SLITRK2, an X-linked modifier of the age at onset in *C9orf72* frontotemporal lobar degeneration

Authors

Mathieu Barbier[1], PhD; Agnès Camuzat[1], MSc; Khalid El Hachimi[1], PhD; Justine Guegan[1], MSc; Daisy Rinaldi[1,2], PhD; Serena Lattante[3], PhD; Marion Houot[1,2,4], MSc; Raquel Sánchez-Valle[5], MD, PhD; Mario Sabatelli[6], MD; Anna Antonell[5], PhD ; Laura Molina-Porcel[5,7] MD, PhD; Fabienne Clot[8], PhD; Philippe Couratier[9], MD, PhD; Emma van der Ende[10], MD; Julie van der Zee[11,12], PhD; Claudia Manzoni[13], PhD; William Camu[14], MD, PhD; Cécile Cazeneuve[8], PharmD, PhD; François Sellal[15,16], MD, PhD; Mira Didic[17], MD, PhD; Véronique Golfier[18], MD, PhD; Florence Pasquier[19], MD, PhD; Charles Duyckaerts[1,20], MD, PhD; Giacomina Rossi[21], PhD; Amalia C. Bruni[22], MD; Victoria Alvarez[23,24], MD; Estrella Gómez-Tortosa[25], MD, PhD; Alexandre de Mendonça[26], MD; PhD; Caroline Graff[27], MD, PhD; Mario Masellis[28], MD, PhD; Benedetta Nacmias[29], PhD; Badreddine Mohan Oumoussa[30], MSc; Ludmila Jornea[1], BSc; Sylvie Forlani[1], PhD; The French clinical and genetic Research network on FTLD/FTLD-ALS and PREVDEMALS*, The International Frontotemporal Dementia Genomics Consortium**, the European Early Onset Dementia (EU -EOD) Consortium***; Brainbank Neuro-CEB Neuropathology Network****; Neurological Tissue Bank of the Biobank Hospital Clinic-IDIBAPS*****; Viviana Van Deerlin[31], MD, PhD; Jonathan D. Rohrer[32], MD, PhD; Ellen Gelpi[7,33], MD, PhD; Rosa Rademakers[11], PhD; John Van Swieten[10], MD, PhD; Eric Le Guern[8], MD, PhD; Christine Van Broeckhoven[11,12], PhD; Raffaele Ferrari[34], PhD; Emmanuelle Génin[35], PhD; Alexis Brice[1], MD; and Isabelle Le Ber[1,2], MD, PhD.

***The French clinical and genetic research network on FTLD/FTLD-ALS and PREVDEMALS study group includes:** Alexis Brice (Hôpital de la Salpêtrière, Paris), Sophie Auriacombe (CHU Pellegrin, Bordeaux), Serge Belliard (CHU Rennes), Anne Bertrand (Hôpital Pitié-Salpêtrière, Paris), Anne Bissery (Hôpital Pitié-Salpêtrière, Paris), Frédéric Blanc (Hôpitaux Civils, Strasbourg), Marie-Paule Boncoeur (CHU Limoges), Stéphanie Bombois (CHU Roger Salengro, Lille), Claire Boutoleau-Bretonnière (CHU Laennec, Nantes), Agnès Camuzat (ICM, Paris), Mathieu Ceccaldi (CHU La Timone, Marseille), Marie Chupin (ICM, Paris), Philippe Couratier (CHU Limoges), Olivier Colliot (ICM, Paris), Vincent Deramecourt (CHU Roger Salengro, Lille), Mira Didic (CHU La Timone, Marseille), Bruno Dubois (Hôpital de la Salpêtrière, Paris), Charles Duyckaerts (Hôpital de la Salpêtrière, Paris), Frédérique Etcharry-Bouyx (CHU Angers), Aurélie Guignebert-Funkiewiez (Hôpital de la Salpêtrière, Paris), Maïté Formaglio (CHU Lyon), Véronique Golfier (CHU Rennes), Marie-Odile Habert (Hôpital Pitié-Salpêtrière, Paris), Didier Hannequin (CHU Charles Nicolle, Rouen), Lucette Lacomblez (Hôpital de la Salpêtrière, Paris), Julien Lagarde (CHU Sainte Anne, Paris), Géraldine Lautrette (CHU Limoges), Isabelle Le Ber (Hôpital de la Salpêtrière, Paris), Benjamin Le Toullec (ICM, Paris), Richard Levy (Hôpital de la Salpêtrière, Paris), Marie-Anne Mackowiak (CHU Roger Salengro, Lille), Bernard-François Michel (CH Sainte-Marguerite, Marseille), Florence Pasquier (CHU Roger Salengro, Lille), Thibaud Lebouvier (CHU Roger Salengro, Lille), Carole Roué-Jagot (CHU Sainte Anne, Paris), Christel Thauvin-Robinet (CHU Dijon), Catherine Thomas-Anterion (Plein-Ciel, Lyon), Jérémie Pariente (CHU Rangueil, Toulouse), François Salachas (Hôpital Pitié-Salpêtrière, Paris), Sabrina Sayah (Hôpital Pitié-Salpêtrière, Paris), François Sellal (CH Colmar), Assi-Hervé Oya (Hôpital Pitié-Salpêtrière, Paris), Daisy Rinaldi (Hôpital Pitié-Salpêtrière, Paris), Adeline Rollin-Sillaire (CHU Roger Salengro, Lille), Martine Vercelletto (CHU Laennec, Nantes) and David Wallon (CHU Rouen), Armelle Rametti-Lacroux (ICM, Paris).

****The International Frontotemporal Dementia Genomics Consortium includes:**

Funding, as well as full locations and affiliations details can be found in the Appendix 1: Raffaele Ferrari, Dena G. Hernandez, Michael A. Nalls, Jonathan D. Rohrer, Adaikalavan Ramasamy, John B. J. Kwok, Carol Dobson-Stone, William S. Brooks, Peter R. Schofield, Glenda M. Halliday, John R. Hodges, Olivier Piguet, Lauren Bartley, Elizabeth Thompson, Isabel Hernández, Agustín Ruiz, Mercè Boada, Barbara Borroni, Alessandro Padovani, Carlos Cruchaga, Nigel J. Cairns, Luisa Benussi, Giuliano Binetti, Roberta Ghidoni, Gianluigi Forloni, Diego Albani, Daniela Galimberti, Chiara Fenoglio, Maria Serpente, Elio Scarpini, Jordi Clarimón, Alberto Lleó, Rafael Blesa; Maria Landqvist Waldö, Karin Nilsson, Christer Nilsson, Ian R. A. Mackenzie, Ging-Yuek R. Hsiung, David M. A. Mann, Jordan Grafman, Christopher M. Morris, Johannes Attems, Timothy D. Griffiths, Ian G. McKeith, Alan J. Thomas, Pietro Pietrini, Edward D. Huey, Eric M. Wassermann, Atik Baborie, Evelyn Jaros, Michael C. Tierney, Pau Pastor, Cristina Razquin, Sara Ortega-Cubero, Elena Alonso, Robert Perneczky, Janine Diehl-Schmid, Panagiotis Alexopoulos, Alexander Kurz, Innocenzo Rainero, Elisa Rubino, Lorenzo Pinessi, Ekaterina Rogaeva, Peter St George-Hyslop, Giacomina Rossi, Fabrizio Tagliavini, Giorgio Giaccone, James B. Rowe, Johannes C. M. Schlachetzki, James Uphill, John Collinge, Simon Mead, Adrian Danek, Vivianna M. Van Deerlin, Murray Grossman, John Q. Trojanowski, Julie van der Zee, Christine Van Broeckhoven, Stefano F. Cappa, Isabelle Leber, Didier Hannequin, Véronique Golfier, Martine Vercelletto, Alexis Brice, Benedetta Nacmias, Sandro Sorbi, Silvia Bagnoli, Irene Piaceri, Jørgen E. Nielsen, Lena E. Hjermind, Matthias Riemenschneider, Manuel Mayhaus, Bernd Ibach, Gilles Gasparoni, Sabrina Pichler, Wei Gu, Martin N Rossor, Nick C. Fox, Jason D. Warren, Maria Grazia Spillantini, Huw R. Morris, Patrizia Rizzu, Peter Heutink, Julie S. Snowden, Sara Rollinson, Anna Richardson, Alexander Gerhard, Amalia C. Bruni, Raffaele Maletta, Francesca Frangipane, Chiara Cupidi, Livia Bernardi, Maria Anfossi, Maura Gallo,

Maria Elena Conidi, Nicoletta Smirne, Rosa Rademakers, Matt Baker, Dennis W. Dickson, Neill R. Graff-Radford, Ronald C. Petersen, David Knopman, Keith A. Josephs, Bradley F. Boeve, Joseph E. Parisi, William W. Seeley, Bruce L. Miller, Anna M. Karydas, Howard Rosen, John C. van Swieten, Elise G. P. Dopper, Harro Seelaar, Yolande A. L. Pijnenburg, Philip Scheltens, Giancarlo Logroscino, Rosa Capozzo, Valeria Novelli, Annibale A. Puca, Massimo Franceschi, Alfredo Postiglione, Graziella Milan, Paolo Sorrentino, Mark Kristiansen, Huei-Hsin Chiang, Caroline Graff, Florence Pasquier, Adeline Rollin, Vincent Deramecourt, Thibaud Lebouvier, Dimitrios Kapogiannis, Luigi Ferrucci, Stuart Pickering-Brown, Andrew B. Singleton, John Hardy, Parastoo Momeni.

***** The European Early Onset Dementia (EU-EOD) Consortium includes:**

Janine Diehl-Schmid (Technische Universität München, München, Germany); Matthis Synofzik (Hertie Institute for Clinical Brain Research and Centre of Neurology, and DZNA, Tübingen, Germany); Alfredo Ramirez (University of Bonn, Bonn, Germany; University of Cologne, Cologne, Germany); Michael Heneka (University of Bonn and DZNE, Bonn, Germany); Frank Jessen (University of Bonn and DZNE, Bonn, Germany; University of Cologne, Cologne, Germany); Raquel Sanchez-Valle, Albert Llado (Hospital Clínic, IDIBAPS, Barcelona, Spain); Jordi Clarimón (Universitat Autònoma de Barcelona, Barcelona, Spain; CIBERNED, Madrid, Spain); Isabel Hernández, Mercè Boada, Agustín Ruiz (Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain); Pau Pastor (University of Barcelona School of Medicine, Terrassa, Barcelona, Spain; CIBERNED, Madrid, Spain); Estrella Gómez-Tortosa (Fundación Jiménez Díaz, Madrid, Spain); Manuel Menéndez Gonzalez, Victoria Álvarez (HUCA and Oviedo University, Oviedo, Spain); Alexandre de Mendonça, Gabriel Miltenberger-Miltényi (Hospital Santa

Maria and University of Lisbon, Lisbon, Portugal); Isabel Santana, Maria Rosário Almeida (University of Coimbra, Coimbra, Portugal); Barbara Borroni, Alessandro Padovani (University of Brescia, Brescia, Italy); Luisa Benussi, Roberta Ghidoni (IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy); Giovanni B Frisoni (IRCCS Fatebenefratelli, Brescia, Italy); Benedetta Nacmias (University of Florence, Florence, Italy), Sandro Sorbi (IRCCS Don Carlo Gnocchi Scandicci, and University of Florence, Florence, Italy); Gian Maria Fabrizi, Silvia Testi (University of Verona, Verona, Italy); Gabor G. Kovacs (Medical University of Vienna, Vienna, Austria); Radoslav Matej (Thomayer Hospital, Prague and Charles University, Prague, Czech Republic); Caroline Graff (Karolinska University Hospital and Karolinska Institutet, KI-Alzheimer Disease Research Center, Stockholm, Sweden); Matthew J. Fraidakis (NeuroRARE Centre for Rare and Genetic Neurological & Neuromuscular Diseases & Neurogenetics Athens, Greece); Magda Tsolaki (Aristotle University of Thessaloniki, Makedonia, Greece) .

****** The Brainbank Neuro-CEB Neuropathology Network includes:**

Dr Franck Letournel (CHU Angers), Dr Marie-Laure Martin-Négrier (CHU Bordeaux), Pr Françoise Chapon (CHU Caen), Pr Catherine Godfraind (CHU Clermont-Ferrand), Pr Claude-Alain Maurage (CHU Lille), Dr Vincent Deramecourt (CHU Lille), Dr David Meyronnet (CHU Lyon), Dr Nathalie Streichenberger (CHU Lyon), Dr André Maues de Paula (CHU Marseille), Pr Valérie Rigau (CHU Montpellier), Dr Fanny Vandenbos-Burel (Nice), Pr Charles Duyckaerts (CHU PS Paris), Pr Danielle Seilhean (CHU PS, Paris), Dr Susana Boluda (CHU PS, Paris), Dr Isabelle Plu (CHU PS, Paris), Dr Serge Milin (CHU Poitiers), Dr Dan Christian Chiforeanu (CHU Rennes), Pr Annie Laquerrière (CHU Rouen), Dr Béatrice Lannes (CHU Strasbourg).

*****Neurological Tissue Bank of the Biobank Hospital Clinic-IDIBAPS includes**:

Full locations and affiliations details can be found in the Appendix 1: Raquel Sánchez-Valle, Albert Lladó, Anna Antonell, Sergi Borrego-Écija, Isabel Hernandez, Miquel Aguilar, Ricardo Rojas-Garcia, Alberto Lleo, Sonia Sirisi, Monica Povedano, Isidre Ferrer, Jordi Gascón, Glòria Garrabou, Dolores Lopez-Villegas, Jose Álvarez Sabin, Lorena Bajo Peñas, Oscar Macho, Isabel Collado, Rosa De Eugenio, Ana Escrig Avellaneda.

Affiliations

[1]Sorbonne Université, Institut du Cerveau-Paris Brain Institute-ICM, Hôpital de la Salpêtrière, Inserm U 1127, CNRS UMR 7225, 75013, Paris, France.

[2]Center for Rare or Early-Onset Dementias, IM2A, Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France.

[3]Sezione di Medicina Genomica, Dipartimento Scienze della Vita e Sanità Pubblica, Facoltà di Medicina e Chirurgia, Università Cattolica Sacro Cuore; U.O.C. Genetica Medica, Dipartimento di Scienze di Laboratorio e Infettivologico, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy.

[4]Centre of Excellence of Neurodegenerative Disease (CoEN), Hôpital Pitié-Salpêtrière, Paris, France.

[5]Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS),University of Barcelona, Barcelona, Catalunya, 08036 Spain.

[6]Adult NEMO Clinical Center, Unit of Neurology, Department of Aging, Neurological, Orthopedic and Head-Neck Sciences, Fondazione Policlinico Universitario A. Gemelli IRCCS, 00168 Rome, Italy; Section of Neurology, Department of Neuroscience, Faculty of Medicine and Surgery, Università Cattolica del Sacro Cuore, 00168 Rome, Italy.

[7]Neurological Tissue Bank of the Biobank-Hospital Clinic-IDIBAPS, Barcelona, Catalunya, Spain.

[8]Unité Fonctionnelle de Neurogénétique Moléculaire et Cellulaire, Département de Génétique et Cytogénétique, AP-HP, Hôpitaux Universitaires La Pitié Salpêtrière-Charles Foix, Paris, France.

[9]Centre Démences rares University Hospital Limoges, Limoges, France.

[10]Department of Neurology, Erasmus University Medical Center, Doctor Molewaterplein 40, 3015 GD Rotterdam, the Netherlands.

[11]Neurodegenerative Brain Diseases Group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium.

[12]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.

[13]School of Pharmacy, University of Reading, Whiteknights, Reading, UK.

[14]Reference Centre for ALS, University Hospital Gui de Chauliac, University of Montpellier, Montpellier, France.

[15]Neurology Department, Hôpitaux Civils de Colmar, France.

[16]INSERM U-1118, Strasbourg University, Strasbourg, France.

[17]APHM, Timone, Service de Neurologie et Neuropsychologie, Hôpital Timone Adultes, Marseille, France ; Aix Marseille Univ, INSERM, INS, Inst Neurosci Syst, Marseille, France.

[18]Service de Neurologie, Centre Hospitalier Yves Le Foll, Saint Brieuc, France.

[19]University of Lille, Inserm UMRS1172, CHU, DISTAlz, LiCEND, F-59000 Lille, France.

[20]Laboratoire de Neuropathologie Escourolle, Hôpital de la Pitié-Salpêtrière, AP-HP, Paris, France.

[21]Division of Neurology V and Neuropathology; Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy.

[22]Regional Neurogenetic Centre, Department of Primary Care, ASP-CZ, Catanzaro, Italy.

[23]Laboratorio de Genética- Hospital Universitario Central de Asturias, Oviedo, Spain.

[24]Instituto de INvestigación Biosanitaria del Principado de Asturias (ISPA); Avda de Roma s/n 33011, Oviedo, Spain.

[25]Department of Neurology, Fundación Jiménez Díaz, Madrid, Spain.

[26]Faculty of Medicine, University of Lisbon, Lisbon, Portugal.

[27]Department of Geriatric Medicine, Karolinska University Hospital-Huddinge, Stockholm, Sweden.

[28]Hurvitz Brain Sciences Program, Sunnybrook Research Institute; Department of Medicine (Neurology), Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Canada.

[29]Department of Neuroscience, Psychology, Drug Research and Child Health - University of Florence – Florence, Italy; IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy.

[30]Sorbonne Université, Inserm, UMS Production et Analyse des données en Sciences de la vie et en Santé, PASS, Plateforme Post-génomique de la Pitié-Salpêtrière, P3S, F-75013, Paris, France.

[31]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.

[32]Dementia Research Centre, Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, University College London, London, UK.

[33]Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Vienna, Austria.

[34]University College London, Institute of Neurology, London, UK.

[35]Génétique, Génomique Fonctionnelle et Biotechnologies, Faculté de Médecine, Univ Brest, Inserm UMR1078, Brest, France.

Word/item count: Title: 94 characters; Abstract: 315 words; Text: 5956 words; References: 42; Figures: 4; Tables: 4; Supplementary materials: supplementary figures: 4; supplementary tables: 4; supplementary Materials and Methods: 1; Appendix: 1.

Corresponding author: Dr Isabelle Le Ber, Institut du Cerveau (ICM), AP-HP - Hôpital Pitié-Salpêtrière, 47, boulevard de l'Hôpital, 75013 Paris, France. Email: isabelle.leber@upmc.fr

Abstract

The G_4C_2-repeat expansion in *C9orf72* is the most common cause of frontotemporal dementia and of amyotrophic lateral sclerosis. The variability of age at onset and phenotypic presentations is a hallmark of *C9orf72* disease. In this study, we aimed to identify modifying factors of disease onset in *C9orf72* carriers using a family-based approach, in pairs of *C9orf72* carrier relatives with concordant or discordant age at onset. Linkage and association analyses provided converging evidences for a *locus* on chromosome Xq27.3. The minor allele A of rs1009776 was associated with an earlier onset ($p=1x10^{e-5}$). The association with onset of dementia was replicated in an independent cohort of unrelated *C9orf72* patients (p=0.009). The protective major allele delayed the onset of dementia from 5 to 13 years on average depending on the cohort considered. The same trend was observed in an independent cohort of *C9orf72* patients with extreme deviation of the age at onset (p=0.055). No association of rs1009776 was detected in *GRN* patients, suggesting that the effect of rs1009776 was restricted to the onset of dementia due to *C9orf72*. The minor allele A is associated with a higher *SLITRK2* expression based on both eQTL databases and *in-house* expression studies performed on *C9orf72* brain tissues. *SLITRK2* encodes for a post-synaptic adhesion protein. We further show that synaptic vesicle glycoprotein 2 and synaptophysin, two synaptic vesicle proteins, were decreased in frontal cortex of *C9orf72* patients carrying the minor allele. Up-regulation of *SLITRK2* might be associated with synaptic dysfunctions and drives adverse effects in *C9orf72* patients that could be modulated in those carrying the protective allele. How the modulation of *SLITRK2* expression affects synaptic functions and influences the disease onset of dementia in *C9orf72* carriers will require further investigations. In summary, this study describes an original approach to detect modifier genes in rare diseases, and reinforces rising links between *C9orf72* and synaptic dysfunctions that might directly influence the occurrence of first symptoms.

Keywords: Frontotemporal Dementia; Amyotrophic lateral sclerosis; C9orf72; TDP-43; SLITRK2.

Introduction

Frontotemporal dementia (FTD) is the second cause of degenerative dementia in the presenium, after Alzheimer's disease. *C9orf72*, *GRN* and *MAPT* are the major genes implicated in autosomal dominant forms of FTD (Hutton *et al.*, 1998; Baker *et al.*, 2006; Cruts *et al.*, 2006; DeJesus-Hernandez *et al.*, 2011; Renton *et al.*, 2011). The GGGGCC (G_4C_2) repeats expansion in *C9orf72* is also a major genetic cause of amyotrophic lateral sclerosis (ALS). Most healthy individuals carry fewer than 24 G_4C_2 units, whereas patients usually have up to thousands of repeats (DeJesus-Hernandez *et al.*, 2011; Renton *et al.*, 2011). Three mechanisms have been proposed to explain the pathogenicity: the formation of nuclear RNA *foci*, the presence of dipeptide repeat proteins generated by repeat-associated non-ATG translation, and C9orf72 loss-of-function (Lagier-Tourenne *et al.*, 2013).

Besides the heterogeneity of clinical phenotypes, the age at onset (AO) remarkably varies in *C9orf72* disease. Individuals carrying repeat expansions can develop behavioral or motor symptoms from the third decade of life to a nearly incomplete penetrance in elderly mutation carriers. This extensive variability of AO remains largely unexplained so far. A high heritability of AO was evidenced in *C9orf72* families, suggesting a strong effect of genetic modifiers in addition to the causative mutation (Barbier *et al.*, 2017).

Identifying environmental and/or genetic factors influencing the age at onset in *C9orf72* carriers is a great challenge to improve genetic counselling, and define the better time lapse to initiate forthcoming therapeutic trials. In contrast with other repeat expansion disorders, the

size of the *C9orf72* expansion in lymphocytes is not a reliable marker to predict AO (Suh *et al.*, 2015; Fournier *et al.*, 2019; Jackson *et al.*, 2020). So far, studies evaluating the influence of other genetic variations on penetrance or AO variability in *C9orf72* carriers remain limited. *TMEM106B* rs6966915 is a known modifier of penetrance in FTD-*GRN* (Van Deerlin *et al.*, 2010). Its role in *C9orf72* carriers is much more controversial, as rs6966915 minor allele has been alternatively associated either with reduced risk to develop FTD-*C9orf72*, or with an earlier age at onset, in contradictory studies (Gallagher *et al.*, 2014; van Blitterswijk *et al.*, 2014). In another study, we evidenced that *TMEM106B* has no major impact on the AO or disease penetrance in *C9orf72* carriers (Lattante *et al.*, 2014). More recently, *C6orf10* rs9357140 was found to be associated with AO in ~~FTD~~ *C9orf72* disease using a DNA methylation-based approach (Zhang *et al.*, 2018). However, this association did not completely explain the extreme variability of AO. Thus, other modifiers of AO remain to be identified (Koçoğlu *et al.*, 2020).

In this study, we searched for new modifying factors of AO in *C9orf72* carriers. A family-based approach, followed by replication analyses in unrelated patients, provided converging evidences of linkage and association with AO of FTD for one polymorphism (rs1009776) on chromosome X. We further showed that this eQTL influences the expression of the nearby gene *SLITRK2* in frontal cortex, and provides functional hypotheses on how this polymorphism, *SLITRK2* and alterations of synaptic vesicular process might influence disease onset.

Materials and Methods

Discovery cohort - Pairs of *C9orf72* relatives with concordant/discordant AO

Patients of the discovery cohort were selected among a large cohort of 590 *C9orf72* patients from 424 families (424 probands, 166 affected relatives). They were enrolled by expert neurologists of a national research network on FTLD/FTLD-ALS (project #RBM02-59) and PREVDEMALS study group, as previously described (Le Ber *et al.*, 2006). Five patients (forming concordant or discordant pairs) from two Dutch families were also included. AO of affected subjects were reviewed by two evaluators based on patient's clinical charts and on caregiver's interviews, as previously described (Le Ber *et al.*, 2006). AO of FTD was defined as the age of occurrence of the first symptom (either behavioral, language, or motor), as reported by the patient or the principal informant. In most cases, a second informant was questioned independently to accurately determine the disease onset. The age of ALS onset was self-reported by patients. Patients/families with inaccurate information were excluded.

A family-based design prioritizing the analysis of related patients with either concordant or discordant AO was adopted (Figure 1). This methodology, originally used in pioneer linkage analyses, has been successfully applied in other rare diseases to identify genetic modifiers (Aubart *et al.*, 2018). Here, AO was defined as concordant between two members of the same family when the difference of AO was ≤3 years. Pairs of relatives with a difference of AO≥9 years were considered as discordant. Thus, 50 concordant or discordant pairs from 34 families were included in linkage and association analyses. The mean difference in the 20 concordant and in the 30 discordant pairs were 1.9±0.9 years and 19.0±7.8 years, respectively, the AO ranging from 30 to 81 years. Their clinical and demographic characteristics are presented in Table 1. Relatedness of each pair, differences of AO, and chronology of onset (early, intermediate, or late onset) are given in supplementary Table S1.

Replication cohort 1– Cohort of unrelated *C9orf72* patients from the International FTD-Genomics Consortium (IFGC)

For replication, genotypes of the 12 candidates SNPs identified in the discovery phase were extracted from an independent cohort of *C9orf72* patients, obtained from the International FTD-Genomics Consortium (IFGC, https://ifgcsite.wordpress.com/). The phase I cohort of the IFGC included 124 unrelated *C9orf72* patients, without selection criteria regarding the AO (Table 1). The AO ranged from 34 to 77 years. All individuals were Caucasians. The absence of relatedness among the 124 patients was checked prior to any analyses. There was no redundancy between patients of the discovery and the replication cohorts. More details about this cohort are provided elsewhere (Ferrari *et al.*, 2014).

Replication cohort 2- Unrelated *C9orf72* patients with extreme deviation of AO

Another independent cohort of 159 unrelated *C9orf72* patients with "extreme" (early or late) AO was studied in the second replication phase. These patients had been enrolled by the French research network on FTLD/FTLD-ALS (n=140) or the EU-EOD European Early-Onset Dementia Consortium (n=19). None of these patients was included in the discovery or the first replication cohort. The selection criterion for early onset (EO) was defined by AO≤53 years, and for late onset (LO) by AO≥67 years. Ninety-five patients presented with an early-onset (45.47 ± 6.20 years) and 64 had late onset (72.03 ± 4.38 years). The patients (86 males and 73 females) were included in association analyses studying AO as a binary phenotype, in absence of relatedness between patients from the same group. Detailed information on this cohort is indicated in Table 1.

Replication cohort 3- Unrelated *C9orf72* patients with isolated ALS

We looked at the association of our most robust candidate SNP with onset of ALS. To this end, we analyzed a new independent cohort of 109 *C9orf72* patients with predominant or isolated ALS without dementia (Policlinico Universitario A. Gemelli in Roma, Italy; French research network on FTD/FTD-ALS) (Table 1). Age of ALS appearance was established on patient reported first symptom onset.

Cohort of FTD patients with mutations in *GRN* ("FTD non-*C9orf72*")

In a further step, we evaluated if identified SNPs were specifically associated with AO in *C9orf72* patients, or may also influence AO in other genetic forms of FTD. For this purpose, patients with *GRN* gene mutations were analyzed, ensuring definite diagnosis of FTD and a pathological homogeneity. Pairs of relatives with concordant or discordant AO were selected out of a large cohort of 181 *GRN* patients from 141 Caucasian families to replicate the approach used in FTD-*C9orf72* (Sellami *et al.*, 2020). The same criterion to select concordant or discordant pairs of relatives were applied. In this cohort, 50 patients from 22 families forming 33 concordant or discordant pairs were selected. Mean difference of AO between relatives of the 13 concordant and 20 discordant pairs were 1.8 ± 1.1 years and 15.5 ± 7.0 years, respectively. None of these patients carried *C9orf72* repeat expansion. Additional information about gender and AO are available in Table 1.

Brain post-mortem material

Brain tissue from 28 *C9orf72* patients (15 males, 13 females) was used for expression studies and immunostaining analyses. Paraffin-embedded sections (5µm thickness) and/or frozen samples (frontal cortex) of 17 *C9orf72* patients were obtained from the Barcelona Neurological Tissue Bank of the Biobank-HC-IDIBAPS. Frozen tissue (frontal cortex) of 11

additional patients was provided by the NeuroCEB brain biobank. Prior to any experiments, the genotypes of rs1009776 were determined on DNA extracted from frozen samples using the TaqMan™ SNP genotyping assay C__8338994_10 (Applied Biosystems). We selected 16 cases with genotypes hemi- or homozygous A or C for further expression studies on frozen tissue to exclude the confounding effect of X-chromosome inactivation (XCI) in heterozygous females. The mean repeat expansion size, determined in 15/16 brain samples as previously described (Fournier *et al.*, 2019),was similar between rs1009776 C/CC carriers (mean=1933 repeats, SD=115.1) and A/AA carriers (mean=1943 repeats, SD=320.5, Mann Whitney U test p=0.76). Repeat expansion sizes for each brain sample are reported in the supplementary Table S2. Among all cases with paraffin-embedded sections available, three were A/AA carriers, and were compared to four C/CC carriers for immunostainings experiments. Both frozen tissue and paraffin sections were available for these seven patients, allowing comparisons of expression and immunostaining experiments on the same patients' samples.

Ethics

All participants were included in research studies after written informed consent was obtained from the patients or their guardians, in agreement with their national bioethics laws. Approvals from Ethics Committees from each cohort regarding patients' biological and brain tissues samples included in this study are detailed in the supplementary Materials and Methods.

Genotyping and Quality Controls (QC)

Whole-Genome-Genotyping (WGG) was performed using Illumina Infinium OmniExpressExome-8 v1.4/1.6 arrays in the 75 *C9orf72* patients of the discovery cohort, and in the 50 *GRN* carriers. Genotyping and downstream QC were done using the same pipeline

described in the supplementary Materials and Methods. Genotypes of *TMEM106B* rs6966915 and *C6orf10* rs9357140 were extracted to create covariates in association analyses, and the whole genotyping dataset was used to compute the kinship matrix. Imputations of non-genotyped SNPs were performed as described in supplementary Materials and Methods.

Genotypes from 12 SNPs were directly obtained from the IFGC as well as genotypes for *TMEM106B* rs6966915 and *C6orf10* rs9357140 to build a replication cohort (Ferrari *et al.*, 2014).

The candidate SNP rs1009776 was also genotyped in the group of 159 unrelated *C9orf72* patients with extreme AO and in the cohort of 109 *C9orf72* patients with ALS using the TaqMan™ SNP genotyping assay C__8338994_10 (Applied Biosystems) following manufacturer's instructions.

Linkage analyses

Linkage analyses were performed on a subset of SNPs obtained after linkage disequilibrium (LD) pruning using PLINK (a r^2 threshold of 0.2 was used). Both parametric (recessive, additive or dominant models) and non-parametric linkage tests (Kong and Cox LOD score from a linear model) were tested using MERLIN 1.1.2 (Abecasis *et al.*, 2002). A disease allele frequency of 0.001 was assumed in parametric analyses. Data from chromosome X were analyzed using the dedicated program MINX from the MERLIN package (Abecasis *et al.*, 2002).

Association tests

Genome-wide Association Studies with univariate linear mixed models

Genome-wide association analyses with AO as a quantitative trait among relatives were conducted in patients carrying *C9orf72* expansions (discovery and ALS patient's cohorts) as

well as in patients carrying *GRN* mutations. We used the Genome-wide Efficient Mixed Model Association algorithm implemented in the software GEMMA v0.94.1 (Zhou and Stephens, 2012). This software notably runs Genome-wide Association Studies with univariate linear mixed models taking into account relatedness between individuals. In particular, GEMMA can fit a univariate linear mixed model to correct for population stratification, cryptic relationship, and kinship to handle familial data. The kinship matrix was created from LD-pruned genotypes of *C9orf72* relatives and applied in the linear mixed model. Genome-wide Association tests were also adjusted for sex, *TMEM106B* rs6966915 and *C6orf10* rs9357140 genotypes as covariates included in the model. Results of the association test included the beta coefficient (β), standard error (se) and adjusted p-value derived from the likelihood ratio test (pLRT) for each variant. Manhattan and Q-Q plots of p-values were constructed using the qqman R package (Figure 2 and supplementary Figure S1). The genomic inflation factor was calculated using the R package GenABEL and did not suggest major confounding factors in the analysis of the discovery cohort (λ_{GC}=1.05) (Aulchenko *et al.*, 2007). Risk of developing ALS was considered as a binary trait in a separate analysis and analyzed with GEMMA, patients who developed ALS or FTD-ALS being coded as affected (Zhou and Stephens, 2012).

Linear and logistic regressions

The association between rs1009776 genotypes and AO or clinical phenotype (risk of developing ALS) was then tested in the independent cohort of 124 unrelated *C9orf72* carriers obtained from the IFGC, as described above. There was no deviation of genotypes frequencies from Hardy-Weinberg equilibrium (p=0.36). Linear and logistic regressions were both performed using PLINK software v1.90b6.2 to test for association with AO as a quantitative trait or risk to develop ALS (all using the --xchr-model command available in PLINK to handle X-linked genotypes). Sex, *TMEM106B* rs6966915 and *C6orf10* rs9357140 genotypes

were included as covariates. Beta coefficient (β) for the linear regression or odds ratio (OR) for the logistic regression and standard error (se) are provided along with corresponding adjusted p-values. Linear regression adjusted for sex was also used to test for the association between rs1009776 and AO of ALS in an independent cohort of 109 unrelated *C9orf72* carriers. There was no deviation of genotypes frequency from Hardy-Weinberg equilibrium (p=0.25).

Additional analyses were conducted on unrelated *C9orf72* patients with early (EO) *vs.* late onset (LO). Patients with EO and LO were considered as affected and unaffected, respectively. No deviation of rs1009776 genotypes frequencies from the Hardy-Weinberg equilibrium was observed in all analyses. PLINK software v1.90b6.2 was used to perform logistic regressions as described above (Purcell *et al.*, 2007; Chang *et al.*, 2015).

Cox proportional hazard regression models

Cox proportional hazard regression models were first applied in the discovery and in the replication cohort 1 (IFGC) to assess for the effect of rs1009776 minor allele on AO, before estimating the pooled Hazard Ratio (HR) from the meta-analysis. The R coxme 2.2-16 package was used for the discovery cohort to adjust for familial belonging. Briefly, this approach can fit Cox proportional hazards models including familial interactions with a kinship matrix using a ``frailty'' model (Ripatti and Palmgren, 2000). Thus, HR are adjusted for familial relationships. The coxph function from survival 3.1-12 package was used in the replication cohort including unrelated individuals to perform the Cox proportional hazards model. All analyses were adjusted on sex, *TMEM106B* rs6966915 and *C6orf10* rs9357140 genotypes. The adjusted HR with 95% confidence interval (CI), and corresponding p-values from likelihood ratio tests based on the risk allele frequency are presented.

A meta-analysis to assess the pooled Cox regression coefficient HR from the discovery and replication 1 cohorts was performed using the R metafor 2.4-0 package with a fixed effect model. Both allele- or genotype-dependent tests were tested.

SLITRK2 expression studies

The functional impact of rs1009776 genotypes on the expression of *SLITRK2* transcript (NM_032539.5) was then evaluated. We quantified *SLIRTK2* transcript levels in brain tissue of 16 *C9orf72* patients, homo- or hemizygous for allele C (n=11) or A (n=5). RNA was extracted from frozen brain tissue using the RNeasy lipid tissue mini kit (Qiagen). RNA quality was evaluated using the Bioanalyser 2100 system (Agilent Technologies). cDNA synthesis was performed on total RNA with a mixture of oligo(dT) and random primers by using the Maxima First Strand cDNA Synthesis kit (ThermoFisher Scientific) following manufacturer instructions. Quantitative PCR (qPCR) was carried out in triplicate on the LC480 LightCycler (Roche) using FastStart Essential DNA Green Master (Roche). Normalized relative quantities were calculated using the $2^{-\Delta\Delta Ct}$ method with *XPNPEP1* and *AARS* as reference genes. *SLITRK2* transcripts levels were compared between C and A carriers using Mann Whitney U test. Primers are provided in supplementary Table S3.

Immunostaining analyses on brain tissue, images acquisition and normalization

To quantify the relationship between rs1009776 genotypes and synaptic vesicles densities, we used SV2 (synaptic vesicle glycoprotein 2) and SYP (synaptophysin) antibodies. Mouse SV2 antibodies were deposited to the DSHB (Developmental Studies Hybridoma Bank) by Buckley, K.M. (DSHB Hybridoma Product SV2). SYP monoclonal antibody was purchased from Dako.

Both immunostainings were achieved on paraffin sections from frontal cortex of patients homo- or hemizygous for allele A (n=3) or C (n=4). Noteworthy, all paraffin sections were obtained from the IDIBAPS brain biobank, and were fixed and treated using the same protocol. Paraffin sections from the seven patients were treated simultaneously for each immunostaining. The SV2 and SYP staining were performed independently in blinded manner, at the Paris Brain Institute and at the Medical University of Vienna, respectively.

Quantification of the two immunostainings was realized using the same protocol as follow. Three regions of interest (ROI) were defined for each patient: two different ROI were selected in the grey matter for duplicates and covered cortical layers III, IV and V; one ROI was defined *per* section in white matter to normalize the signal. Finally, two normalized measures of cortical staining were available *per* patient to calculate a mean. Immunostaining intensities and *SLITRK2* transcript levels were correlated using Spearman correlation tests. The complete protocol for immunostaining, images acquisition and normalization of signals is available in the supplementary Materials and Methods. No commercial antibodies for SLITRK2 provided satisfactory results.

Data availability

Data are available upon reasonable requests.

Results

Linkage analyses

Linkage analyses in the discovery cohort of relatives with concordant or discordant AO provided suggestive evidence of linkage with a LOD score >2 in 2 *loci*, on chromosome 9 and X (Figure 2A).

The X-linked region lies on chromosome Xq27.3. A max LOD score = 2.13 was observed. Nine genes map to this *locus*: *MAGEC3*, *MAGEC1*, *SPANXN4*, *SPANXN3*, *SLITRK4*, *SPANXN2*, *UBE2NL*, *SPANXN1*, and *SLITRK2*. As model-dependent linkage analyses hardly take into account the influence of random X-chromosome inactivation, we also performed a model-free linkage analysis (non-parametric linkage). A suggestive signal of linkage (max LOD score=2.11) was detected at the same location, restricted to the *SLITRK2 locus* (supplementary Figure S2).

On the autosome, a suggestive evidence for linkage was observed on chromosome 9 (region 9p21.2) under an additive model (max LOD score=2.32). All other models (different mode of inheritance and allele frequencies) did not improve the results. The large region of positive linkage (around 20 Mb) includes the *C9orf72 locus*, at the centromeric limit. However, the peak of linkage lies the *PTPRD locus* on chromosome 9p23, at the opposite telomeric side of the region (Figure 2A). There was no linkage disequilibrium between these two distant regions (data not shown).

Association analyses with AO in concordant/discordant pairs of *C9orf72* relatives

A mixed linear model taking into account the kinship between relatives was performed to evaluate the association of frequent SNPs (MAF>1%) with AO as a quantitative trait (Figure 2B). No SNP displayed a p-value reaching the genome-wide significant threshold of $1x10^{-8}$. However, a suggestive association with AO ($p \leq 1x10^{-5}$) was detected for 12 SNPs localized in eight different *loci* (Table 2). These SNPs are located in or near seven genes: *CTNNA2*, *LRRTM1*, *UMAD1*, *RPA3*, *OXR1*, *DAAM1*, and *SLITRK2*.

Among these variants, rs1009776, which lies 10 Kb upstream of *SLITRK2* on chromosome X, was the only one located in a region of positive linkage. The minor allele A was associated with an earlier AO. The median AO for hemizygous males and homozygous females for the

major allele C was 62.5 years, 47.5 years for heterozygous females, and 46.0 years for hemizygous males and females homozygous for the allele A (Table 3).

Association analyses using imputed genotypes did not allow the detection of any other signal in the X-linked region. No significant LD was detected between rs1009776 and other SNPs in this region. No association was found with genotyped or imputed SNPs in the chr9p23 region highlighted in linkage analyses.

Replication of association in unrelated *C9orf72* patients

In this replication stage, the 12 SNPs with a suggestive signal of association in the discovery cohort ($p \leq 1x10^{e-5}$, Table 2) were selected, and corresponding genotypes from 124 unrelated *C9orf72* patients were used in linear regressions for association tests (replication cohort 1, Table 1). Only rs1009776 was significantly associated with AO (β=-2.94; se=1.11; adjusted p=0.009). As in the discovery cohort, mean and median AO were earlier in carriers of the minor allele A (Table 2). The median AO of hemizygous males and homozygous females carrying the C allele was 58.0 years. It was earlier in carriers of the A allele: 55.5 years for heterozygous females, and in 50.5 years in hemizygous males/homozygous females (Table 3).

Meta-analysis of rs1009776 hazard ratios

Given the different design of sampling, HR were first estimated in each cohort using mix Cox proportional hazard regression in the discovery cohort and Cox proportional hazard regression in the replication cohort before pooling HR. Carrying minor allele A increased significantly hazards in the discovery cohort (adjusted p=0.0003, HR=2.42, 95% CI [1.49; 3.93]), and in the replication cohort (adjusted p=0.0141, HR=1.45, 95% CI [1.10; 1.93]), in line with the abovementioned association tests (Figure 3A and 3B). The combined effect of rs1009776 in the two cohort was investigated by the estimation of the pooled HR. Meta-analysis (n=199)

revealed that allele A increased risk by 66% (adjusted p=4.97.10^{e}-5, pooled HR=1.66, 95% CI [1.30; 2.11]) (Figure 3B).

Meta-analyses also revealed that HR was greater when patients homo or hemizygous for C or A alleles were compared (adjusted p=0.0004, HR=2.58; 95% CI [1.53; 4.36]), than when heterozygotes CA were compared to homo or hemizygous for the major allele C (adjusted p=0.0142, HR=2.00, 95% CI [1.15; 3.47]) suggesting that the effect was more moderate in heterozygous females.

Association of rs1009776 with AO according to gender

As rs1009776 is located on chromosome X, we considered males and females separately in association analyses. Both the minor allele frequency (MAF) and AO were similar between males and females in the discovery population and replication cohort 1 (Table 1 and Supplementary Table S4). The sex-ratio was also comparable (Table 1). In the discovery cohort, the association of rs1009776 was restrained to males (adjusted p=7.76x10^{-5} in males *vs*. p=0.11 in females). In males, the median AO was 60.0 years in C-carriers and 45.5 years in A-carriers. In the replication cohort 1, the association was also significant in males, and non-significant in females, (adjusted p=0.01 *vs*. p=0.48, respectively). The median AO in C-carriers males was 58.0 years and 50.0 years in A-carriers males.

Association of rs1009776 in unrelated *C9orf72* carriers with extreme deviation of AO

A complementary approach was performed, considering AO as a binary trait in a third independent population of 159 unrelated *C9orf72* carriers with extreme deviation of AO, either EO (≤53 y, n=95) or LO (≥67 y, n=64). Logistic regressions models were fitted to assess the association between rs1009776 alleles and groups of extreme AO (Table 4). The

allele A frequency was much higher in patients with EO than in those with LO especially when patients with isolated FTD were considered (0.20 *vs*. 0.07 respectively; OR=3.37; se=0.63, adjusted *p*=0.055). This trend became significant when adding unrelated patients from the discovery cohort who fitted criterion of extreme deviation of AO and isolated FTD without introducing relatedness inside each group (adjusted p=0.006). The signal of association was further reinforced in a final meta-analysis of 134 patients with FTD, after adding patients from the replication cohort 1 with extreme EO or LO (MAF: 0.25 *vs*. 0.06 respectively; adjusted p=0.002). Interestingly, when analyzing these patients together with isolated ALS patients and FTD-ALS, the signal of association decreased (Table 4). Again, this suggests the specificity of the genetic association of rs1009776 with the onset of FTD rather than ALS in *C9orf72* carriers.

No association of rs1009776 with AO of ALS

As ALS occurs at a younger age than FTD in the discovery cohort (54.0 years on average for ALS compared to 58.0 in FTD), that raised the possibility that the observed association could be driven by the risk of developing ALS in some patients, rather than by the variability of AO. However, no association of rs1009776 with the risk to develop ALS was detected, neither in the discovery nor in the replication cohorts (adjusted p=0.42 and p=0.68, respectively).

Moreover, no association of rs1009776 with AO as a quantitative trait was found in an independent cohort of 109 *C9orf72* carriers with ALS (β=-1.94; se=1.39; adjusted p=0.17). The meta-analysis of patients with ALS (n=150) did not yield any significant result (data not shown).

Absence of linkage and association in the X-linked locus among non-*C9orf72* FTD patients

Both parametric and non-parametric linkages analyses were performed in pairs of FTD-*GRN* relatives with concordant or discordant AO. No significant or suggestive LOD score was obtained on chromosome X (data not shown). The SNP rs1009776 was detected with nearly the same MAF than in the *C9orf72* discovery cohort. However, no trend of association between rs1009776 and AO could be observed in patients carrying *GRN* gene mutations (β=1.15; se=1.74; p=0.78). The separate analysis of males and females did not lead to detect any signal of association (data not shown).

Functional impact of rs1009776 on *SLITRK2* transcript levels

A slightly higher *SLITRK2* expression level was found in the frontal cortex of *C9orf72* patients carrying rs1009776 minor allele A compared with carriers of the C allele (n=16, Mann Whitney U test p=0.024) (Figure 4A), irrespectively of the *C9orf72* repeats number estimated in these tissues. Even if this effect was moderate in our series, this polymorphism is a known eQTL of *SLITRK2*, the minor allele A being associated with a higher *SLITRK2* expression according to the GTEX database (V8; https://www.gtexportal.org/home).

Synaptic vesicles markers were reduced in rs1009776 allele A carriers

Both SV2 and SYP staining intensities were reduced in brain tissue from hemi- or homozygous patients for allele A compared to patients carrying allele C (2.43 and 2.14-fold decrease, respectively) (Figures 4B and 4C; supplementary Figures S3 and S4). This was not explained by confounding factors such as disease duration, age at death, associated ALS, sex, and *post-mortem* delay (p>0.10 for all correlation tests, data not shown), or with brain *C9orf72* repeats size between A and C carriers (Mann Whitney U test p=0.76).

SLITRK2 transcript levels tended to be inversely correlated with SV2 staining intensity (Spearman r=-0.54; p=0.24) as well as with SYP (Spearman r=-0.57; p=0.20) in the seven samples with tissues preparations suitable for both qPCR and immunostaining analyses. Conversely, SV2 and SYP intensities were highly correlated (Spearman r=0.93; p=0.007).

Discussion

In this study, we searched for genetic modifiers of the age at disease onset using a family-based approach including pairs of *C9orf72* relatives with concordant or discordant AO. We detected suggestive and converging evidences of linkage and association for a chromosome X *locus*. Initially, the linkage analyses highlighted two regions with a LOD score >2, on chromosomes 9, and X. Linkage peaks lied in the *PTPRD* and *SLITRK2* loci, respectively. These two proteins encoded by the murine ortholog genes *Ptprd* and *Slitrk2* were described to interact as synaptic adhesion proteins (Yamagata *et al.*, 2015). However, we failed to detect a signal of association in the *PTPRD* locus, even after imputation of genotyping data, suggesting that this region deserves more investigation.

Only one SNP, rs1009776, provided robust evidences of association as we detected the genetic association in the discovery cohort, and replicated the association independently. Interestingly, this polymorphism is located in the aforementioned region of positive linkage on chromosome X, and lies 10Kb upstream of *SLITRK2*. In both the discovery and replication cohort 1, in which the AO was considered as a quantitative trait, the C-allele conferred a mean of 5 to 13 years delay in disease onset. The association was further confirmed by a third different methodological approach considering the AO as a dichotomous trait using an extreme phenotype sampling (EO vs. LO) on another distinct population of unrelated *C9orf72* patients. All together, these converging findings supports a protective role of the C-allele.

No association of rs1009776 was found with the risk of developing ALS symptoms. In addition, the association with AO was not replicated in an independent cohort of ALS *C9orf72* carriers. However, since our study was primarily focused on the onset of FTD, the impact of rs1009776 in larger cohorts of patients with ALS is warranted. Alternatively, a different effect of rs1009776 on AO in patients with FTD or ALS might also reflect different pathogenic mechanisms in cortical frontal or motor neurons.

The effect of rs1009776 on AO appeared to be specific to FTD-*C9ORF72*, as neither linkage nor association analyses pointed out the *SLITRK2 locus* in FTD-*GRN*. All the more, this *locus* has not been detected in a prior GWAS study searching for genetic modifiers of *GRN* disease that looked at the chromosome X (Van Deerlin *et al.*, 2010). Highlighting X-linked *loci* in association studies is uncommon although this chromosome carries up to 800 protein-coding genes. Only 20-30% of published GWAS studies include chromosome X results, which subsequently limits the discovery of X-linked associated markers (Wise *et al.*, 2013). This is particularly true in FTD-*C9orf72* since all prior studies did not include chromosome X markers (Gallagher *et al.*, 2014; van Blitterswijk *et al.*, 2014; Zhang *et al.*, 2018).

Generally, statistics for association studies on chromosome X suffer from two principal and potential biases: the difference of allele frequencies for a SNP of interest, and the difference of mean values for quantitative traits between males and females (Özbek *et al.*, 2018). These biases did not affect the current study since both allele frequencies and mean of AO were similar in males and females, in the discovery and in the replication cohorts (Table 1; supplementary Table S4). In addition, sex was considered as a covariate in all analyses, and males' hemizygous X-linked genotypes were coded as homozygous. Another way to get around the difference of chromosome X copy number between males and females is to perform a gender-dependent analysis. Here, genetic associations were statistically significant only in males, in both the discovery and replication stages. Female carriers showed

intermediate AO between homozygous carriers and non-carriers. This phenomenon is well described in X-linked dominant diseases, in which heterozygous carriers often present mild or intermediate symptoms. XCI can modulate the effect of pathogenic mutations in carriers, and the effect of an X-linked modifier could be altered as well. This X-linked genetic factor might contribute to the sex-dependent penetrance evidenced before and specific to the FTD-*C9orf72* disease, males being affected earlier than females (Le Ber *et al.*, 2013; Murphy *et al.*, 2017). It is also in line with the pattern of intra-familial correlation of AO in *C9orf72* families, evocative of the influence of X-linked modifiers on the AO (Barbier *et al.*, 2017). Genotyping of this SNP in larger cohorts should allow confirming this hypothesis.

SLITRK2 encodes for the SLIT and TRK Like Family Member 2 protein, a leucine-rich repeat protein. The Slitrk family comprises six vertebrate members (Slitrk1–6) that are highly expressed in the central nervous system (Won *et al.*, 2019). SLITRK2 is a postsynaptic cell-adhesion molecule which promotes neurite outgrowth and excitatory synapse development (Aruga and Mikoshiba, 2003; Han *et al.*, 2019; Salesse *et al.*, 2020). The expression of *SLITRK2* was slightly higher in brain samples of patients carrying rs1009776 allele A in our study, and not correlated with the *C9orf72* repeats size. Transcript levels tended to be more variable among allele A carriers, which limits the interpretation of results as the number of brain tissues from homo/hemizygous patients for allele A was limited (n=5). However; this is consistent with data from the GTEx database, which classify rs1009776 as an eQTL of *SLITRK2*. Noteworthy, *SLITRK2* was one of the most up-regulated genes in iPSC from *C9orf72* patients (Sareen *et al.*, 2013; Satoh *et al.*, 2014). Therefore, the up-regulation of *SLITRK2* could drive adverse effects in *C9orf72* patients that might be modulated in those carrying the protective allele.

Recent works highlighted the strong relationship between *C9orf72*, vesicular transport, synaptic signaling, and regulation of post-synaptic glutamate receptor 1 levels (Dickson *et al.*,

2019; Xiao *et al.*, 2019). In particular, the detection of the C9orf72 protein in the presynapse, and the reduction of SV2 associated with an increased Ca^{2+} influx and finally cellular toxicity in *C9orf72*-iPS derived neurons have highlighted the potential contribution of a synaptic dysfunction in the disease pathogenesis (Frick *et al.*, 2018; Selvaraj *et al.*, 2018; Jensen *et al.*, 2020). We therefore pushed further the functional investigations regarding to the impact of rs1009776 at the synaptic level in brain tissues from *C9orf72* patients. The immunohistochemical detection of SV2, and of Synaptophysin, two well-known markers of synaptic vesicles, could be considered as surrogates of the condition of synaptic vesicular trafficking, reflecting the deleterious effect of C9orf72 which could be worsened in allele carriers of the rs1009776 risk allele A. SV2 and SYP staining intensities were weaker in patients carrying the allele A associated with an earlier onset than in those with allele C. This suggests that a synaptic vesicles defect may be exacerbated in patients carrying the A allele. Importantly, the *C9orf72* repeats size was not statistically different carriers and non-carriers of the modifying allele which excluded a cofounding effect of repeats number in immunostaining analyses. These results could bring a new piece of evidence linking *C9orf72* with a synaptic dysfunction. Thus, the aberrant cellular calcium influx, and the subsequent neuronal toxicity in the context of pathogenic *C9orf72* expansion could be modulated by a lower expression of SLITRK2, preventing the neuronal loss and the appearance of first symptoms in carriers of the protective C allele. However, direct links between rs1009776, *SLITRK2* transcript levels, synaptic vesicles defect and a potential impact on neuron excitability and Ca^{2+} influx requires more in-depth functional investigations to be confirmed.

In conclusion, this work not only describes the discovery and replication of the association between an X-linked SNP with AO in *C9orf72* patients, but also suggests that synaptic dysfunctions in *C9orf72* carriers may contribute in part to the variability of AO. Our approach

illustrates that coming back to family-based analyses could represent a powerful method to detect new genetic modifying factors in rare diseases.

Acknowledgements

We are very grateful to patients and their families who were enrolled in this study, and to the French patients' association France DFT. The Brainbank Neuro-CEB Neuropathology Network is associated with the following patients' associations: "France DFT, ARSLA, Connaître les Syndromes Cérébelleux, Fondation ARSEP, Fondation Vaincre Alzheimer, France Parkinson." We also thank the DNA and cell bank (BADN, ICM, Paris), the genotyping (P3S, Paris), the bioinformatics (ICONICS, ICM, Paris), and the histology (Histomics, ICM, Paris) facilities for their technical assistance. We also thank the Neurological Tissue Bank of the Bionbank-Hospital Clinic-IDIBAPS, Barcelona, Spain, for data and sample procurement. Several authors of this publication are members of the European Reference Network for Rare Neurological Diseases (ERN-RND, Project ID No 739510).

Fundings

The research leading to these results received funding from the "Investissements d'avenir" ANR-11-INBS-0011. This work was funded by the Programme Hospitalier de Recherche Clinique (PHRC) FTLD-exome (to ILB, promotion by Assistance Publique – Hôpitaux de Paris), and by ANR/DGOS PREVDEMALS (to ILB, promotion by Assistance Publique – Hôpitaux de Paris); Fondation Maladies Rares Grant FONDATION-WES-20161202 (MB); Association pour la Recherche sur la Sclérose Latérale Amyotrophique –ARSLA- research

funding (MB); Alzheimer's Society grant # 284 (RF); fondi per la ricerca 2019 (BN); Fundació Marató de TV3, Barcelona, Spain grant # 20143810 (RSV); The Flemish Government initiated Impulse Program on Networks for Dementia Research (VIND), the Methusalem Excellence Program, the Research Foundation Flanders (FWO) and the University of Antwerp Research Fund (CVB, JvdZ); Instituto de Salud Carlos III and FEDER funds grant # FIS14/00099 (EGT); Memorabel grants from Deltaplan Dementie (the Netherlands Organisation for Health Research and Development and Alzheimer Nederland grant numbers 7330550813 and 733050103), the Bluefield Project to Cure Frontotemporal Dementia, the Dioraphte foundation (grant number 1402 1300), and the European Joint Programme – Neurodegenerative Disease Research (JPND, PreFrontALS) (ELvdE and JCvS).

Competing interests

The authors declare no conflicts of interest.

Figure legends

Figure 1. Flow chart of the study design and genetic analyses. Main results are presented. ALS: amyotrophic lateral sclerosis; AO: age at onset; EO: early onset; LO: late onset; FTD: frontotemporal dementia; GWAS: Genome-Wide Association Study; IFGC: International Frontotemporal Dementia Genetics Consortium.

Figure 2. Linkage analysis and Genome-wide association study in *C9orf72* relatives with concordant or discordant AO. A) LOD score plots from linkage analyses on chromosome 9 and chromosome X; **B**) Manhattan plots from the linear mixed model association analysis

with AO in *C9orf72* relatives. Negative $\log_{10}$-transformed p-values are shown for each variant genotyped on the y axis in function of the chromosomal position. The blue line represents the p-value threshold of $1x10^{e-5}$ for suggestive associations.

Figure 3. rs1009776 minor allele A associated with an earlier disease onset in FTD-*C9orf72*. **A**) Kaplan-Meier curve of cumulative incidence of disease onset in the discovery cohort according to rs1009776 genotypes. CC or AA include homo/hemizygous for the corresponding allele; **B**) Hazard ratios (HR) from Cox proportional hazard regressions in the discovery and replication cohorts based of the risk-associated minor allele count, and pooled HR from the meta-analysis between the two cohorts are presented.

Figure 4. *SLITRK2* transcript levels and synaptic vesicles markers staining in *C9orf72* brain tissue according to rs1009776. **A**) *SLITRK2* transcript levels in *C9orf72* brain tissue **B**) Example of SV2 and (SYP) immunostaining (frontal cortex) from *C9orf72* patients carrying allele C (left) or A (right). Black (SV2) and brown (SYP) scales (100µm) are indicated. Regions of interest (ROI) cover cortical layers III, IV and V. All ROIs are available on supplementary Figure S3 and S4. *p<0.05. Quantification of SV2 (**C**) and SYP (**D**) staining intensities in *C9orf72* brain tissue from patients carrying alleles C or A; mean and error bars (SD) are reported.

Tables.

Table 1. Description of cohorts.

	Discovery cohort concordant/discordant pairs of relatives	Replication cohort 1 IFGC	Replication cohort 2 early onset (EO) vs. late onset (LO)		Cohort ALS	Concordant/discordant pairs of relatives in non-*C9orf72* FTD patients
Gene mutated	*C9orf72*	*C9orf72*	*C9orf72*		*C9orf72*	*GRN*
n	75	124	159		109	50
			EO n=95	LO n=64		
FTD	52	83	49	31	-	50
FTD-ALS	5	41	22	14	54	-
ALS	18	-	24	19	55	-
males (%)	44 (59)	70 (57)	54 (57)	32 (50)	53 (49)	26 (52)
[min-max AO]	[30-81]	[34-77]	[29-53]	[67-87]	[38-74]	[46-86]
mean AO ± SD (y)						
all	56.99 ± 12.82	56.44 ± 8.75	45.47 ± 6.20	71.89 ± 4.39	58.15 ± 8.4	60.64 ± 8.12
males	56.57 ± 12.05	56.16 ± 9.15	45.70 ± 6.09	71.88 ± 4.58	58.06 ± 7.83	61.92 ± 7.69
females	57.58 ± 14.03	56.80 ± 8.27	45.15 ± 6.46	71.91 ± 4.27	58.23 ± 8.98	59.25 ± 8.50
Overlap with other cohorts (n)	no	No	No		no	no

Table 2. *Loci* identified in the *C9orf72* discovery cohort displaying suggestive associations with AO ($p \leq 1x10^{-5}$) and corresponding p-values in the *C9orf72* replication cohort.

	Discovery cohort concordant/discordant pairs of relatives										Replication cohort 1 IFGC			
Linkage	Association										Association			
	chr	rsID	Location (GRCh38)	Nearby gene	Allele 1	Allele 2	MAF	β	se	P	MAF	β	se	p
	2	rs17017537	79681381	*CTNNA2 / LRRTM1*	G	T	0.08	-17.13	3.87	$4.9x10^{-6}$	0.11	0.11	1.74	0.95
	2	rs12471455	79683223		A	G	0.08	-17.13	3.87	$4.9x10^{-6}$	0.10	0.79	1.85	0.67
	4	rs10012732	30003812	NA	T	C	0.347	10.03	2.30	$6.4x10^{-6}$	0.26	0.10	1.13	0.93
	7	rs10952069	7735053	*UMAD1 / RPA3*	T	C	0.28	-11.31	2.25	$5.7x10^{-6}$	0.18	-1.94	1.47	0.19
	7	rs13237260	7739460		T	G	0.28	-11.31	2.25	$5.7x10^{-6}$	0.18	-1.94	1.47	0.19
no	8	rs16920973	54967126	NA	C	T	0.193	-12.53	2.31	$8.1x10^{-6}$	0.24	0.25	1.26	0.84
	8	rs10108020	106512196	*OXR1*	G	A	0.093	15.21	3.57	$9.4x10^{-6}$	0.13	-1.29	1.66	0.44
	14	rs17255311	59132213		T	C	0.147	14.17	3.05	$6.9x10^{-6}$	0.11	-2.06	1.74	0.24
	14	rs4901902	59150362	*DAAM1*	C	A	0.147	14.17	3.05	$6.9x10^{-6}$	0.09	-0.83	1.83	0.65
	14	rs1252914	59169691		G	A	0.28	11.68	2.50	$3.7x10^{-6}$	0.24	-1.14	1.22	0.35
	20	rs6116309	4315003	NA	G	T	0.14	11.65	2.66	$4.9x10^{-6}$	0.17	-0.06	1.51	0.97
yes	**X**	**rs1009776**	**145807459**	***SLITRK2***	**A**	**C**	**0.24**	**-8.44**	**2.02**	**$1x10^{-5}$**	**0.17**	**-2.94**	**1.11**	**0.009**

Table 3. Mean age at onset (AO) in the discovery and replication cohorts according to rs1009776 genotypes.

	AO	C/C*	C/A	A/A*
Discovery cohort	mean (SD)	60.5 (10.8)	52.1 (14.5)	47.3 (13.2)
	median	62.5	47.5	46.0
Replication cohort 1 IFGC	mean (SD)	57.2 (8.5)	55.1 (7.9)	52.4 (10.3)
	median	58.0	55.5	50.5

*includes both homozygous females and hemizygous males.

Table 4. Association of rs1009776 with extreme deviation of the age at onset (AO) in unrelated *C9orf72* patients with early-onset (EO, AO≤53y) *vs.* late-onset (LO, AO≥67y). Number of patients (n) and rs1009776 minor allele A frequency (maf) are indicated.

	C9orf72 patients with extreme AO						+ patients with extreme AO from the discovery cohort[a]						+ patients with extreme AO from the replication cohort 1[a]					
	N	maf EO	maf LO	OR[b]	se[b]	*p*[b]	N	maf EO	maf LO	OR[b]	se[b]	*p*[b]	n	maf EO	maf LO	OR[b]	se[b]	*p*[b]
FTD	80	0.20	0.07	3.37	0.63	*0.055*	101	0.26	0.07	4.54	0.55	*0.006*	134	0.25	0.06	4.64	0.50	*0.002*
FTD+FTD-ALS	117	0.17	0.09	1.88	0.40	*0.12*	139	0.22	0.09	2.59	0.37	*0.011*	195	0.23	0.09	2.40	0.30	*0.003*
FTD+FTD-ALS+ALS	159	0.17	0.13	1.32	0.28	*0.32*	191	0.22	0.12	1.73	0.25	*0.029*	247	0.23	0.11	1.80	0.23	*0.008*

[a]Only unrelated patients were added.

[b]Odds ratio for the minor allele (OR), standard error (se) and adjusted p-value (p) were calculated from logistic regressions including sex as covariate.

References

Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 2002; 30: 97–101.

Aruga J, Mikoshiba K. Identification and characterization of Slitrk, a novel neuronal transmembrane protein family controlling neurite outgrowth. Mol Cell Neurosci 2003; 24: 117–29.

Aubart M, Gazal S, Arnaud P, Benarroch L, Gross M-S, Buratti J, et al. Association of modifiers and other genetic factors explain Marfan syndrome clinical variability. Eur J Hum Genet 2018; 26: 1759–72.

Aulchenko YS, Ripke S, Isaacs A, van Duijn CM. GenABEL: an R library for genome-wide association analysis. Bioinformatics 2007; 23: 1294–6.

Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006; 442: 916–9.

Barbier M, Camuzat A, Houot M, Clot F, Caroppo P, Fournier C, et al. Factors influencing the age at onset in familial frontotemporal lobar dementia: Important weight of genetics. Neurol Genet 2017; 3: e203.

van Blitterswijk M, Mullen B, Nicholson AM, Bieniek KF, Heckman MG, Baker MC, et al. TMEM106B protects C9ORF72 expansion carriers against frontotemporal dementia. Acta Neuropathol 2014; 127: 397–406.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 2015; 4: 7.

Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 2006; 442: 920–4.

DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011; 72: 245–56.

Dickson DW, Baker MC, Jackson JL, DeJesus-Hernandez M, Finch NA, Tian S, et al. Extensive transcriptomic study emphasizes importance of vesicular transport in C9orf72 expansion carriers. Acta Neuropathol Commun 2019; 7: 150.

Ferrari R, Hernandez DG, Nalls MA, Rohrer JD, Ramasamy A, Kwok JBJ, et al. Frontotemporal dementia and its subtypes: a genome-wide association study. Lancet Neurol 2014; 13: 686–99.

Fournier C, Barbier M, Camuzat A, Anquetil V, Lattante S, Clot F, et al. Relations between C9orf72 expansion size in blood, age at onset, age at collection and transmission across

generations in patients and presymptomatic carriers. Neurobiol Aging 2019; 74: 234.e1-234.e8.

Frick P, Sellier C, Mackenzie IRA, Cheng C-Y, Tahraoui-Bories J, Martinat C, et al. Novel antibodies reveal presynaptic localization of C9orf72 protein and reduced protein levels in C9orf72 mutation carriers. Acta Neuropathol Commun 2018; 6: 72.

Gallagher MD, Suh E, Grossman M, Elman L, McCluskey L, Van Swieten JC, et al. TMEM106B is a genetic modifier of frontotemporal lobar degeneration with C9orf72 hexanucleotide repeat expansions. Acta Neuropathol 2014; 127: 407–18.

Han KA, Kim J, Kim H, Kim D, Lim D, Ko J, et al. Slitrk2 controls excitatory synapse development via PDZ-mediated protein interactions. Sci Rep 2019; 9: 17094.

Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998; 393: 702–5.

Jackson JL, Finch NA, Baker MC, Kachergus JM, DeJesus-Hernandez M, Pereira K, et al. Elevated methylation levels, reduced expression levels, and frequent contractions in a clinical cohort of C9orf72 expansion carriers. Mol Neurodegener 2020; 15: 7.

Jensen BK, Schuldi MH, McAvoy K, Russell KA, Boehringer A, Curran BM, et al. Synaptic dysfunction induced by glycine-alanine dipeptides in C9orf72-ALS/FTD is rescued by SV2 replenishment. EMBO Mol Med 2020; 12: e10722.

Koçoğlu C, Gossye H, Dillen L, Van Mossevelde S, De Bleecker JL, Vandenberghe R, et al. No association of CpG SNP rs9357140 with onset age in Belgian C9orf72 repeat expansion carriers. Neurobiol Aging 2020

Lagier-Tourenne C, Baughn M, Rigo F, Sun S, Liu P, Li H-R, et al. Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc Natl Acad Sci USA 2013; 110: E4530-4539.

Lattante S, Le Ber I, Galimberti D, Serpente M, Rivaud-Péchoux S, Camuzat A, et al. Defining the association of TMEM106B variants among frontotemporal lobar degeneration patients with GRN mutations and C9orf72 repeat expansions. Neurobiol Aging 2014; 35: 2658.e1-5.

Le Ber I, Camuzat A, Guillot-Noel L, Hannequin D, Lacomblez L, Golfier V, et al. C9ORF72 repeat expansions in the frontotemporal dementias spectrum of diseases: a flow-chart for genetic testing. J Alzheimers Dis 2013; 34: 485–99.

Le Ber I, Guedj E, Gabelle A, Verpillat P, Volteau M, Thomas-Anterion C, et al. Demographic, neurological and behavioural characteristics and brain perfusion SPECT in frontal variant of frontotemporal dementia. Brain 2006; 129: 3051–65.

Murphy NA, Arthur KC, Tienari PJ, Houlden H, Chiò A, Traynor BJ. Age-related penetrance of the C9orf72 repeat expansion. Sci Rep 2017; 7: 2116.

Özbek U, Lin H-M, Lin Y, Weeks DE, Chen W, Shaffer JR, et al. Statistics for X-chromosome associations. Genet Epidemiol 2018; 42: 539–50.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559–75.

Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011; 72: 257–68.

Ripatti S, Palmgren J. Estimation of multivariate frailty models using penalized partial likelihood. Biometrics 2000; 56: 1016–22.

Salesse C, Charest J, Doucet-Beaupré H, Castonguay A-M, Labrecque S, De Koninck P, et al. Opposite Control of Excitatory and Inhibitory Synapse Formation by Slitrk2 and Slitrk5 on Dopamine Neurons Modulates Hyperactivity Behavior. Cell Rep 2020; 30: 2374-2386.e5.

Sareen D, O'Rourke JG, Meera P, Muhammad AKMG, Grant S, Simpkinson M, et al. Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion. Sci Transl Med 2013; 5: 208ra149.

Satoh J-I, Yamamoto Y, Kitano S, Takitani M, Asahina N, Kino Y. Molecular network analysis suggests a logical hypothesis for the pathological role of c9orf72 in amyotrophic lateral sclerosis/frontotemporal dementia. J Cent Nerv Syst Dis 2014; 6: 69–78.

Sellami L, Rucheton B, Ben Younes I, Camuzat A, Saracino D, Rinaldi D, et al. Plasma progranulin levels for frontotemporal dementia in clinical practice: a 10-year French experience. Neurobiol Aging 2020; 91: 167.e1-167.e9.

Selvaraj BT, Livesey MR, Zhao C, Gregory JM, James OT, Cleary EM, et al. C9ORF72 repeat expansion causes vulnerability of motor neurons to Ca2+-permeable AMPA receptor-mediated excitotoxicity. Nat Commun 2018; 9: 347.

Suh E, Lee EB, Neal D, Wood EM, Toledo JB, Rennert L, et al. Semi-automated quantification of C9orf72 expansion size reveals inverse correlation between hexanucleotide repeat number and disease duration in frontotemporal degeneration. Acta Neuropathol 2015; 130: 363–72.

Van Deerlin VM, Sleiman PMA, Martinez-Lage M, Chen-Plotkin A, Wang L-S, Graff-Radford NR, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. Nat Genet 2010; 42: 234–9.

Wise AL, Gyi L, Manolio TA. eXclusion: toward integrating the X chromosome in genome-wide association analyses. Am J Hum Genet 2013; 92: 643–7.

Won SY, Lee P, Kim HM. Synaptic organizer: Slitrks and type IIa receptor protein tyrosine phosphatases. Curr Opin Struct Biol 2019; 54: 95–103.

Xiao S, McKeever PM, Lau A, Robertson J. Synaptic localization of C9orf72 regulates post-synaptic glutamate receptor 1 levels. Acta Neuropathol Commun 2019; 7: 161.

Yamagata A, Sato Y, Goto-Ito S, Uemura T, Maeda A, Shiroshima T, et al. Structure of Slitrk2-PTPδ complex reveals mechanisms for splicing-dependent trans-synaptic adhesion. Sci Rep 2015; 5: 9686.

Zhang M, Ferrari R, Tartaglia MC, Keith J, Surace EI, Wolf U, et al. A C6orf10/LOC101929163 locus is associated with age of onset in C9orf72 carriers. Brain 2018; 141: 2895–907.

Zhou X, Stephens M. Genome-wide efficient mixed-model analysis for association studies. Nat Genet 2012; 44: 821–4.

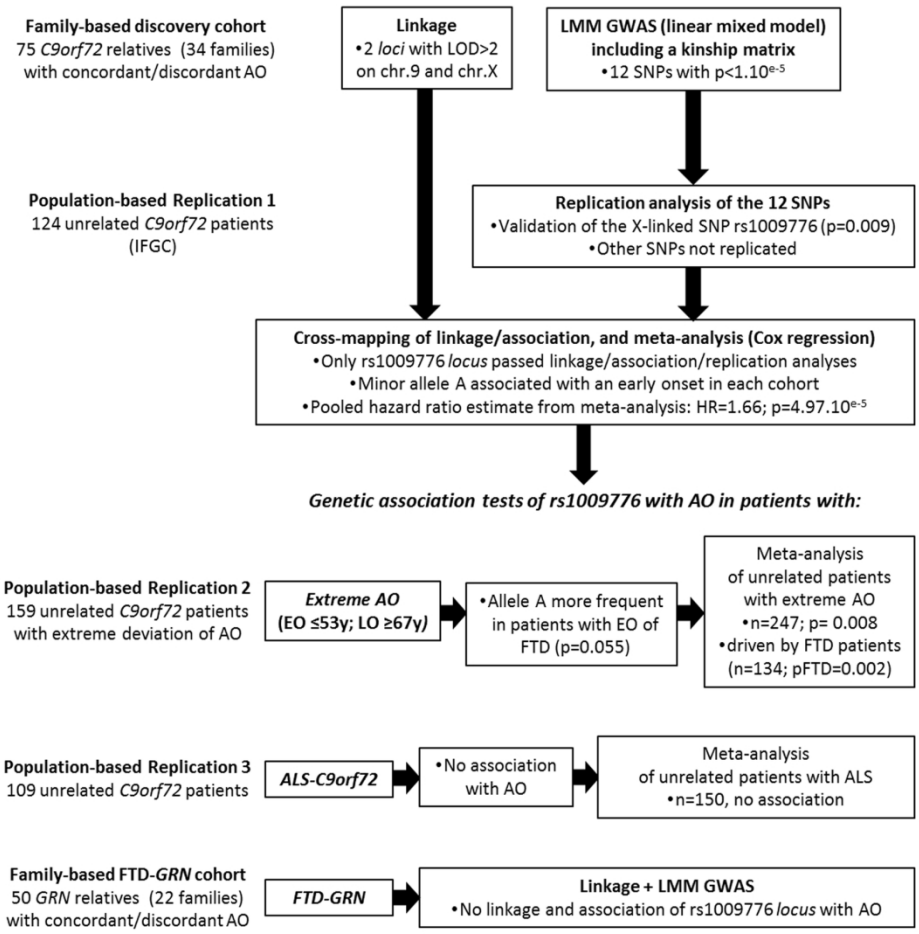


Figure 1. Flow chart of the study design and genetic analyses. Main results are presented. ALS: amyotrophic lateral sclerosis; AO: age at onset; EO: early onset; LO: late onset; FTD: frontotemporal dementia; GWAS: Genome-Wide Association Study; IFGC: International Frontotemporal Dementia Genetics Consortium.

159x157mm (300 x 300 DPI)

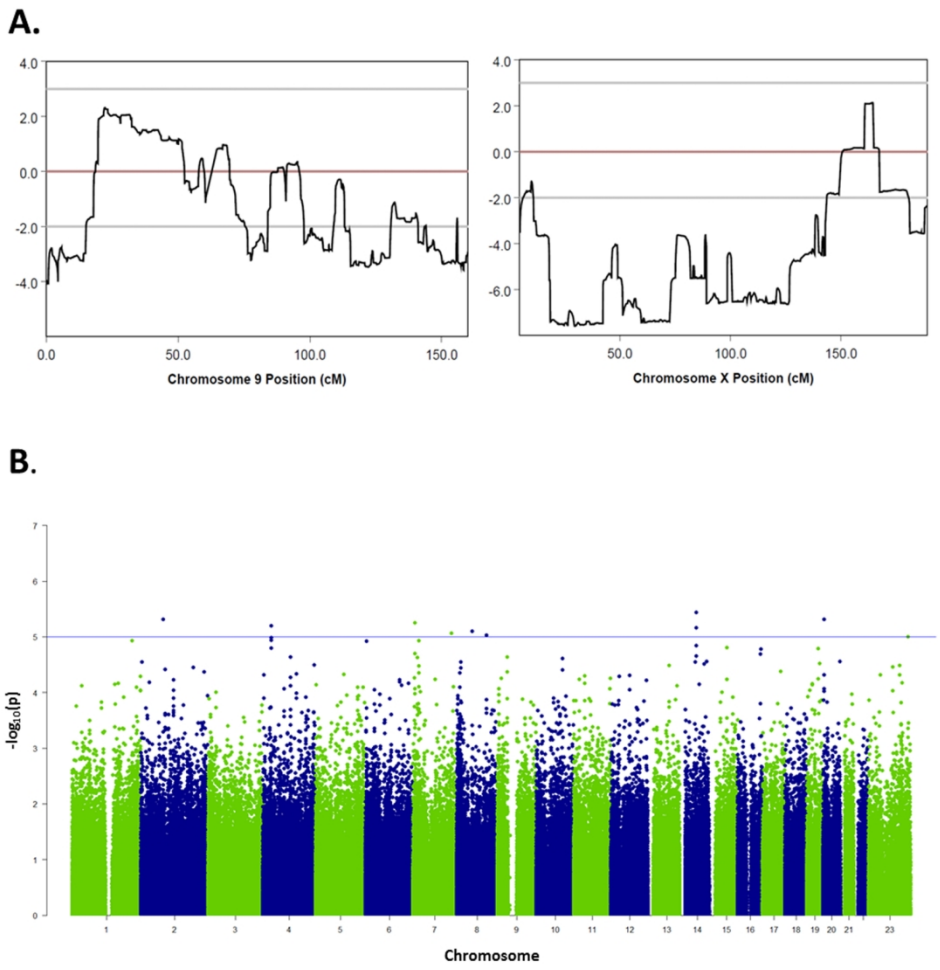


Figure 2. Linkage analysis and Genome-wide association study in C9orf72 relatives with concordant or discordant AO. A) LOD score plots from linkage analyses on chromosome 9 and chromosome X; B) Manhattan plots from the linear mixed model association analysis with AO in C9orf72 relatives. Negative log10-transformed p-values are shown for each variant genotyped on the y axis in function of the chromosomal position. The blue line represents the p-value threshold of 1x10e-5 for suggestive associations.

149x152mm (300 x 300 DPI)

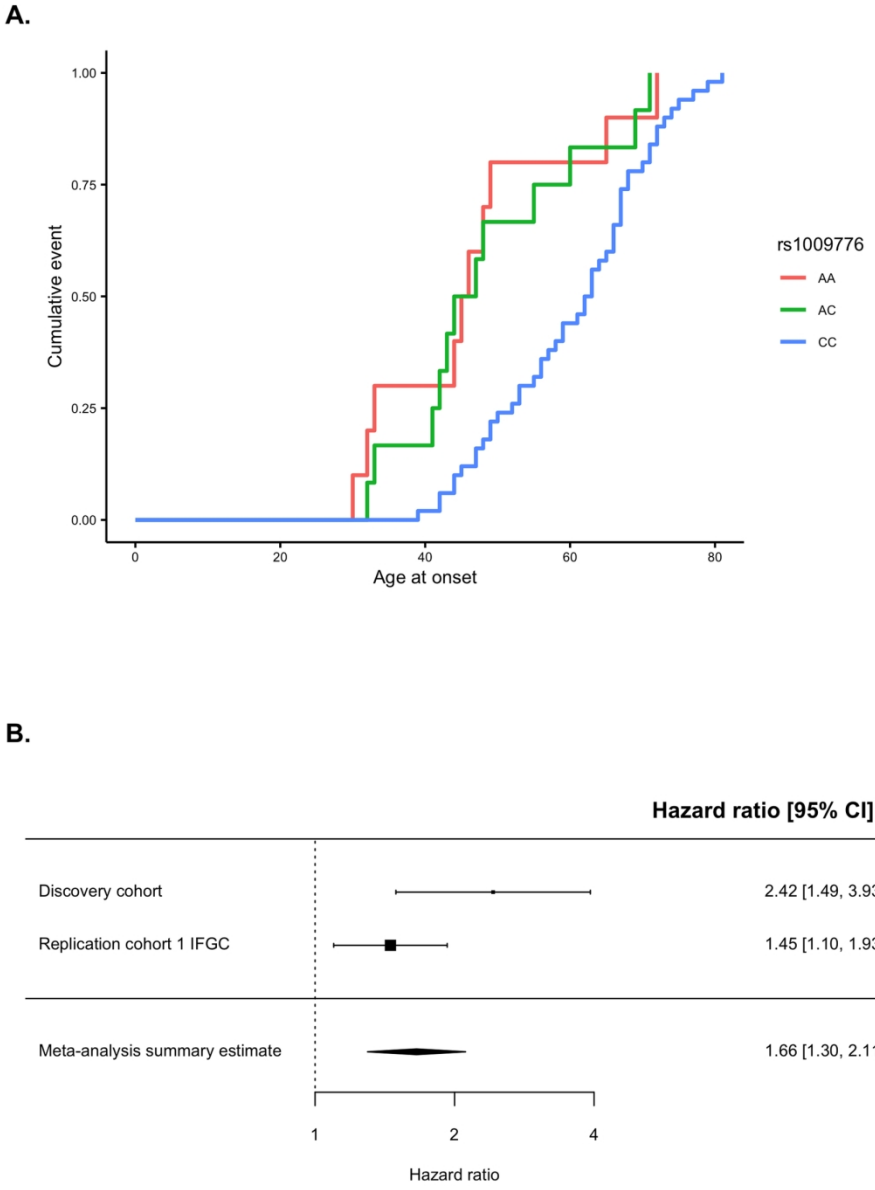


Figure 3. rs1009776 minor allele A associated with an earlier disease onset in FTD-C9orf72. A) Kaplan-Meier curve of cumulative incidence of disease onset in the discovery cohort according to rs1009776 genotypes. CC or AA include homo/hemizygous for the corresponding allele; B) Hazard ratios (HR) from Cox proportional hazard regressions in the discovery and replication cohorts based of the risk-associated minor allele count, and pooled HR from the meta-analysis between the two cohorts are presented.

149x192mm (300 x 300 DPI)

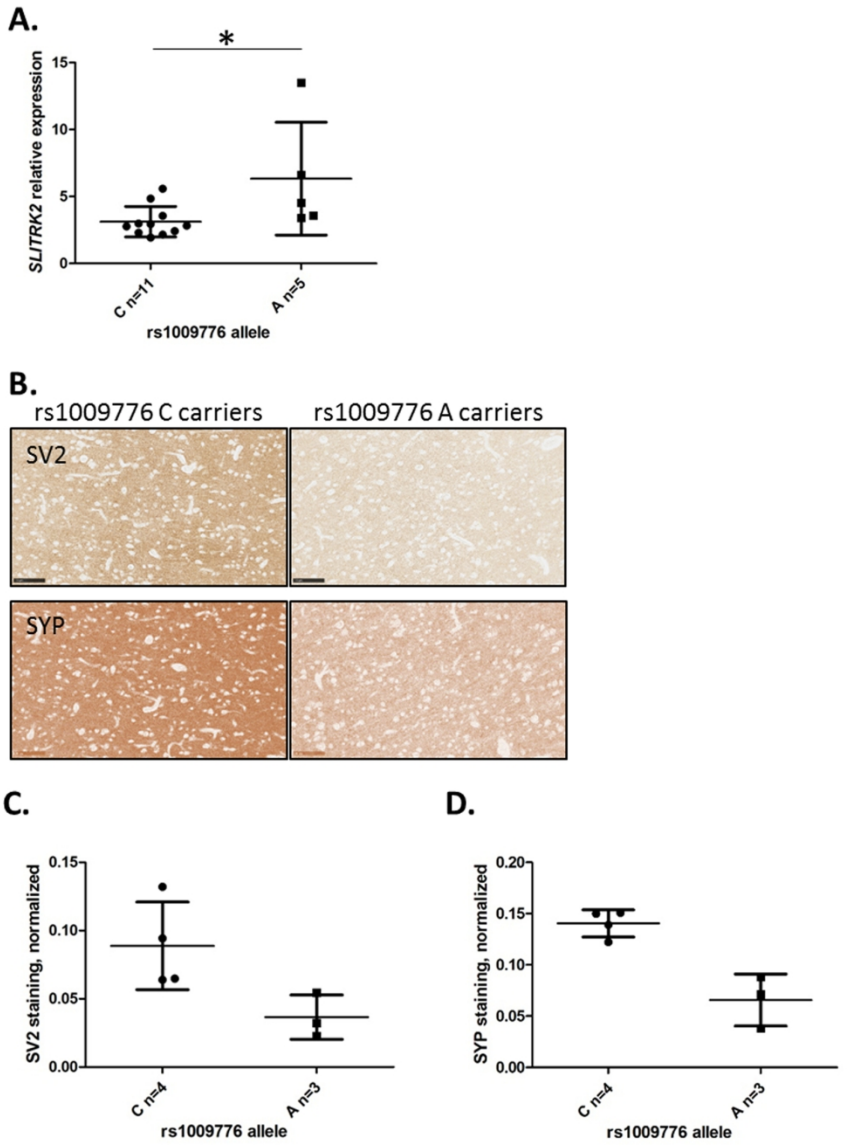


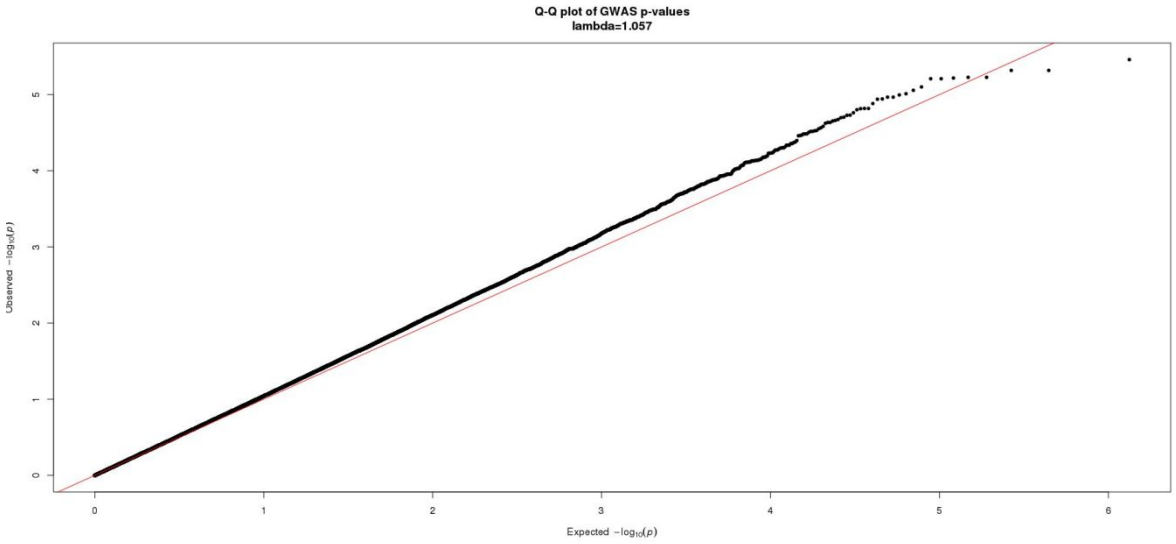
Figure 4. SLITRK2 transcript levels and synaptic vesicles markers staining in C9orf72 brain tissue according to rs1009776. A) SLITRK2 transcript levels in C9orf72 brain tissue B) Example of SV2 and (SYP) immunostaining (frontal cortex) from C9orf72 patients carrying allele C (left) or A (right). Black (SV2) and brown (SYP) scales (100µm) are indicated. Regions of interest (ROI) cover cortical layers III, IV and V. All ROIs are available on supplementary Figure S3 and S4. *p<0.05. Quantification of SV2 (C) and SYP (D) staining intensities in C9orf72 brain tissue from patients carrying alleles C or A; mean and error bars (SD) are reported.

149x198mm (300 x 300 DPI)

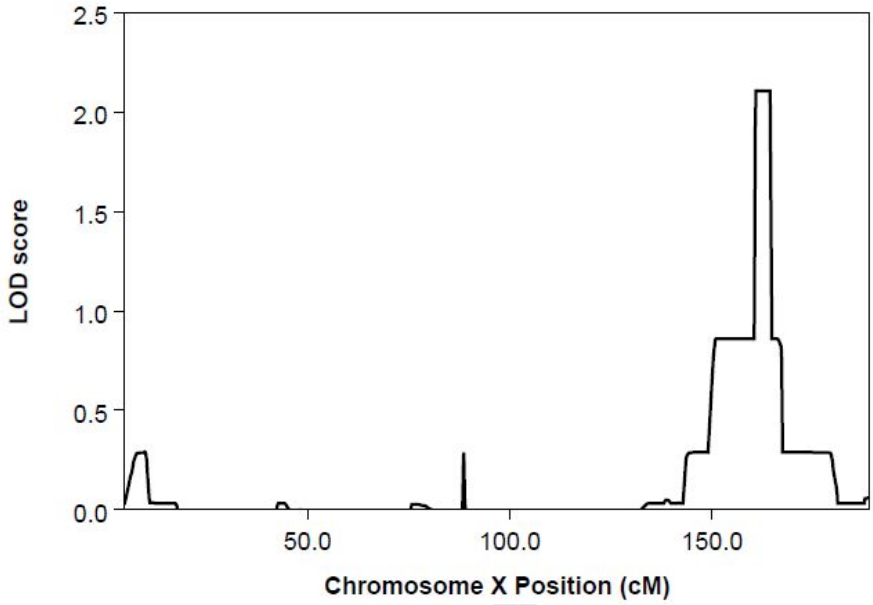
Supplementary Figures and Tables

Supplementary Figures

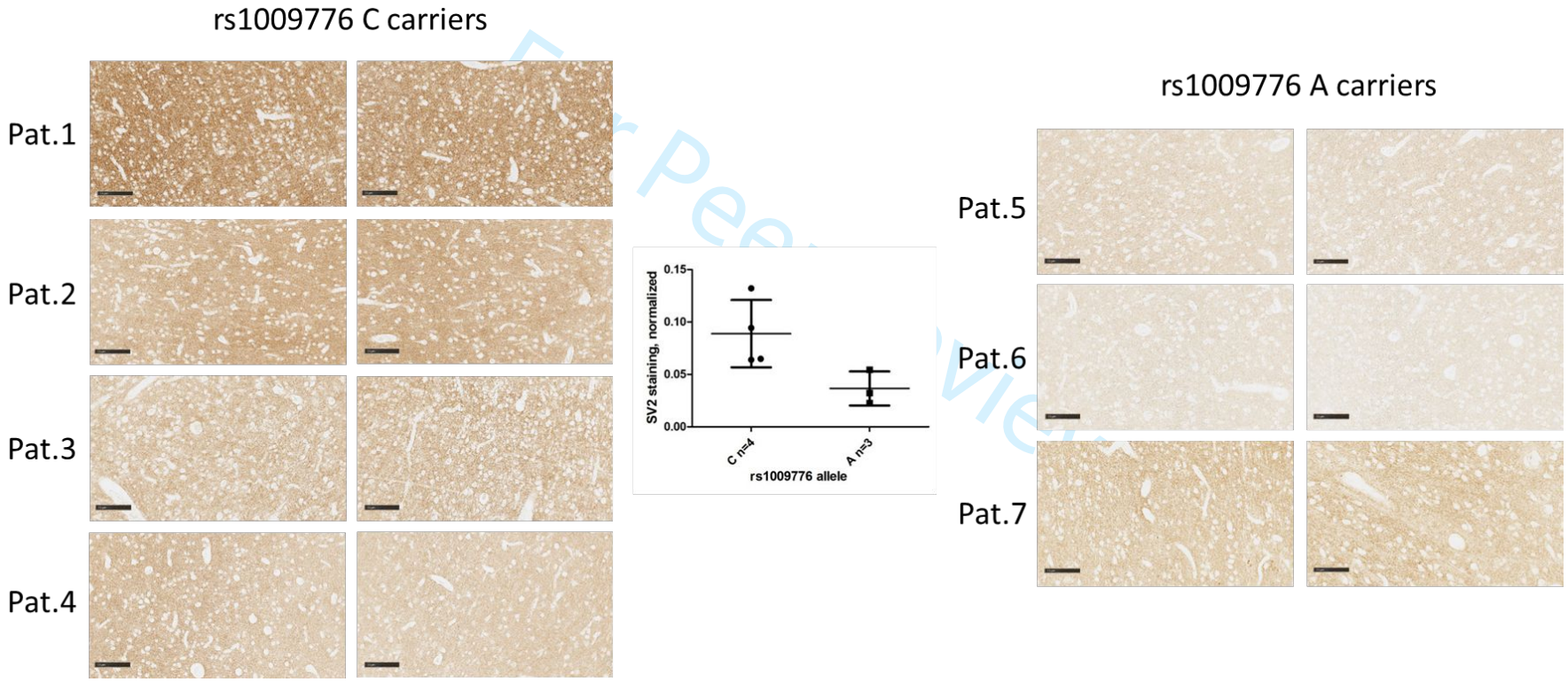
Supplementary Figure S1. Q-Q plots of adjusted p-values from the Genome-wide association test with AO in the discovery cohort (FTD-*C9orf72* relatives).



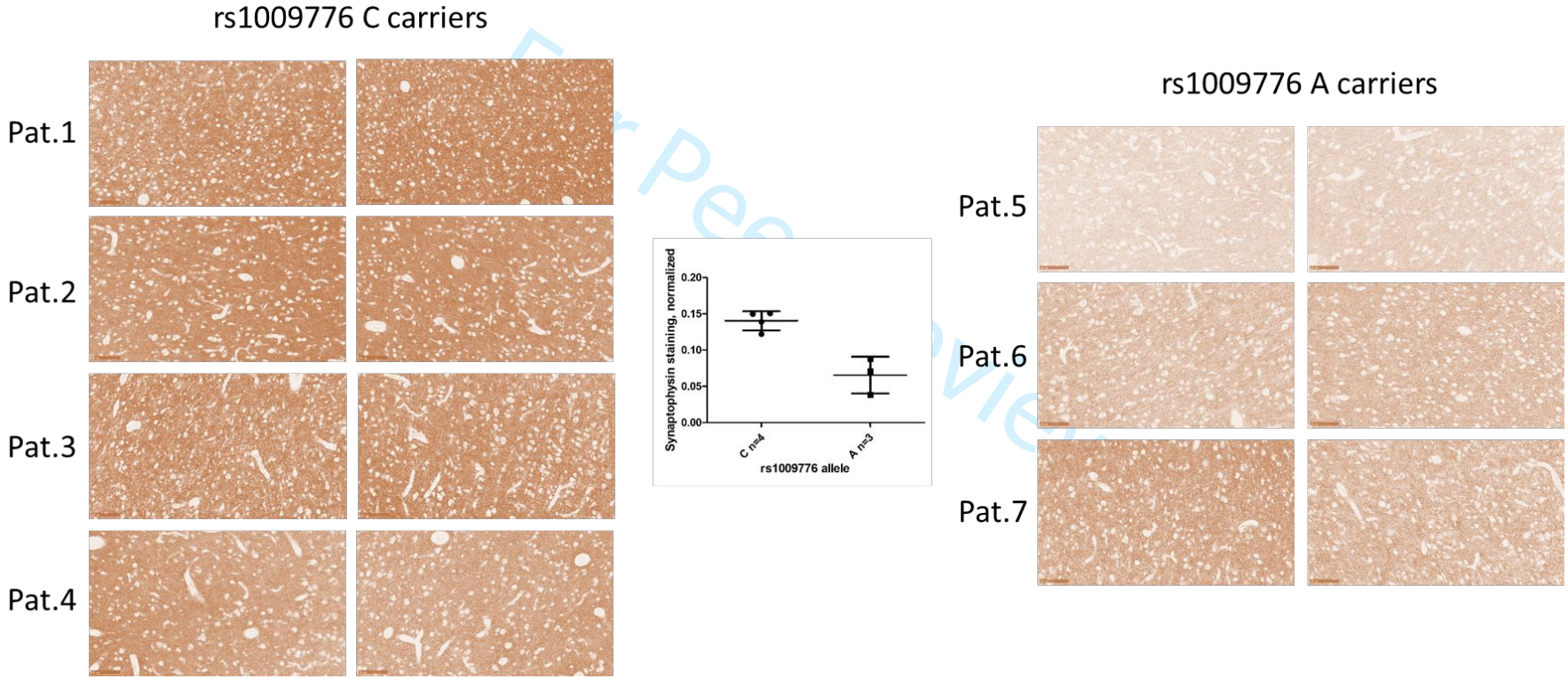
Supplementary Figure S2. LOD score plots from non-parametric linkage analyses performed in FTD-*C9orf72* relatives on chromosome X.



Supplementary Figure S3. Synaptic vesicle glycoprotein 2 (SV2) immunostaining in *C9orf72* brain tissue from patients carrying the candidate modifier rs1009776 alleles C or A. Two regions of interest (ROI) were defined *per* patients after immunostaining for image acquisition and analysis. Measure of SV2 staining normalized corresponding to white-matter signal for each section is resumed on the central graph. ROIs cover cortical layers III, IV and V. Black scales (100µm) is represented on each ROI.



Supplementary Figure S4. Synaptophysin (SYP) immunostaining in *C9orf72* brain tissue from patients carrying the candidate modifier rs1009776 alleles C or A. Two regions of interest (ROI) were defined *per* patients after immunostaining for image acquisition and analysis. Measure of SYP staining normalized corresponding to white-matter signal for each section is resumed on the central graph. Mean ±SD are indicated. ROIs cover cortical layers III, IV and V. Brown scales (100µm) is represented on each ROI.



Supplementary Tables

Supplementary Table S1. List of *C9orf72* pairs of relatives with relatedness, difference of AO and subtype (early, mild or late AO) for each pair.

pairs #	kinship	delta AO	type
1	sib	3	concordant early
2	sib	11	discordant
3	sib	1	concordant early
4	sib	12	discordant
5	cousin	3	concordant early
6	cousin	9	discordant
7	cousin	2	concordant early
8	sib	1	concordant early
9	sib	2	concordant mild
10	sib	2	concordant mild
11	avuncular	24	discordant
12	avuncular	22	discordant
13	sib	1	concordant mild
14	parent-offspring	20	discordant
15	sib	3	concordant mild
16	sib	3	concordant mild
17	sib	13	discordant
18	sib	10	discordant
19	sib	14	discordant
20	sib	12	discordant
21	sib	1	concordant early
22	sib	3	concordant early
23	sib	2	concordant early
24	cousin	1	concordant late
25	cousin	15	discordant
26	cousin	16	discordant
27	sib	14	discordant
28	parent-offspring	21	discordant
29	sib	2	concordant mild
30	sib	2	concordant mild
31	sib	0	concordant mild
32	sib	15	discordant
33	sib	15	discordant
34	parent-offspring	40	discordant
35	parent-offspring	11	discordant
36	sib	14	discordant
37	sib	17	discordant
38	sib	3	concordant late
39	avuncular	27	discordant

40	parent-offspring	27	discordant
41	cousin	2	concordant late
42	parent-offspring	31	discordant
43	parent-offspring	29	discordant
44	avuncular	33	discordant
45	sib	20	discordant
46	sib	22	discordant
47	avuncular	25	discordant
48	parent-offspring	26	discordant
49	sib	1	concordant late
50	sib	27	discordant

Supplementary Table S2. Mean *C9orf72* repeats numbers estimated in brain samples.

rs1009776 allele	
C	A
2041.71	2346.738
1894.321	1501.637
2059.626	1918
2049	2133
2041	1815
1911	
1914	
1914	
1767	
1743	

Supplementary Table S3. Primers used for quantitative PCR.

Gene	Forward 5'>3'	Reverse 5'>3'
SLITRK2	GTCTTCTCCTGATGTCGATTGC	AATCCTGCCCATCTCCTCCT
XPNPEP1	CAGACAAAGAGTGCGACTGG	TTGGAGATGGGTTGCGTCTC
AARS	GTGATCGTGACGGAAGAAGC	CTTTCCTGAGGGCCTTCTGG

Supplementary Table S4. rs1009776 minor allele frequencies according to gender and first clinical symptoms.

	rs1009776 minor allele frequency		
	overall	Male	Female
C9orf72 Discovery cohort n=75	0.24	0.23	0.26
C9orf72 Replication cohort 1 (IFGC) n=124	0.17	0.16	0.19
C9orf72 Replication cohort 2 (Extreme AO) n=159	0.15	0.13	0.16
C9orf72 ALS n=109	0.17	0.11	0.21
GRN (non-*C9orf72* patients) n=50	0.19	0.27	0.15

SLITRK2, an X-linked modifier of the age at onset in *C9orf72* frontotemporal lobar degeneration

Supplementary Materials and Methods

Authors

Mathieu Barbier[1], PhD; Agnès Camuzat[1], MSc; Khalid El Hachimi[1], PhD; Justine Guegan[1], MSc; Daisy Rinaldi[1,2], PhD; Serena Lattante[3], PhD; Marion Houot[1,2,4], MSc; Raquel Sánchez-Valle[5], MD, PhD; Mario Sabatelli[6], MD; Anna Antonell[5], PhD ; Laura Molina-Porcel[5,7] MD, PhD; Fabienne Clot[8], PhD; Philippe Couratier[9], MD, PhD; Emma van der Ende[10], MD; Julie van der Zee[11,12], PhD; Claudia Manzoni[13], PhD; William Camu[14], MD, PhD; Cécile Cazeneuve[8], PharmD, PhD; François Sellal[15,16], MD, PhD; Mira Didic[17], MD, PhD; Véronique Golfier[18], MD, PhD; Florence Pasquier[19], MD, PhD; Charles Duyckaerts[1,20], MD, PhD; Giacomina Rossi[21], PhD; Amalia C. Bruni[22], MD; Victoria Alvarez[23,24], MD; Estrella Gómez-Tortosa[25], MD, PhD; Alexandre de Mendonça[26], MD; PhD; Caroline Graff[27], MD, PhD; Mario Masellis[28], MD, PhD; Benedetta Nacmias[29], PhD; Badreddine Mohan Oumoussa[30], MSc; Ludmila Jornea[1], BSc; Sylvie Forlani[1], PhD; The French clinical and genetic Research network on FTLD/FTLD-ALS and PREVDEMALS*, The International Frontotemporal Dementia Genomics Consortium**, the European Early Onset Dementia (EU-EOD) Consortium***; Brainbank Neuro-CEB Neuropathology Network****; Neurological Tissue Bank of the Biobank Hospital Clinic-IDIBAPS*****; Viviana Van Deerlin[31], MD, PhD; Jonathan D. Rohrer[32], MD, PhD; Ellen Gelpi[7,33], MD, PhD; Rosa Rademakers[11], PhD; John Van Swieten[10], MD, PhD; Eric Le Guern[8], MD, PhD; Christine Van Broeckhoven[11,12], PhD; Raffaele Ferrari[34], PhD; Emmanuelle Génin[35], PhD; Alexis Brice[1], MD; and Isabelle Le Ber[1,2], MD, PhD.

Ethics

All participants were included in research studies after written informed consent was obtained from the patients or their guardians, in agreement with their national bioethics laws.

All French patients recruited by the French clinical and genetic research network on FTLD/FTLD-ALS (Inserm project #RBM 02-59) were enrolled in agreement with the French bioethics laws (Institutional Review Board: CPP Ile de France II). The 15 Dutch individuals (5 with *C9orf72* expansions and 10 with mutations in *GRN*) were part of study approved by the Medical Ethics Committee (METC) of the Erasmus Medical Center in the Netherlands (MEC-2009-409) according to the code of conduct of the Dutch Medical Research Involving Human Subjects Act (WMO) and the principles of the Declaration of Helsinki (version 7, 2013). Ethical issues about the International cohorts of FTD-Genomics Consortium (https://ifgcsite.wordpress.com/) and of the European EU -EOD were described elsewhere (van der Zee *et al.*, 2013; Ferrari *et al.*, 2014).

B tissues from French and Spanish individuals were obtained as part of a program of "Brain Donation for Research" (National Neuro-CEB Brain Bank, GIE Neuro-CEB BB-0033-00011, IDIBAPS Biobank). Brain donations were obtained after the patients or their legal representatives have signed informed consent in their name, as allowed by the French and Spanish laws and approved by local ethics committees.

Genotyping and Quality Controls (QC)

Whole-Genome-Genotyping (WGG) was performed in the 75 *C9orf72* patients (50 pairs of relatives) of the discovery cohort using Illumina Infimium OmniExpressExome-8 v1.4 arrays, at the local genotyping facility (Plateforme P3S, UMS-2 US29 Omique, Paris). Genotypes were assigned using the Genome Studio 2.0 (Illumina) and exported for downstream QC and genetic analyses. Genotyping data from 960011 SNPs were first handled with the PLINK

software v1.90b6.2 (Purcell *et al.*, 2007; Chang *et al.*, 2015). Individuals or SNPs with more than 2% of missing information were excluded. All *C9orf72* carriers have a genotyping rate >98% and passed QC. 12172 variants were removed due to missing genotyping rate >2%. Thirty-three variants did not pass the Hardy-Weinberg exact test ($p < 0.0001$). Variants with a minor allele frequency (MAF) <0.01 were excluded. Finally, 668066 variants and 75 individuals were retained for subsequent analyses. The mean genotyping rate was 0.99. Genotyping data were pruned for Linkage Disequilibirum (LD) with a r^2 threshold of 0.2 prior to estimate kinship. Sex and relatedness of patients inferred from the genotyping data in the discovery cohort were in accordance with expected results. Genotypes from rs1009776, *TMEM106B* rs6966915 and *C6orf10* rs9357140 were extracted and used to include covariates in association analyses for adjustment.

The same genotyping arrays and filtering pipeline were applied to the cohort of *GRN carriers*. Fifty individuals were genotyped. All of them had less than 2% of missing genotyping information. Eighty-nine, 5596 and 239973 variants were removed due to deviation from the Hardy-Weinberg equilibrium, more than 2% of missing information per SNP, and MAF < 0.01, respectively. Finally, 714120 SNPS passed QC for subsequent analyses. We did not observe sex or relatedness discordance. Genotypes from *TMEM106B* rs6966915 and *C6orf10* rs9357140 were extracted to create covariates. The whole genotyping data were used to compute the kinship matrix as described below.

Genotypes from 12 SNPs were directly obtained from the IFGC as well as clinical data and genotypes from *TMEM106B* rs6966915, *C6orf10* rs9357140 to build a replication cohort (Ferrari *et al.*, 2014).

Imputation of data was performed to further explore the association of un-genotyped SNPs in the regions of interest. Genotyping data were imputed to the Haplotype Reference Consortium

(HRC) panel (39.2 million variants) using the University of Michigan Imputation Server with « pre-phase with EAGLE2 and IMPUTE » pipeline (Howie *et al.*, 2009; Loh *et al.*, 2016). Only variants with an imputation accuracy $r^2>0.7$ were kept for further analysis, resulting in 35,129,846 SNPs (1,030,597 on chr.X).

The candidate SNP rs1009776 was also genotyped in the group of 159 unrelated *C9orf72* patients with extreme AO and in the cohort of 109 *C9orf72* patients with ALS using the TaqMan™ SNP genotyping assay C__8338994_10 (Applied Biosystems) following manufacturer's instructions. The same method of genotyping was used on frozen brain tissues.

Immunostaining analyses on brain tissue

For SV2 staining, paraffin sections were first dewaxed. Endogenous peroxidase was inhibited using MethOH 40% + H_2O_2 1%. Tissue was permeabilized with Triton 0.2%. Normal horse serum was used to improve the specificity of antigen recognition. Tissue sections were incubated with SV2 antibodies (1/100) overnight. Secondary antibody (1/200; Biotinylated Horse Anti-Mouse IgG Antibody, Maravai LifeSciences) was incubated and the VECTASTAIN amplification kit (peroxidase-streptavidin, Maravai LifeSciences) was used to reveal the immunostaining. Anti-synaptophysin antibody (Dako, Clone DAK-SYNAP, mouse monoclonal) was diluted at 1:500 and incubated for 30 minutes. Tissue section pretreatment and visualization of immunoreaction was performed applying the Envision-FLEX system at low pH. The immunostaining procedure was performed automatically on an autostainer (DAKO autostainer plus). The use of DAB (3,3′-Diaminobenzidine) allowed the visualization of staining with microscopy (white-light).

Image acquisition and analysis

Stained sections were scanned on Nanozoomer (Hamamatsu Photonics, Hamamatsu, Japan). Three regions of interest (ROI) were defined for each patient. Two were located in the grey matter for duplicates and covered cortical layers III, IV and V). One ROI was defined per section in white matter to normalize the signal). Digital pictures of each ROI (870x488µm) were exported in TIFF format with the NDP view 2 software (Hamamatsu Photonics, Hamamatsu, Japan). The mean gray value of the pixels was then assessed in a surface area (330750 pixels) for each ROI using the Icy software (http://icy.bioimageanalysis.org) and converted to optical density (OD) using the formula OD = Log (256/[gray value + 1]). OD was normalized relative to the staining of the white matter in each corresponding section, which served as baseline value to obtain relative optical densities (RODs) of SV2 staining. So, two normalized measures of cortical SV2 staining were available *per* patients and the mean of values was used for statistical comparisons. The intensity of immunostaining was compared between three cases hemi-or homozygous for minor allele A and four cases hemi-or homozygous for the allele C.

References

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 2015; 4: 7.

Ferrari R, Hernandez DG, Nalls MA, Rohrer JD, Ramasamy A, Kwok JBJ, et al. Frontotemporal dementia and its subtypes: a genome-wide association study. Lancet Neurol 2014; 13: 686–99.

Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 2009; 5: e1000529.

Loh P-R, Danecek P, Palamara PF, Fuchsberger C, A Reshef Y, K Finucane H, et al. Reference-based phasing using the Haplotype Reference Consortium panel. Nat Genet 2016; 48: 1443–8.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559–75.

van der Zee J, Gijselinck I, Dillen L, Van Langenhove T, Theuns J, Engelborghs S, et al. A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. Hum Mutat 2013; 34: 363–73.

Appendix 1

The International Frontotemporal Dementia Genomics Consortium (IFGC) members and affiliations:

Raffaele Ferrari (UCL, Department of Molecular Neuroscience, Russell Square House, 9-12 Russell Square House, London, WC1B 5EH), Dena G Hernandez (Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Building 35, Room 1A215, 35 Convent Drive, Bethesda, MD 20892, USA; Reta Lila Weston Research Laboratories, Department of Molecular Neuroscience, UCL Institute of Neurology, London WC1N 3BG, UK), Michael A Nalls (Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health Building 35, Room 1A215, 35 Convent Drive, Bethesda, MD 20892, USA), Jonathan D Rohrer (Reta Lila Weston Research Laboratories, Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK; Dementia Research Centre, Department of Neurodegenerative Disease, UCL Institute of Neurology), Adaikalavan Ramasamy (Reta Lila Weston Research Laboratories, Department of Molecular Neuroscience, UCL Institute of Neurology, London WC1N 3BG, UK; Department of Medical and Molecular Genetics, King's College London Tower Wing, Guy's Hospital, London SE1 9RT, UK; The Jenner Institute, University of Oxford, Roosevelt Drive, Oxford OX3 7BQ, UK), John BJ Kwok (Neuroscience Research Australia, Sydney, NSW 2031, Australia; School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia), Carol Dobson-Stone (Neuroscience Research Australia, Sydney, NSW 2031, Australia; School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia), William S Brooks Neuroscience Research Australia, Sydney, NSW 2031, Australia; Prince of Wales Clinical School, University of New South Wales, Sydney, NSW 2052, Australia), Peter R Schofield (Neuroscience Research Australia, Sydney, NSW 2031, Australia; School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia), Glenda M Halliday (Neuroscience Research Australia, Sydney, NSW 2031, Australia; School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia), John R Hodges (Neuroscience Research Australia, Sydney, NSW 2031, Australia; School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia), Olivier Piguet (Neuroscience Research Australia, Sydney, NSW 2031, Australia; School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia), Lauren Bartley (Neuroscience Research Australia, Sydney, NSW 2031, Australia), Elizabeth Thompson (South Australian Clinical Genetics Service, SA Pathology (at Women's and Children's Hospital), North Adelaide, SA 5006, Australia; Department of Paediatrics, University of Adelaide, Adelaide, SA 5000, Australia), Isabel Hernández (Research Center and Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain), Agustín Ruiz (Research Center and Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain), Mercè Boada (Research Center and Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain), Barbara Borroni (Neurology Clinic, University of Brescia, Brescia, Italy), Alessandro Padovani (Neurology Clinic, University of Brescia, Brescia, Italy), Carlos Cruchaga (Department of Psychiatry, Washington University, St. Louis, MO, USA; Hope Center, Washington University School of Medicine, St. Louis, MO, USA), Nigel J Cairns (Hope Center, Washington University School of Medicine, St. Louis, MO, USA; Department of Pathology and Immunology, Washington University, St. Louis, MO, USA), Luisa Benussi (Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli,Brescia, Italy), Giuliano Binetti (MAC Memory Clinic, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy), Roberta Ghidoni (Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy), Gianluigi Forloni (Biology of Neurodegenerative Disorders, IRCCS Istituto di Ricerche Farmacologiche, "Mario Negri", Milano, Italy), Diego Albani (Biology of Neurodegenerative Disorders, IRCCS Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy), Daniela Galimberti (University of Milan, Milan, Italy; Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, via F. Sforza 35, 20122, Milan, Italy), Chiara Fenoglio (University of Milan, Milan, Italy; Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, via F. Sforza 35, 20122, Milan, Italy), Maria Serpente (University of Milan, Milan, Italy; Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, via F. Sforza 35, 20122, Milan, Italy), Elio Scarpini (University of Milan, Milan, Italy; Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, via F. Sforza 35, 20122, Milan, Italy), Jordi Clarimón (Memory Unit, Neurology Department and Sant Pau Biomedical Research Institute, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain; Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain), Alberto Lleó (Memory Unit, Neurology Department and Sant Pau Biomedical Research Institute, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain; Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain), Rafael Blesa (Memory Unit, Neurology Department and Sant Pau Biomedical Research Institute, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona,

Barcelona, Spain; Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain); Maria Landqvist Waldö (Unit of Geriatric Psychiatry, Department of Clinical Sciences, Lund University, Lund, Sweden), Karin Nilsson (Unit of Geriatric Psychiatry, Department of Clinical Sciences, Lund University, Lund, Sweden), Christer Nilsson (Clinical Memory Research Unit, Department of Clinical Sciences, Lund University, Lund, Sweden), Ian RA Mackenzie (Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada), Ging-Yuek R Hsiung (Division of Neurology, University of British Columbia, Vancouver, Canada), David MA Mann (Institute of Brain, Behaviour and Mental Health, University of Manchester, Salford Royal Hospital, Stott Lane, Salford, M6 8HD, UK), Jordan Grafman (Rehabilitation Institute of Chicago, Departments of Physical Medicine and Rehabilitation, Psychiatry, and Cognitive Neurology & Alzheimer's Disease Center; Feinberg School of Medicine, Northwestern University, Chicago, USA; Department of Psychology, Weinberg College of Arts and Sciences, Northwestern University, , Chicago, USA), Christopher M Morris (Newcastle Brain Tissue Resource, Institute for Ageing, Newcastle University, NE4 5PL, Newcastle upon Tyne, UK; Newcastle University, Institute of Neuroscience and Institute for Ageing, Campus for Ageing and Vitality, NE4 5PL, Newcastle upon Tyne, UK; Institute of Neuroscience, Newcastle University Medical School, Framlington Place NE2 4HH, Newcastle upon Tyne, UK), Johannes Attems (Newcastle University, Institute of Neuroscience and Institute for Ageing, Campus for Ageing and Vitality, NE4 5PL, Newcastle upon Tyne, UK; Timothy D Griffiths (Institute of Neuroscience, Newcastle University Medical School, Framlington Place, NE2 4HH, Newcastle upon Tyne, UK), Ian G McKeith (Newcastle University, Institute of Neuroscience and Institute for Ageing, Campus for Ageing and Vitality, Newcastle University, NE4 5PL, Newcastle upon Tyne, UK), Alan J Thomas (Newcastle University, Institute of Neuroscience and Institute for Ageing, Campus for Ageing and Vitality, NE4 5PL, Newcastle upon Tyne, UK), Pietro Pietrini (IMT School for Advanced Studies, Lucca, Lucca, Italy), Edward D Huey (Taub Institute, Departments of Psychiatry and Neurology, Columbia University, 630 West 168th Street New York, NY 10032), Eric M Wassermann (Behavioral Neurology Unit, National Insititute of Neurological Disorders and Stroke, National Insititutes of Health, 10 CENTER DR MSC 1440, Bethesda, MD 20892-1440), Atik Baborie (Department of Laboratory Medicine & Pathology, Walter Mackenzie Health Sciences Centre, 8440 - 112 St, University of Alberta Edmonton, Alberta T6G 2B7, Canada), Evelyn Jaros (Newcastle University, Institute for Ageing and Health, Campus for Ageing and Vitality, NE4 5PL, Newcastle upon Tyne, UK), Michael C Tierney (Behavioral Neurology Unit, National Insititute of Neurological Disorders and Stroke, National Insititutes of Health, 10 CENTER DR MSC 1440, Bethesda, MD 20892-1440), Pau Pastor (Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain; Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, Universidad de Navarra, Pamplona, Spain; Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain), Cristina Razquin (Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, Universidad de Navarra, Pamplona, Spain), Sara Ortega-Cubero (Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain; Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, Universidad de Navarra, Pamplona, Spain), Elena Alonso (Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, Universidad de Navarra, Pamplona, Spain), Robert Perneczky (Neuroepidemiology and Ageing Research Unit, School of Public Health, Faculty of Medicine, The Imperial College of Science, Technology and Medicine, London W6 8RP, UK; West London Cognitive Disorders Treatment and Research Unit, West London Mental Health Trust, London TW8 8 DS, UK; Department of Psychiatry and Psychotherapy, Technische Universität München, Munich, 81675 Germany), Janine Diehl-Schmid (Department of Psychiatry and Psychotherapy, Technische Universität München, Munich, 81675 Germany), Panagiotis Alexopoulos (Department of Psychiatry and Psychotherapy, Technische Universität München, Munich, 81675 Germany), Alexander Kurz (Department of Psychiatry and Psychotherapy, Technische Universität München, Munich, 81675 Germany), Innocenzo Rainero (Neurology I, Department of Neuroscience, University of Torino, Italy, A.O. Città della Salute e della Scienza di Torino, Torino, Italy), Elisa Rubino (Neurology I, Department of Neuroscience, University of Torino, Italy, A.O. Città della Salute e della Scienza di Torino, Torino, Italy), Lorenzo Pinessi (Neurology I, Department of Neuroscience, University of Torino, Italy, A.O. Città della Salute e della Scienza di Torino, Torino, Italy), Ekaterina Rogaeva (Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto 60 Leonard Street, Toronto, Ontario, Canada, M5T 2S8), Peter St George-Hyslop (Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, 60 Leonard Street, Toronto, Ontario, Canada, M5T 2S8; Cambridge Institute for Medical Research, and the Department of Clinical Neurosciences, University of Cambridge, Hills Road, Cambridge, UK CB2 0XY), Giacomina Rossi (Division of Neurology V and Neuropathology, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milano, Italy), Fabrizio Tagliavini (Division of Neurology V and Neuropathology, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milano, Italy), Giorgio Giaccone (Division of Neurology V and Neuropathology, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milano, Italy), James B Rowe Cambridge University Department of Clinical

Neurosciences, Cambridge, CB2 0SZ, UK; MRC Cognition and Brain Sciences Unit, Cambridge, CB2 7EF, UK; Behavioural and Clinical Neuroscience Institute, Cambridge, CB2 3EB, UK), Johannes CM Schlachetzki (University of California San Diego, Department of Cellular & Molecular Medicine, 9500 Gilman Drive, La Jolla, CA, 92093), James Uphill (MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square House, Queen Square, London, WC1N 3BG), John Collinge (MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology Queen Square House, Queen Square, London, WC1N 3BG), Simon Mead (MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square House, Queen Square, London, WC1N 3BG), Adrian Danek (Neurologische Klinik und Poliklinik, Ludwig-Maximilians-Universität, Munich, Germany, German Center for Neurodegenerative Diseases (DZNE)), Vivianna M Van Deerlin (University of Pennsylvania Perelman School of Medicine, Department of Pathology and Laboratory Medicine, Philadelphia, PA, USA), Murray Grossman (University of Pennsylvania Perelman School of Medicine, Department of Neurology and Penn Frontotemporal Degeneration Center, Philadelphia, PA, USA), John Q Trojanowski (University of Pennsylvania Perelman School of Medicine, Department of Pathology and Laboratory Medicine, Philadelphia, PA, USA), Julie van der Zee (Neurodegenerative Brain Diseases group, VIB-UAntwerp Center of Molecular Neurology, Antwerp, Belgium; Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium), Christine Van Broeckhoven (Neurodegenerative Brain Diseases group, VIB-UAntwerp Center of Molecular Neurology, Antwerp, Belgium; Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium), Stefano F Cappa (Neurorehabilitation Unit, Dept. Of Clinical Neuroscience, Vita-Salute University and San Raffaele Scientific Institute, Milan, Italy), Isabelle Leber (Inserm, UMR_S975, CRICM; UPMC Univ Paris 06, UMR_S975; CNRS UMR 7225, F-75013, Paris, France; AP-HP, Hôpital de la Salpêtrière, Département de neurologie-centre de références des démences rares, F-75013, Paris, France), Didier Hannequin (Service de Neurologie, Inserm U1079, CNR-MAJ, Rouen University Hospital, Rouen, France), Véronique Golfier (Service de neurologie, CH Saint Brieuc, France), Martine Vercelletto (Service de neurologie, CHU Nantes, France), Alexis Brice (Inserm, UMR_S975, CRICM; UPMC Univ Paris 06, UMR_S975; CNRS UMR 7225, F-75013, Paris, France; AP-HP, Hôpital de la Salpêtrière, Département de neurologie-centre de références des démences rares, F-75013, Paris, France), Benedetta Nacmias (Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA) University of Florence, Florence, Italy), Sandro Sorbi (Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence and IRCCS "Don Carlo Gnocchi" Firenze, Florence, Italy), Silvia Bagnoli (Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Florence, Italy), Irene Piaceri (Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Florence, Italy), Jørgen E Nielsen (Danish Dementia Research Centre, Neurogenetics Clinic, Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; Department of Cellular and Molecular Medicine, Section of Neurogenetics, The Panum Institute, University of Copenhagen, Copenhagen, Denmark), Lena E Hjermind (Danish Dementia Research Centre, Neurogenetics Clinic, Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; Department of Cellular and Molecular Medicine, Section of Neurogenetics, The Panum Institute, University of Copenhagen, Copenhagen, Denmark), Matthias Riemenschneider (Saarland University Hospital, Department for Psychiatry & Psychotherapy, Kirrberger Str.1, Bld.90, 66421 Homburg/Saar, Germany; Saarland University, Laboratory for Neurogenetics, Kirrberger Str.1, Bld.90, 66421 Homburg/Saar, Germany), Manuel Mayhaus (Saarland University, Laboratory for Neurogenetics, Kirrberger Str.1, Bld.90, 66421 Homburg/Saar, Germany), Bernd Ibach (University Regensburg, Department of Psychiatry, Psychotherapy and Psychosomatics, Universitätsstr. 84, 93053 Regensburg, Germany), Gilles Gasparoni (Saarland University, Laboratory for Neurogenetics, Kirrberger Str.1, Bld.90, 66421 Homburg/Saar, Germany), Sabrina Pichler (Saarland University, Laboratory for Neurogenetics, Kirrberger Str.1, Bld.90, 66421 Homburg/Saar, Germany), Wei Gu (Saarland University, Laboratory for Neurogenetics, Kirrberger Str.1, Bld.90, 66421 Homburg/Saar, Germany; Luxembourg Centre For Systems Biomedicine (LCSB), University of Luxembourg 7, avenue des Hauts-Fourneaux, 4362 Esch-sur-Alzette, Luxembourg), Martin N Rossor (Dementia Research Centre, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, WC1N 3BG), Nick C Fox (Dementia Research Centre, Department of Neurodegenerative Disease, UCL Institute of Neurology Queen Square, London, WC1N 3BG), Jason D Warren (Dementia Research Centre, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, WC1N 3BG), Maria Grazia Spillantini (University of Cambridge, Department of Clinical Neurosciences, John Van Geest Brain Repair Centre, Forvie Site, Robinson way, Cambridge CB2 0PY), Huw R Morris (UCL, Department of Molecular Neuroscience, Russell Square House, 9-12 Russell Square House, London, WC1B 5EH), Patrizia Rizzu (German Center for Neurodegenerative Diseases-Tübingen, Otfried Muellerstrasse 23, Tuebingen 72076, Germany), Peter Heutink (German Center for Neurodegenerative Diseases-Tübingen, Otfried Muellerstrasse 23, Tuebingen 72076, Germany), Julie S Snowden (Institute of Brain, Behaviour

and Mental Health, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK), Sara Rollinson (Institute of Brain, Behaviour and Mental Health, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK), Anna Richardson (Salford Royal Foundation Trust, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK), Alexander Gerhard (Institute of Brain, Behaviour and Mental Health, The University of Manchester, 27 Palatine Road, Withington, Manchester, M20 3LJ, UK), Amalia C Bruni (Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Raffaele Maletta (Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Francesca Frangipane (Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Chiara Cupidi (Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Livia Bernardi (Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Maria Anfossi (Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Maura Gallo (Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Maria Elena Conidi Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Nicoletta Smirne (Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy), Rosa Rademakers (Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224), Matt Baker (Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224), Dennis W Dickson (Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224), Neill R Graff-Radford (Department of Neurology, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224), Ronald C Petersen (Department of Neurology, Mayo Clinic Rochester, 2001st street SW Rochester MN 5905), David Knopman (Department of Neurology, Mayo Clinic Rochester, 2001st street SW Rochester MN 5905), Keith A Josephs (Department of Neurology, Mayo Clinic Rochester, 2001st street SW Rochester MN 5905), Bradley F Boeve (Department of Neurology, Mayo Clinic Rochester, 2001st street SW Rochester MN 5905), Joseph E Parisi (Department of Pathology, Mayo Clinic Rochester, 2001st street SW Rochester MN 5905), William W Seeley (Department of Neurology, Box 1207, University of California, San Francisco, CA 94143, USA), Bruce L Miller (Memory and Aging Center, Department of Neurology, University of California, San Francisco, CA 94158, USA), Anna M Karydas (Memory and Aging Center, Department of Neurology, University of California, San Francisco, CA 94158, USA), Howard Rosen (Memory and Aging Center, Department of Neurology, University of California, San Francisco, CA 94158, USA), John C van Swieten (Department of Neurology, Erasmus Medical Centre, Rotterdam, The Netherlands; Department of Medical Genetics, VU university Medical Centre, Amsterdam, The Netherlands), Elise GP Dopper (Department of Neurology, Erasmus Medical Centre, Rotterdam, The Netherlands), Harro Seelaar (Department of Neurology, Erasmus Medical Centre, Rotterdam, The Netherlands), Yolande AL Pijnenburg (Alzheimer Centre and department of neurology, VU University medical centre, Amsterdam, The Netherlands), Philip Scheltens (Alzheimer Centre and department of neurology, VU University medical centre, Amsterdam, The Netherlands), Giancarlo Logroscino (Department of Basic Medical Sciences, Neurosciences and Sense Organs of the "Aldo Moro" University of Bari, Bari, Italy), Rosa Capozzo (Department of Basic Medical Sciences, Neurosciences and Sense Organs of the "Aldo Moro" University of Bari, Bari, Italy), Valeria Novelli (Medical Genetics Unit, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy), Annibale A Puca (Cardiovascular Research Unit, IRCCS Multimedica, Milan, Italy; Department of Medicine and Surgery, University of Salerno, Baronissi (SA), Italy), Massimo Franceschi (Neurology Dept, IRCCS Multimedica, Milan, Italy), Alfredo Postiglione (Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy), Graziella Milan (Geriatric Center Frullone- ASL Napoli 1 Centro, Naples, Italy), Paolo Sorrentino (Geriatric Center Frullone- ASL Napoli 1 Centro, Naples, Italy), Mark Kristiansen (UCL Genomics, Institute of Child Health (ICH), UCL, London, UK), Huei-Hsin Chiang (Karolinska Institutet, Dept NVS, Alzheimer Research Center, Novum, SE-141 57, Stockholm, Sweden; Dept of Geriatric Medicine, Genetics Unit, M51, Karolinska University Hospital, SE-14186, Stockholm), Caroline Graff (Karolinska Institutet, Dept NVS, Alzheimer Research Center, Novum, SE-141 57, Stockholm, Sweden; Dept of Geriatric Medicine, Genetics Unit, M51, Karolinska University Hospital, SE-14186, Stockholm), Florence Pasquier (Univ Lille, Inserm 1171, DISTALZ, CHU 59000 Lille, France), Adeline Rollin (Univ Lille, Inserm 1171, DISTALZ, CHU 59000 Lille, France), Vincent Deramecourt (Univ Lille, Inserm 1171, DISTALZ, CHU 59000 Lille, France), Thibaud Lebouvier (Univ Lille, Inserm 1171, DISTALZ, CHU 59000 Lille, France), Dimitrios Kapogiannis (National Institute on Aging (NIA/NIH), 3001 S. Hanover St, NM 531, Baltimore, MD, 21230), Luigi Ferrucci (Clinical Research Branch, National Institute on Aging, Baltimore, MD, USA), Stuart Pickering-Brown (Institute of Brain, Behaviour and Mental Health, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK), Andrew B Singleton (Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Building 35, Room 1A215, 35 Convent Drive, Bethesda, MD 20892, USA), John Hardy (UCL, Department of Molecular Neuroscience, Russell Square House, 9-12 Russell Square House, London, WC1B 5EH), Parastoo Momeni (Laboratory of Neurogenetics, Department of Internal Medicine, Texas Tech University Health Science Center, 4th street, Lubbock, Texas 79430, USA).

IFGC Acknowledgments

Intramural funding from the National Institute of Neurological Disorders and Stroke (NINDS) and National Institute on Aging (NIA), the Wellcome/MRC Centre on Parkinson's disease, Alzheimer's Research UK (ARUK, Grant ARUK-PG2012-18) and by the office of the Dean of the School of Medicine, Department of Internal Medicine, at Texas Tech University Health Sciences Center.

We thank Mike Hubank and Kerra Pearce at the Genomic core facility at the Institute of Child Health (ICH), University College of London (UCL), for assisting RF in performing Illumina genotyping experiments (FTD-GWAS genotyping). This study utilized the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health, Bethesda, Md. (http://biowulf.nih.gov). North American Brain Expression Consortium (NABEC) - The work performed by the North American Brain Expression Consortium (NABEC) was supported in part by the Intramural Research Program of the National Institute on Aging, National Institutes of Health, part of the US Department of Health and Human Services; project number ZIA AG000932-04. In addition this work was supported by a Research Grant from the Department of Defense, W81XWH-09-2-0128. UK Brain Expression Consortium (UKBEC) - This work performed by the UK Brain Expression Consortium (UKBEC) was supported by the MRC through the MRC Sudden Death Brain Bank (C.S.), by a Project Grant (G0901254 to J.H. and M.W.) and by a Fellowship award (G0802462 to M.R.). D.T. was supported by the King Faisal Specialist Hospital and Research Centre, Saudi Arabia. Computing facilities used at King's College London were supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. We would like to thank AROS Applied Biotechnology AS company laboratories and Affymetrix for their valuable input. RF's work is supported by Alzheimer's Society (grant number 284), UK; JBJK was supported by the National Health and Medical Resarch Council (NHMRC) Australia, Project Grants 510217 and 1005769; CDS was supported by NHMRC Project Grants 630428 and 1005769; PRS was supported by NHMRC Project Grants 510217 and 1005769 and acknowledges that DNA samples were prepared by Genetic Repositories Australia, supported by NHMRC Enabling Grant 401184; GMH was supported by NHMRC Research Fellowship 630434, Project Grant 1029538, Program Grant 1037746; JRH was supported by the Australian Research Council Federation Fellowship, NHMRC Project Grant 1029538, NHMRC Program Grant 1037746; OP was supported by NHMRC Career Development Fellowship 1022684, Project Grant 1003139. IH, AR and MB acknowledge the patients and controls who participated in this project and the Trinitat Port-Carbó and her family who are supporting Fundació ACE research programs. CC was supported by Grant P30-NS069329-01 and acknowledges that the recruitment and clinical characterization of research participants at Washington University were supported by NIH P50 AG05681, P01 AG03991, and P01 AG026276. LB and GB were supported by the Ricerca Corrente, Italian Ministry of Health; RG was supported by Fondazione CARIPLO 2009-2633, Ricerca Corrente, Italian Ministry of Health; GF was supported by Fondazione CARIPLO 2009-2633. ES was supported by the Italian Ministry of Health; CF was supported by Fondazione Cariplo; MS was supported from the Italian Ministry of Health (Ricerca Corrente); MLW was supported by Government funding of clinical research within NHS Sweden (ALF); KN was supported by Thure Carlsson Foundation; CN was supported by Swedish Alzheimer Fund. IRAM and GYRH were supported by CIHR (grant 74580) PARF (grant C06-01). JG was supported by the NINDS intramural research funds for FTD research. CMM was supported by Medical Research Council UK, Brains for Dementia Research, Alzheimer's Society, Alzheimer's Research UK, National Institutes for Health Research, Department of Health, Yvonne Mairy Bequest and acknowledges that tissue made available for this study was provided by the Newcastle Brain Tissue Resource, which was funded in part by grants G0400074 and G1100540 from the UK MRC, the Alzheimer's Research Trust and Alzheimer's Society through the Brains for Dementia Research Initiative and an NIHR Biomedical Research Centre Grant in Ageing and Health, and NIHR Biomedical Research Unit in Lewy Body Disorders. CMM was supported by the UK Department of Health and Medical Research Council and the Research was supported by the National Institute for Health Research Newcastle Biomedical Research Centre based at Newcastle Hospitals Foundation Trust and Newcastle University and acknowledges that the views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health; JA was supported by MRC, Dunhill Medical Trust, Alzheimer's Research UK; TDG was supported by Wellcome Trust Senior Clinical Fellow; IGM was supported by NIHR Biomedical Research Centre and Unit on Ageing Grants and acknowledges the National Institute for Health Research Newcastle Biomedical Research Centre based at Newcastle Hospitals Foundation Trust and Newcastle University. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health; AJT was supported by Medical Research Council, Alzheimer's Society, Alzheimer's Research UK, National Institutes for Health Research. EJ was supported by NIHR, Newcastle Biomedical Research Centre. PP, CR, SOC and EA were supported partially by FIMA (Foundation for Applied Medical Research); PP acknowledges Manuel Seijo-Martínez (Department of Neurology, Hospital do Salnés, Pontevedra, Spain), Ramon Rene, Jordi Gascon and

Jaume Campdelacreu (Department of Neurology, Hospital de Bellvitge, Barcelona, Spain) for providing FTD DNA samples. RP, JDS, PA and AK were supported by German Federal Ministry of Education and Research (BMBF; grant number FKZ 01GI1007A – German FTLD consortium). IR was supported by Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) of Italy. PStGH was supported by the Canadian Institutes of Health Research, Wellcome Trust, Ontario Research Fund. FT was supported by the Italian Ministry of Health (ricerca corrente) and MIUR grant RBAP11FRE9; GR and GG were supported by the Italian Ministry of Health (ricerca corrente). JBR was supported by Camrbidge NIHR Biomedical Research Centre and Wellcome Trust (088324). JU, JC, SM were supported by the MRC Prion Unit core funding and acknowledge MRC UK, UCLH Biomedical Research Centre, Queen Square Dementia BRU; SM acknowledges the work of John Beck, Tracy Campbell, Gary Adamson, Ron Druyeh, Jessica Lowe, Mark Poulter. AD acknowledges the work of Benedikt Bader and of Manuela Neumann, Sigrun Roeber, Thomas Arzberger and Hans Kretzschmar†; VMVD and JQT were supported by Grants AG032953, AG017586 and AG010124; MG was supported by Grants AG032953, AG017586, AG010124 and NS044266; VMVD acknowledges EunRan Suh, PhD for assistance with sample handling and Elisabeth McCarty-Wood for help in selection of cases; JQT acknowledges Terry Schuck, John Robinson and Kevin Raible for assistance with neuropathological evaluation of cases. CVB and the Antwerp site were in part funded by the MetLife Foundation for Medical Research Award (to CVB); the Belgian Science Policy Office (BELSPO) Interuniversity Attraction Poles program; the Flemish Government initiated Methusalem Excellence Program (to CVB); the Flemish government initiated Impulse Program on Networks for Dementia Research (VIND); the Research Foundation Flanders (FWO) and the University of Antwerp Research Fund. CVB and JvdZ acknowledge the neurologists S Engelborghs, PP De Deyn, A Sieben, R Vandenberghe and the neuropathologist JJ Martin for the clinical and pathological diagnoses. CVB and JvdZ further thank the personnel of the Neuromics Support Facility of the VIB Center for Molecular Neurology and the Antwerp Biobank of the Institute Born-Bunge for their expert support. IL and AB were supported by the program "Investissements d'avenir" ANR-10-IAIHU-06 and acknowledges the contribution of The French research network on FTLD/FTLD-ALS for the contribution in samples collection. BN is founded by Fondazione Cassa di Risparmio di Pistoia e Pescia (grant 2014.0365), SS is founded by the Cassa di Risparmio di Firenze (grant 2014.0310) and a grant from Ministry of Health n° RF-2010-2319722. JEN was supported by the Novo Nordisk Foundation, Denmark. MR was supported by the German National Genome Network (NGFN); German Ministry for Education and Research Grant Number 01GS0465. JDR, MNR, NCF and JDW were supported by an MRC programme grant and the Dementia Platform UK, the NIHR Queen Square Dementia Biomedical Research Unit (BRU) and the Leonard Wolfson Experimental Neurology Centre. MGS was supported by MRC grant n G0301152, Cambridge Biomedical Research Centre and acknowledges Mrs K Westmore for extracting DNA. HM was supported by the Motor Neuron Disease Association (Grant 6057). RR was supported by P50 AG016574, R01 NS080882, R01 NS065782, P50 NS72187 and the Consortium for Frontotemporal Dementia; DWD was supported by P50NS072187, P50AG016574, State of Florida Alzheimer Disease Initiative, & CurePSP, Inc.; NRGR, JEP, RCP, DK, BFB were supported by P50 AG016574; KAJ was supported by R01 AG037491; WWS was supported by NIH AG023501, AG019724, Consortium for Frontotemporal Dementia Research; BLM was supported by P50AG023501, P01AG019724, Consortium for FTD Research; HR was supported by AG032306. JCvS was supported by Stichting Dioraphte Foundation (11 02 03 00), Nuts Ohra Foundation (0801-69), Hersenstichting Nederland (BG 2010-02) and Alzheimer Nederland. CG and HHC acknowledge families, patients, clinicians including Dr Inger Nennesmo and Dr Vesna Jelic, Professor Laura Fratiglioni for control samples and Jenny Björkström, Håkan Thonberg, Charlotte Forsell, Anna-Karin Lindström and Lena Lilius for sample handling. CG was supported by Swedish Brain Power (SBP), the Strategic Research Programme in Neuroscience at Karolinska Institutet (StratNeuro), the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet, Swedish Alzheimer Foundation, Swedish Research Council, Karolinska Institutet PhD-student funding, King Gustaf V and Queen Victoria's Free Mason Foundation. FP, AR, VD and FL acknowledge Labex DISTALZ. RF acknowledges the help and support of Mrs. June Howard at the Texas Tech University Health Sciences Center Office of Sponsored Programs for tremendous help in managing Material Transfer Agreement at TTUHSC.

Neurological Tissue Bank of the Biobank Hospital Clinic-IDIBAPS members and affiliations:

Raquel Sánchez-Valle, Albert Lladó, Anna Antonell, Sergi Borrego-Écija (Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona), Isabel Hernandez (Research Center and Memory Clinic, Fundació ACE, Institut Català de Neurociències Aplicades, Universitat Internacional de Catalunya, Barcelona, Spain. Networking Research Center on

Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain), Miquel Aguilar (Movement Disorders and Memory Unit, Department of Neurology, University Hospital Mutua de Terrassa, Fundació per la Recerca Biomèdica i Social Mútua Terrassa, Terrassa, Barcelona, Spain), Ricardo Rojas-Garcia, Alberto Lleo (Neurology Department, Institut d'Investigacions Biomèdiques-Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain; Networking Research Center on Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain), Ramon Reñé, Monica Povedano, Isidre Ferrer (Hospital Universitari de Bellvitge, University of Barcelona, Spain), Francesc Cardellach (Laboratory of Muscle Research and Mitochondrial Function, Department of Internal Medicine-Hospital Clínic of Barcelona (HCB), Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Faculty of Medicine and Health Science, University of Barcelona (UB), 08036 Barcelona, Spain; Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Raras (CIBERER), 28029 Madrid, Spain), Dolores Lopez-Villegas (EAIA trastorns cognitius. Neuropsychiatry and Drug addiction Institute. Centre Emili Mira, Parc de Salut Mar, Barcelona, Spain), Jose Álvarez Sabin (Neurology Department, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Spain), Lorena Bajo Peñas (Department of Geriatry, Hospital Universitari de la Santa Creu de Vic, Barcelona, Spain), Oscar Macho, Isabel Collado (Àrea de recerca, Consorci Sanitari de l'Alt Penedès i Garraf, Vilafranca del Penedès, Spain), Rosa De Eugenio (Hospital de Palamós, Integrated Health Services Baix Empordà (SSIBE), Catalonia, Spain), Ana Escrig (Hospital General Sant Boi, Barcelona, Spain), Maria de los Llanos Mira García-Gutiérrez (Hospital Sagrat Cor, Barcelona, Spain)