luigi Ruiz[168], Delfina Ferrandi[169], Eugenia Rota[170], Marco Aguggia[171], Nicoletta Di Vito[171], Piero Meineri[172], Paolo Ghiglione[173], Nicola Launaro[174], Michele Dotta[175], Alessia Di Sapio[176] & Guido Giardini[177]

Affiliations

40: "Rita Levi Montalcini" Department of Neuroscience, ALS Centre, University of Torino, Turin, Italy.

41: Neurologia 1, Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Turin, Italy.

148: Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, Neurology Unit 1U, Turin, Italy.

149: Department of Medical Genetics, Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Turin, Italy.

150: Neurologia 3, Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino, Turin, Italy.

151: Istituto Auxologico Italiano, IRCCS, Piancavallo, Italy.

152: Department of Neurology, 'Amedeo Avogadro' University of Piemonte Orientale, Novara, Italy.

153: Azienda Ospedaliero Universitaria 'Maggiore della Carità', Novara, Italy.

154: Department of Health Sciences, 'Amedeo Avogadro' University of Piemonte Orientale, Novara, Italy.

155: Department of Neurology and Multiple Sclerosis Center, Azienda Ospedaliero Universitaria San Luigi, Orbassano, Italy.

156: Department of Neurology, Azienda Ospedaliera 'Ordine Mauriziano' di Torino, Turin, Italy.

157: Department of Neurology, Ospedale Martini, ASL Città di Torino, Turin, Italy.

158: Department of Neurology, Ospedale Maria Vittoria, ASL Città di Torino, Turin, Italy.

159: Department of Neurology, Ospedale San Giovanni Bosco, ASL Città di Torino, Turin, Italy.

160: Ospedale Humanitas Gradenigo, Turin, Italy.

161: Department of Neurology, Ospedale 'Santa Croce' di Moncalieri, ASL Torino 5, Moncaliari, Italy.

162: Department of Neurology, Ospedale Civile di Ivrea, ASL Torino 4, Ivrea, Italy.

163: Department of Neurology, Presidio Ospedaliero di Ciriè, ASL Torino 4, Ciriè, Italy.

164: Department of Neurology, Ospedale 'Degli Infermi' di Biella, ASL Biella, Ponderano, Italy.

165: Department of Neurology, Ospedale 'Sant'Andrea' di Vercelli, ASL Vercelli, Vercelli, Italy.

166: Department of Clinical and Experimental Medicine, 'Amedeo Avogadro' University of Piemonte Orientale, Novara, Italy.

167: Department of Neurology, Ospedale Civile 'Edoardo Agnelli' di Pinerolo, ALS Torino 2, Pinerolo, Italy.

168: Department of Neurology, Azienda Ospedaliera 'Santi Antonio e Biagio' di Alesssandria, Alessandria, Italy.

169: Department of Neurology, Ospedale 'Santo Spirito' di Casale Monferrato, ASL Alessandria, Casale Monferrato, Italy.

170: Department of Neurology, Ospedale 'San Giacomo' di Novi Ligure, ASL Alesssandria, Novi Ligure, Italy.

171: Department of Neurology, Ospedale 'Cardinal Massia' di Asti, ASL Asti, Asti, Italy.

172: Department of Neurology, Azienda Ospedaliera 'Santa Croce e Carle' di Cuneo, Cuneo, Italy.

173: Department of Neurology, Ospedale 'Maggiore Santissima Annuziata' di Savigliano, ASL Cuneo 1, Savigliano, Italy.

174: Department of Anestesiology, Ospedale 'Maggiore Santissima Annuziata' di Savigliano, ASL Cuneo 1, Savigliano, Italy.

175: Department of Neurology, Ospedale 'Michele e Pietro Ferrero" di Verduno, ASL Cuneo 2, Verduno, Italy.

176: Department of Neurology, Ospedale 'Regina Montis Regalis' di Mondovì, ASL Cuneo 1, Italy.

177: Department of Neurology, Ospedale Regionale 'Umberto Parini' di Aosta, Aosta, Italy.

SLAGEN Consortium

Vincenzo Silani[17,18], Nicola Ticozzi[17,18], Antonia Ratti[17,30], Isabella Fogh[14], Cinzia Tiloca[17], Silvia Peverelli[17], Cinzia Gellera[31], Giuseppe Lauria Pinter[32,33], Franco Taroni[178], Viviana Pensato[178], Barbara Castellotti[178], Giacomo P. Comi[34,18], Stefania Corti[34,18], Roberto Del Bo[34,18], Cristina Cereda[35], Mauro Ceroni[179,180], Stella Gagliardi[35], Sandra D'Alfonso[36], Lucia Corrado[36], Letizia Mazzini[181], Gianni Sorarù[37], Flavia Raggi[37], Gabriele Siciliano[38], Costanza Simoncini[38], Annalisa Lo Gerfo[38], Massimiliano Filosto[39], Maurizio Inghilleri[182] & Alessandra Ferlini[183],

Affiliations

14: Maurice Wohl Clinical Neuroscience Institute, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK.

17: Department of Neurology-Stroke Unit and Laboratory of Neuroscience, Istituto Auxologico Italiano IRCCS, Milan, Italy.

18: Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy.

30: Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy.

31: Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy.

32: 3rd Neurology Unit, Motor Neuron Diseases Center, Fondazione IRCCS Istituto Neurologico "Carlo Besta", MIlan, Italy.

33: 'L. Sacco' Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan, Italy.

34: Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

35: Genomic and Post-Genomic Center, IRCCS Mondino Foundation, Pavia, Italy.

36: Department of Health Sciences, University of Eastern Piedmont, Novara, Italy.

37: Department of Neurosciences, University of Padova, Padova, Italy.

38: Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.

39: Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.

178: Unit of Genetics of Neurodegenerative and Metabolic Diseases, Fondazione IRCCS Istituto Neurologico 'Carlo Besta', Milan, Italy.

179: Unit of General Neurology, IRCCS Mondino Foundation, Pavia, Italy.

180: Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy

181: ALS Center, Department of Neurology, Azienda Ospedaliero Universitaria Maggiore della Carità, Novara, Italy

182: Rare Neuromuscular Diseases Centre, Department of Human Neuroscience, Sapienza University, Rome, Italy

183: Unit of Medical Genetics, Department of Medical Science, University of Ferrara, Ferrara, Italy.

SLAP Consortium

Giancarlo Logroscino[43], Ettore Beghi[42], Isabella L. Simone[184], Bruno Passarella[185], Vito Guerra[186], Stefano Zoccolella[187], Cecilia Nozzoli[185], Ciro Mundi[188], Maurizio Leone[189], Michele Zarrelli[189], Filippo Tamma[190], Francesco Valluzzi[191], Gianluigi Calabrese[192], Giovanni Boero[193] & Augusto Rini[185]

Affiliations

42: Laboratory of Neurological Diseases, Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.

43: Department of Clinical Research in Neurology, University of Bari at "Pia Fondazione Card G. Panico" Hospital, Bari, Italy.

184: Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari, Bari, Italy.

185: Neurological Department, Antonio Perrino's Hospital, Brindisi, Italy.

186: National Institute of Digestive Diseases. IRCCS S. de Bellis Research Hospital, Castellana Grotte, Italy.

187: ASL Bari, San Paolo Hospital, Milan, Italy.

188: Department of Neuroscience, United Hospital of Foggia, 71100 Foggia, Italy.

189: Unit of Neurology, Department of Medical Sciences, IRCCS Casa Sollievo della Sofferenza, 71013 San Giovanni Rotondo, Italy.

190: Neurology Unit, Miulli Hospital, Acquaviva delle Fonti, BA, Italy.

191: Unit of Neurology, "S. Giacomo" Hospital, Bari, Italy.

192: Department of Neurology, ASL (Local Health Authority) at the "V Fazzi" hospital, 73100 Lecce, Italy.

193: Department of Neurology, ASL (Local Health Authority) at the "SS. Annunziata" hospital, Taranto, Italy.

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Author contributions

Sample ascertainment: W.v.R, R.A.A.v.d.S, M.M, A.M.D, H-J. Westeneng, G.H.P.T, N.T, J.C-K, B.N.S, M.G, S.C, S.P, K.E.M, P.J.S, J.H, R.W.O, M.S, T.M, N.B, A.J.v.d.K, A.R, C.G, G.L.P, G.P.C, C.C, D.S, S.D'A, G. Sorarù, G. Siciliano, M.F, A.P, A.C, A. Calvo, C.M, M.B, A. Canosa, M. Grassano, E.B, E.P, G.L, B.N, A.O, A.N, Y.L, M.Z, M. Gotkine, R.H.B, S.B, P.V'h, P.C, P. Couratier, S.M, V.M, F.S, J.S.M.P, A.A, R.R-G, P. Dion, J.P.R, A.C.L, J.H.W, D. Brenner, A.F, G.B, A.B, A.D, C.A.M.P, S.S-D, N.W, S.T, R. Rademakers, A. Braun, J.K, D.C.W, C.M.O, A.G.U, A.H, M.R, S. Cichon, M.M.N, P.A, B.T, A.B.S, M. Mitne Neto, R.J.C, R.A.O, M.W-P, C.L-H, V.M.v.D, J.G, A. Rödiger, N.G, A.J, T.B, E.T, B.I, B.S, O.W.W, R.S, C.A.H, C. Graff, L.B, V.F, V.D, A. Ataulina, B.R, B.K, J.Z, M.R-G, D.G, Z.S, V. Drory, M.P, I.P.B, M.C.K, R.D.H, S. Mathers, P.A.M, M.N, G.A.N, R.P, D.B.R, K.A.M, P.S.S, M.d.C, S. Pinto, S. Petri, M.W, G.A.R, V.S, J. Glass, R.H. Brown, J.E.L, C.E.S, P.M.A, D.F, F.C.G, A.F.M, R.L.M, O.H, A.A-C, P.V.D, L.H.v.d.B, J.H.V, SLALOM consortium, PARALS consortium, SLAGEN consortium & SLAP consortium. **SNP-array genotyping:** W.v.R, R.A.A.v.d.S, A.M.D,

A.S, I.F, G.B, A.B, A.D, C.A.M.P, S.S-D, N.W, L.T, W.L, A. Franke, S.R, A. Braun, J.K, D.C.W, C.M.O, A.G.U, A.H, M.R, S. Cichon, M.M.N, P.A, B.T, A.B.S, B.B, S.F, S.T.N, F.J.S, K.L.W, A.K.H, L.W, C. Curtis, G. Breen, D.F, F.C.G, A.F.M, N.R.W, A.A-C, P.V.D, L.H.v.d.B & J.H.V **GWAS quality control:** W.v.R, R.A.A.v.d.S, M.K.B, R.R, R.L.M, N.R.W and J.H.V. **GWAS data analysis:** W.v.R, R.A.A.v.d.S, M.K.B, R.R, R.P.B, M.D, M.H, A.A.K, A.I, A.S, N.T, B.N.S, B.B, D.F, A.F.M, R.L.M, N.R.W and J.H.V. **Whole-genome sequencing:** W.v.R, R.A.A.v.d.S, P.J.H, R.A.J.Z, M.M, A.M.D, G.H.P.T, K.R.v.E, M.K, J.C-K, B.N.S, K.P.K, A.A-C, P.V.D, L.H.v.d.B and J.H.V. **WGS quality-control:** W.v.R, R.A.A.v.d.S, J.J.F.A.v.V, P.J.H, R.A.J.Z, M.M, K.P.K, P.V.D and J.H.V. **WGS rare-variant burden analyses:** W.v.R, R.A.A.v.d.S, P.J.H, R.A.J.Z, K.R.v.E, K.P.K, P.V.D and J.H.V. **WGS STR-analyses:** W.v.R, J.J.F.A.v.V, R.A.J.Z, E.D, M.A.E and J.H.V. **eQTL analyses:** W.v.R, R.A.A.v.d.S, M.K.B, N.d.K, H-J.W, O.B.B, P.D, J.M, L.F and J.H.V. **mQTL analyses:** W.v.R, M.K.B, P.J.H, R.A.J.Z, G.S, E.H, A.M.D and J.H.V. **Cross-disorder analyses:** W.v.R, R.A.A.v.d.S, M.K.B, N.d.K, H-J.W, O.B.B, P.D, E.J.N.G, M.A.v.E, R.J.P, A.F.M, N.R.W, E. Tsai, H.R, L.F and J.H.V. **MR analyses:** W.v.R, R.A.A.v.d.S, M.K.B, D.B, H.J.W, G.D.S, T.R.G, E. Tsai, H.R, and J.H.V. **Writing of the manuscript:** W.v.R, M.K.B, D.B, J.M, E. Tsai and J.H.V. **Revising the manuscript:** W.v.R, R.A.A.v.d.S, M.K.B, J.J.F.A.v.V, G.S, E.H, D.B, R.R, E.D, H.J.W, G.H.P.T, K.R.v.E, E.J.N.G, M.A.v.E, R.J.P, G.D.S, T.R.G, R.L.M, K.P.K, N.R.W, E. Tsai, H.R, L.F, L.H.v.d.B and J.H.V. **Acquired funding and supervised the study:** L.H.v.d.B and J.H.V.

Competing Interests

J.H.V has sponsored research agreements with Biogen Idec. L.H.v.d.B receives personal fees from Cytokinetics, outside the submitted work. A.A-C. has served on scientific advisory boards for Mitsubishi Tanabe Pharma, OrionPharma, Biogen Idec, Lilly, GSK, Apellis, Amylyx, and Wave Therapeutics. A.C. serves on scientific advisory boards for Mitsubishi Tanabe, Roche, Biogen, Denali, and Cytokinetics

Figures

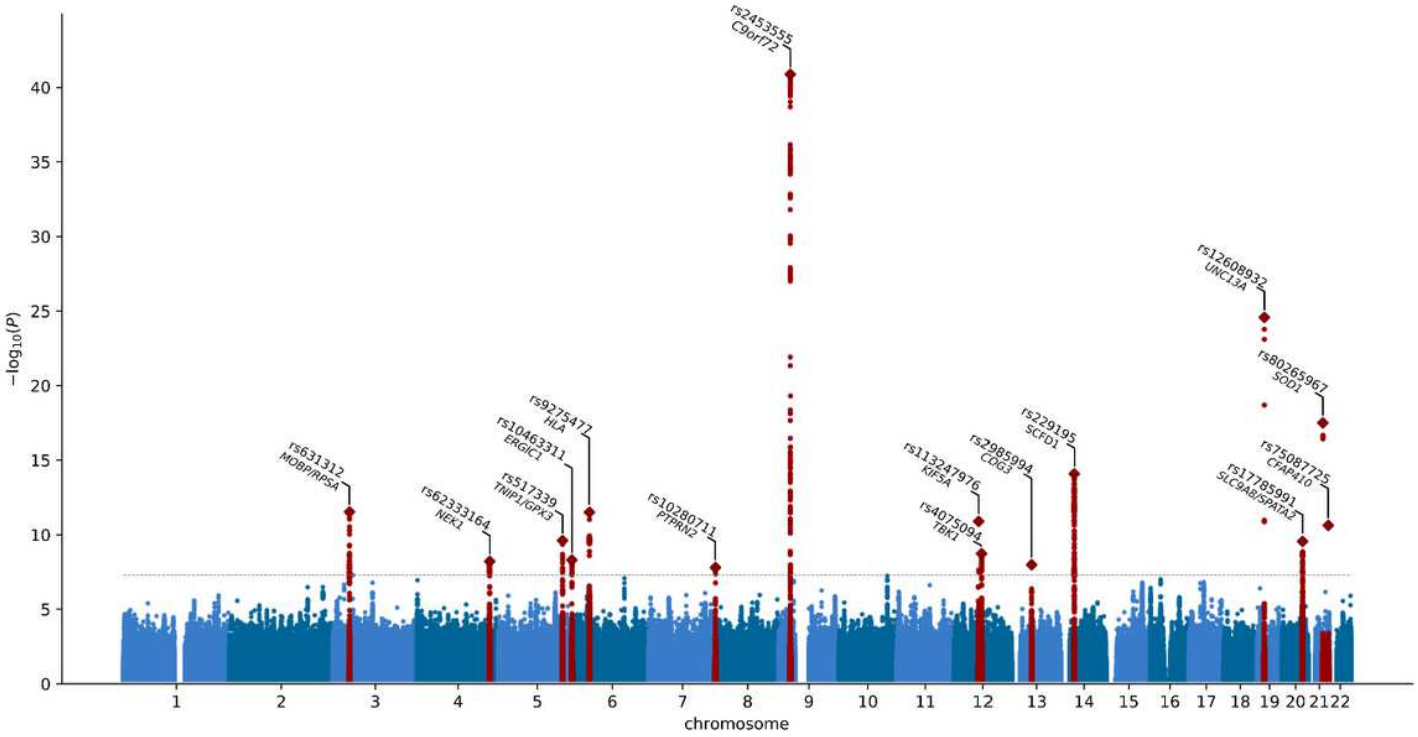

Figure 1

Manhattan plot of cross-ancestry meta-analysis. Horizontal dotted line reflects threshold for calling SNPs genome-wide significant (P = 5 × 10-8). Gene labels reflect those prioritized by gene prioritization analysis.

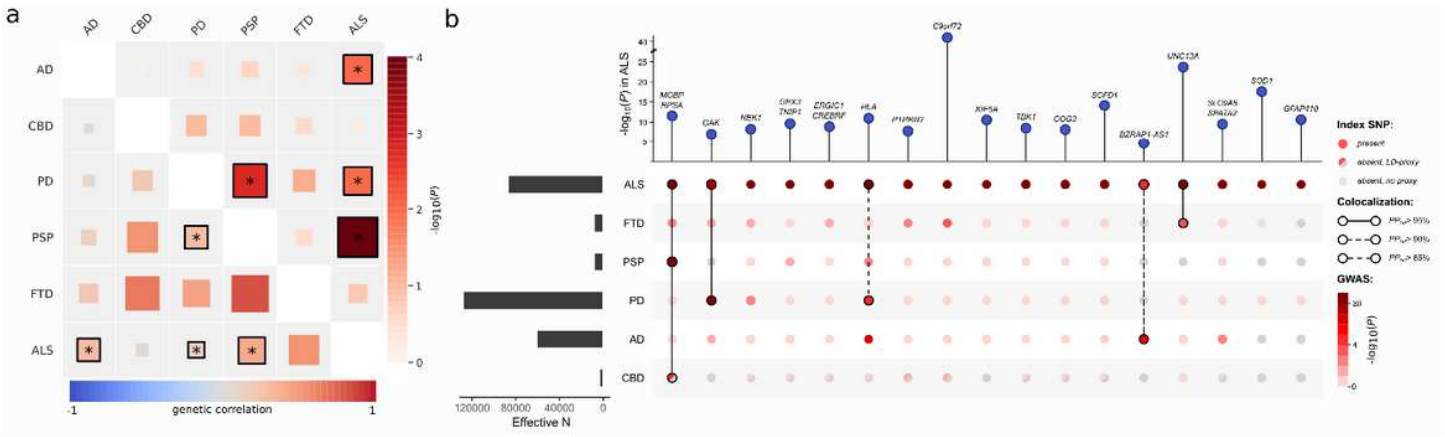

Figure 2

Shared genetic risk among ALS and neurodegenerative diseases. (a) Genetic correlation analysis. Genetic correlation was estimated with LD-score regression between each pair of neurodegenerative diseases being ALS, Alzheimer's disease (AD), corticobasal degeneration (CBD), Parkinson's disease (PD),

progressive supranuclear palsy (PSP), and frontotemporal dementia (FTD). Lower left triangle shows correlation estimate and upper right triangle shows -log10(P-value). Correlations marked with an asterisk were statistically significant P < 0.05. (b) SNP associations of ALS lead SNPs or LD-proxies in neurodegenerative diseases. Effective sample size is shown on the left. Posterior probabilities of the same causal SNP affecting two diseases were estimated through colocalization analysis and highlighted as connections.

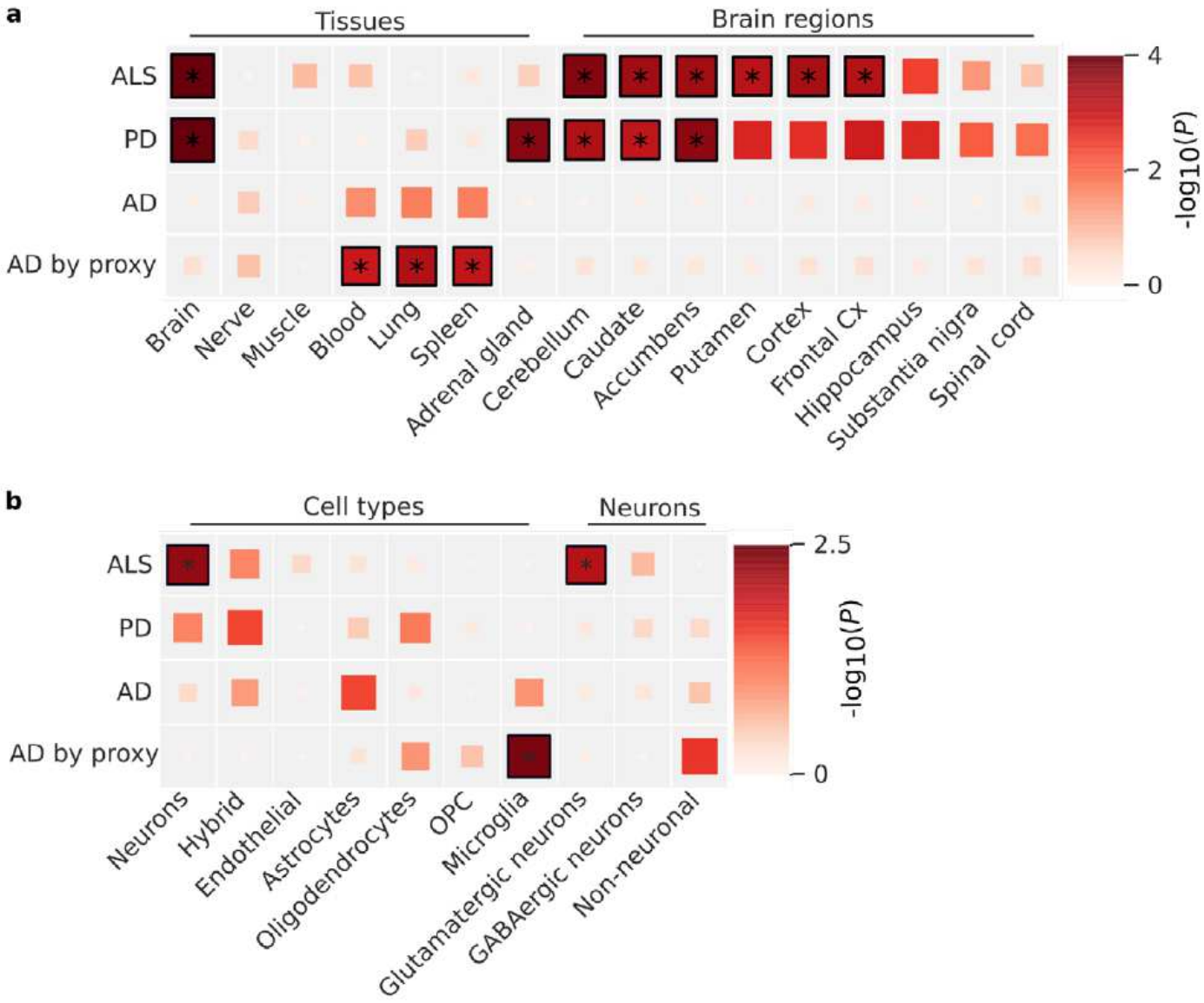

Figure 3

Tissue and cell-type enrichment analysis. (a) Enrichment of tissues and brain regions included in the GTEx v8 illustrates a brain-specific enrichment pattern in ALS, similar to Parkinson's disease but contrasting Alzheimer's disease. (b) Cell-type enrichment analyses indicate neuron-specific enrichment for glutamatergic neurons. No enrichment was found for microglia or other non-neuronal cell-types, contrasting the pattern observed in Alzheimer's disease. Statistically significant enrichments after

correction for multiple testing with a false discovery rate (FDR) < 0.05 are marked with an asterisk. ALS = amyotrophic lateral sclerosis, PD = Parkinson's disease, AD = Alzheimer's disease, Cx = cortex, OPC = oligodendrocyte progenitor cells.

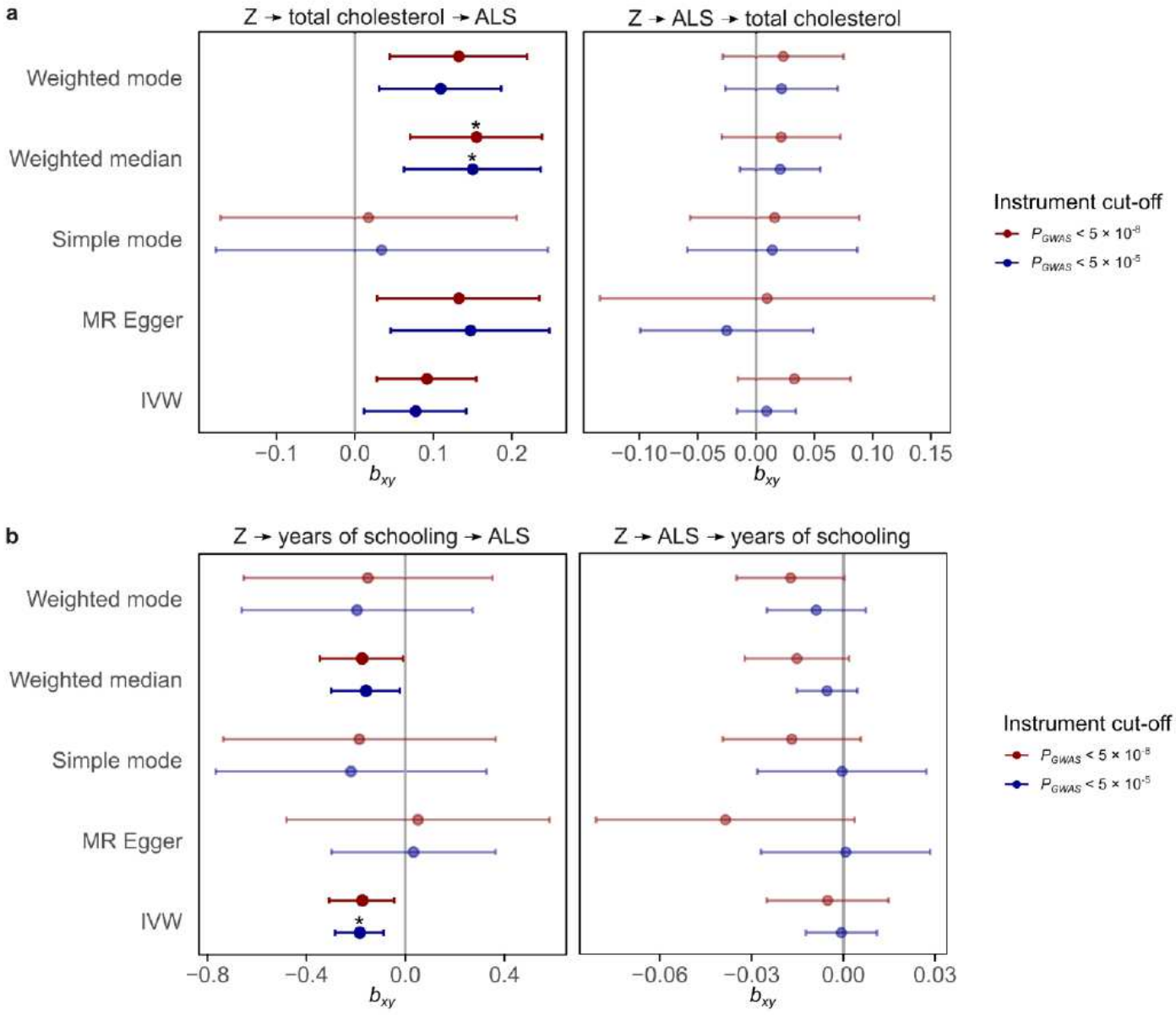

Figure 4

Causal inference of total cholesterol and years of schooling in ALS. (a) Mendelian randomization results for ALS and total cholesterol. Results for the five different Mendelian Randomization methods for two different P-value cut-offs for SNP instrument selection. All methods show a consistent positive effect for an increased risk of ALS with higher total cholesterol levels. There is no evidence for reverse causality. (b) Mendelian randomization results for ALS and years of schooling. Error-bars reflex 95% confidence intervals. Statistically significant effects that pass Bonferroni correction for multiple testing for all tested traits and MR methods are marked with an asterisk. Z = genetic instrument, MR = Mendelian

Randomization, IVW = inverse-variance weighted, bxy = estimated causal effect for one standard deviation increase in genetically predicted exposure.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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