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Plasma Neurofilament Light for Prediction of Disease Progression in Familial Frontotemporal Lobar Degeneration

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Julio C. Rojas, MD, PhD; Ping Wang, MS; Adam M. Staffaroni, PhD; Carolin Heller, BSc; Yann Cobigo, PhD; Amy Wolf, BS; Sheng-Yang M. Goh, BS; Peter A. Ljubenkov, MD; Hilary W. Heuer, PhD; Jamie C. Fong, MS; Joanne B. Taylor, MS; Eliseo Veras, BS; Linan Song; Andreas Jeromin, PhD; David Hanlon, PhD; Lili Yu; Arvind Khinikar; Rajeev Sivasankaran, PhD; Agnieszka Kieloch; Marie-Anne Valentin; Anna M. Karydas; Laura L. Mitic, PhD; Rodney Pearlman; John Kornak, PhD; Joel H. Kramer, PsyD; Bruce L. Miller, MD; Kejal Kantarci, MD; David S. Knopman, MD; Neill Graff-Radford, MD; Leonard Petrucelli, PhD; Rosa Rademakers, PhD; David J. Irwin, MD; Murray Grossman, MD, EdD; Eliana Marisa Ramos, PhD; Giovanni Coppola, MD; Mario F. Mendez, MD, PhD; Yvette Bordelon, MD, PhD; Bradford C. Dickerson, MD; Nupur Ghoshal, MD, PhD; Edward D. Huey, MD; Ian R. Mackenzie, MD; Brian S. Appleby, MD; Kimiko Domoto-Reilly, MD; Ging-Yuek R. Hsiung, MD; Arthur W. Toga, PhD; Sandra Weintraub, PhD; +Daniel I. Kaufer, MD; Diana Kerwin, MD; Irene Litvan, MD; Chiadikaobi U. Onyike, MD; Alexander Pantelyat, MD; Erik D. Roberson, MD, PhD; Maria C. Tartaglia, MD; Tatiana Foroud, PhD; Weiping Chen, MS; Julie Czerkowicz, MS; Danielle L. Graham, PhD; John C. van Swieten, PhD; Barbara Borroni, MD; Raquel Sanchez-Valle, PhD; Fermin Moreno, MD, PhD; Robert Laforce, MD, PhD; Caroline Graff, MD, PhD; Matthis Synofzik, MD; Daniela Galimberti, PhD; James B. Rowe, MD, PhD; Mario Masellis, MD, PhD; Elizabeth Finger, MD; Rik Vandenberghe, MD, PhD; Alexandre de Mendonça, MD, PhD; Fabrizio Tagliavini, MD; Isabel Santana, MD, PhD; Simon Ducharme, MD; Chris R. Butler, PhD, FRCP; Alexander Gerhard, MD, MRCP; Johannes Levin, MD; Adrian Danek, MD; Markus Otto, MD; Sandro Sorbi, PhD; David M. Cash, PhD; Rhian S. Convery, MSc; Martina Bocchetta, PhD; Martha Foiani, MRes; Caroline V. Greaves, BSc; Georgia Peakman, MSc; Lucy Russell, PhD; Imogen Swift, MSc; Emily Todd, MRes; Jonathan D. Rohrer, PhD, FRCP; Bradley F. Boeve, MD; Howard J. Rosen, MD; Adam L. Boxer, MD, PhD on behalf of the ALLFTD and GENFI consortia

+ Author is deceased

Corresponding Author:
Julio C. Rojas
jrojasmartinez@memory.ucsf.edu

Affiliation Information for All Authors:
Julio C. Rojas, University of California, San Francisco, San Francisco, CA, USA
Ping Wang, University of California, San Francisco, San Francisco, CA, USA
Adam M. Staffaroni, University of California, San Francisco, San Francisco, CA, USA
Carolin Heller, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Yann Cobigo University of California, San Francisco, San Francisco, CA, USA
Amy Wolf, University of California, San Francisco, San Francisco, CA, USA
Sheng-Yang M. Goh, University of California, San Francisco, San Francisco, CA, USA
Peter A. Ljubenkov, University of California, San Francisco, San Francisco, CA, USA
Hilary W. Heuer, University of California, San Francisco, San Francisco, CA, USA
Jamie C. Fong, University of California, San Francisco, San Francisco, CA, USA
Joanne B. Taylor, University of California, San Francisco, San Francisco, CA, USA
Eliseo Veras, Quanterix Corporation, Lexington, MA, USA
Linan Song, Quanterix Corporation, Lexington, MA, USA
Andreas Jeromin, Quanterix Corporation, Lexington, MA, USA

David Hanlon, Quanterix Corporation, Lexington, MA, USA
Lili Yu, Novartis Institutes for Biomedical Research Inc, Cambridge, MA, USA
Arvind Kinhikar, Novartis Institutes for Biomedical Research Inc, Cambridge, MA, USA
Rajeev Sivasankaran, Novartis Institutes for Biomedical Research Inc, Cambridge, MA, USA
Agnieszka Kieloch, Novartis Pharma AG, Basel, Switzerland
Marie-Anne Valentin, Novartis Pharma AG, Basel, Switzerland
Anna M. Karydas, University of California, San Francisco, San Francisco, CA, USA
Laura L. Mitic, University of California, San Francisco, San Francisco, CA, USA and Bluefield Project to Cure Frontotemporal Dementia, San Francisco, CA, USA
Rodney Pearlman, Bluefield Project to Cure Frontotemporal Dementia, San Francisco, CA, USA
John Kornak, University of California, San Francisco, San Francisco, CA, USA
Joel H. Kramer, University of California, San Francisco, San Francisco, CA, USA
Bruce L. Miller, University of California, San Francisco, San Francisco, CA, USA
Kejal Kantarci, Mayo Clinic, Rochester, MN, USA
David S. Knopman, Mayo Clinic, Rochester, MN, USA
Neill Graff-Radford, Mayo Clinic, Jacksonville, FL, USA
Leonard Petrucelli, Mayo Clinic, Jacksonville, FL, USA
Rosa Rademakers, Mayo Clinic, Jacksonville, FL, USA
David J. Irwin, University of Pennsylvania, Philadelphia, PA, USA
Murray Grossman, University of Pennsylvania, Philadelphia, PA, USA
Eliana Marisa Ramos, University of California, Los Angeles, Los Angeles, CA, USA
Giovanni Coppola, University of California, Los Angeles, Los Angeles, CA, USA
Mario F. Mendez, University of California, Los Angeles, Los Angeles, CA, USA
Yvette Bordelon, University of California, Los Angeles, Los Angeles, CA, USA
Bradford C. Dickerson, Harvard University/Massachusetts General Hospital, Boston, MA, USA
Nupur Ghoshal, Washington University, St. Louis, MO, US
Edward D. Huey, Columbia University, New York, NY, USA
Ian R. Mackenzie, University of British Columbia, Vancouver, British Columbia, Canada
Brian S. Appleby, Case Western Reserve University, Cleveland, OH, USA
Kimiko Domoto-Reilly, University of Washington, Seattle, WA, USA
Ging-Yuek R. Hsiung, University of British Columbia, Vancouver, British Columbia, Canada
Arthur W. Toga, Laboratory of Neuroimaging, University of Southern California, Los Angeles, CA, USA
Sandra Weintraub, Northwestern University, Chicago, IL, USA
Daniel I. Kaufer, University of North Carolina, Chapel Hill, NC, USA
Diana Kerwin, Texas Health Presbyterian Hospital Dallas, Dallas, TX, USA
Irene Litvan, University of California, San Diego, San Diego, CA, USA
Chiadikaobi U. Onyike, Johns Hopkins Hospital, Baltimore, MD, USA
Alexander Pantelyat, Johns Hopkins Hospital, Baltimore, MD, USA
Erik D. Roberson, University of Alabama, Birmingham, AL, USA
Maria C. Tartaglia, University of Toronto, ON, Canada
Tatiana Foroud, Indiana University School of Medicine, Indianapolis, IN, USA
Weiping Chen, Biogen Inc., Cambridge, MA, USA
Julie Czerkowicz, Biogen Inc., Cambridge, MA, USA
Danielle L. Graham, Biogen Inc., Cambridge, MA, USA
John C. van Swieten, Erasmus Medical Centre, Rotterdam, Netherlands
Barbara Borroni, University of Brescia, Brescia, Italy
Raquel Sanchez-Valle, University of Barcelona, Barcelona, Spain
Fermin Moreno, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain

Robert Laforce, Clinique Interdisciplinaire de Mémoire, Département des Sciences Neurologiques, CHU de Québec, and Faculté de Médecine, Université Laval, QC, Canada
Caroline Graff, Center for Alzheimer Research, Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Solna, Sweden and Unit for Hereditary Dementias, Theme Aging, Karolinska University Hospital, Solna, Sweden
Matthis Synofzik, University of Tübingen, Tübingen, Germany and Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
Daniela Galimberti, Fondazione IRCCS Ospedale Policlinico, Milan, Italy and University of Milan, Centro Dino Ferrari, Milan, Italy
James B. Rowe, Department of Clinical Neurosciences and Cambridge University Hospital, University of Cambridge, Cambridge, UK
Mario Masellis, University of Toronto, ON, Canada
Elizabeth Finger, University of Western Ontario, London, ON, Canada
Rik Vandenberghe, KU Leuven, Leuven, Belgium and Neurology Service, University Hospitals Leuven, Belgium
Alexandre de Mendonça, University of Lisbon, Lisbon, Portugal
Fabrizio Tagliavini, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
Isabel Santana, University of Coimbra, Coimbra, Portugal
Simon Ducharme, McGill University, Montreal, Québec, Canada
Chris R. Butler, University of Oxford, Oxford, UK
Alexander Gerhard, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK and University of Duisburg-Essen, Germany
Johannes Levin, Ludwig-Maximilians-Universität München, Munich, Germany and German Center for Neurodegenerative Diseases, Munich Cluster for Systems Neurology (SyNergy), Munich, Germany
Adrian Danek, Ludwig-Maximilians-Universität München, Munich, Germany
Markus Otto, University of Ulm, Ulm, Germany
Sandro Sorbi, University of Florence, Florence, Italy
David M. Cash, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Rhian S. Convery, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Martina Bocchetta, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Martha Foiani, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Caroline V. Greaves, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Georgia Peakman, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Lucy Russell, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Imogen Swift, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Emily Todd, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Jonathan D. Rohrer, UK Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
Bradley F. Boeve, Mayo Clinic, Rochester, MN, USA
Howard J. Rosen, University of California, San Francisco, San Francisco, CA, USA

Adam L. Boxer, MD, University of California, San Francisco, San Francisco, CA, USA

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Statistical Analysis performed by: Julio C. Rojas, MD, PhD (University of California, San Francisco) John Kornak, PhD (University of California, San Francisco)

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S.M. Goh reports no disclosures relevant to the manuscript
P.A. Ljubenkov reports no disclosures relevant to the manuscript
H.W. Heuer reports no disclosures relevant to the manuscript
J.C. Fong reports no disclosures relevant to the manuscript
J.B. Taylor reports no disclosures relevant to the manuscript
E. Veras is a Quanterix employee
L. Song is a Quanterix employee
A. Jeromin is an advisor to Quanterix
D.Hanlon is a Quanterix employee
L. Yu is a Novartis employee
A. Kinhikar is a Novartis employee
R. Sivasankaran is a Novartis employee
A. Kieloch is a Novartis employee
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M. Grossman receives grant support from NIH, Avid and Piramal, participates in clinical trials sponsored by Biogen, TauRx, and Alector, serves as a consultant to Bracco and UCB, and serves on the Editorial Board of Neurology
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I. Litvan reports no disclosures relevant to the manuscript
C.U. Onyike reports no disclosures relevant to the manuscript
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M.C. Tartaglia reports no disclosures relevant to the manuscript
T. Foroud reports no disclosures relevant to the manuscript
W. Chen is a Biogen employee
J. Czerkowicz is a Biogen employee
D.L. Graham is a Biogen employee
J.C. van Swieten reports no disclosures relevant to the manuscript
B. Borroni reports no disclosures relevant to the manuscript
R. Sanchez-Valle reports no disclosures relevant to the manuscript
F. Moreno reports no disclosures relevant to the manuscript
R. Laforce reports no disclosures relevant to the manuscript
C. Graff reports no disclosures relevant to the manuscript
M. Synofzik reports no disclosures relevant to the manuscript
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E. Finger reports no disclosures relevant to the manuscript
R. Vandenberghe reports no disclosures relevant to the manuscript
A. de Mendonça reports no disclosures relevant to the manuscript
F. Tagliavini reports no disclosures relevant to the manuscript
I. Santana reports no disclosures relevant to the manuscript
S. Ducharme reports no disclosures relevant to the manuscript
C.R. Butler reports no disclosures relevant to the manuscript
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M. Otto reports no disclosures relevant to the manuscript
S. Sorbi reports no disclosures relevant to the manuscript
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C.V. Greaves reports no disclosures relevant to the manuscript
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L. Russell reports no disclosures relevant to the manuscript
I. Swift reports no disclosures relevant to the manuscript
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Coinvestigator Appendix 2 - http://links.lww.com/WNL/B350

Coinvestigator Appendix 3 - http://links.lww.com/WNL/B351

Abstract

Objective: We tested the hypothesis that plasma neurofilament light chain (NfL) identifies asymptomatic carriers of familial frontotemporal lobar degeneration (FTLD)-causing mutations at risk of disease progression.

Methods: Baseline plasma NfL concentrations were measured with Simoa in original (n = 277) and validation (n = 297) cohorts. *C9orf72*, *GRN* and *MAPT* mutation carriers and non-carriers from the same families were classified by disease severity [asymptomatic, prodromal and full phenotype] using the CDR® Dementia Staging Instrument plus behavior and language domains from the National Alzheimer's Disease Coordinating Center FTLD module (CDR®+NACC-FTLD). Linear mixed effect models related NfL to clinical variables.

Results: In both cohorts, baseline NfL was higher in asymptomatic mutation carriers who showed phenoconversion or disease progression compared to non-progressors (original: 11.4 ± 7 pg/mL vs. 6.7 ± 5 pg/mL, $p = 0.002$; validation: 14.1 ± 12 pg/mL vs. 8.7 ± 6 pg/mL, $p = 0.035$). Plasma NfL discriminated symptomatic from asymptomatic mutation carriers or prodromal disease (original cutoff: 13.6 pg/mL, 87.5% sensitivity, 82.7% specificity; validation cutoff: 19.8 pg/mL, 87.4% sensitivity, 84.3% specificity). Higher baseline NfL correlated with worse longitudinal CDR®+NACC-FTLD sum of boxes scores, neuropsychological function and atrophy, regardless of genotype or disease severity, including asymptomatic mutation carriers.

Conclusions: Plasma NfL identifies asymptomatic carriers of FTLD-causing mutations at short-term risk of disease progression, and is a potential tool to select participants for prevention clinical trials.

Classification of evidence: This study provides Class I evidence that in carriers of FTLD-causing mutations, elevation of plasma NfL predicts short-term risk of clinical progression.

Abbreviations: AUC = area under the curve, ARTFL = Advancing Research and Treatment in Frontotemporal Lobar Degeneration, bvFTD = behavioral variant frontotemporal dementia,

CDR®+NACC-FTLD = CDR® Dementia Staging Instrument plus Behavior and Language domains from the National Alzheimer's Disease Coordinating Center Frontotemporal Lobar Degeneration module, CBS = corticobasal syndrome, CGI-S = Clinical Global Impression of Severity, FAS = Functional Assessment Scale, FTLD = frontotemporal lobar degeneration, f-FTLD = familial frontotemporal lobar degeneration, FTD/ALS = frontotemporal dementia with amyotrophic lateral sclerosis, LEFFTDS = Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects, MoCA = Montreal Cognitive Assessment, MBI/MCI = mild behavioral or cognitive impairment, NPI = Neuropsychiatric Inventory, NfL = neurofilament light chain, p-NfH = phosphorylated neurofilament heavy chain, PPA = primary progressive aphasia, ROC = receiver operating characteristic, SEADL = Schwab and England Activities of Daily Living, UPDRS = Unified Parkinson's Disease Rating Scale Motor Section

Introduction

Blood-based biomarkers are uniquely valuable for therapeutic development, because they are easily obtainable and relatively inexpensive.[1] Frontotemporal lobar degeneration (FTLD) produces behavioral, cognitive, language and motor deficits that impair the quality of life of patients and caregivers more severely than other forms of dementia.[2] About 20-30% of FTLD cases are familial and about 60% of those are caused by autosomal dominant mutations in three genes:[3] chromosome 9 open reading frame 72 (*C9orf72)*,[4] progranulin (*GRN)*[5] and microtubule-associated protein tau (*MAPT)*.[6] Several therapies are poised to begin clinical trials for familial FTLD (f-FTLD) due to these mutations.[7] Planning such studies is challenging due to the low f-FTLD prevalence and the lack of good clinical endpoints to monitor disease severity and therapeutic response.

Neurofilament light chain (NfL) is a sensitive marker of neurodegeneration.[8] CSF NfL is elevated in patients with FTLD, compared to Alzheimer's disease and healthy controls,[9-12] with concentrations that correlate with disease severity, cognitive function and disease progression.[13, 14] CSF NfL concentrations normalize upon effective treatment in multiple sclerosis[15] and spinal muscle atrophy,[16] suggesting that NfL is sensitive to treatment effects. Serum NfL is elevated in FTLD[17] and, in symptomatic carriers of f-FTLD-causing mutations, concentrations correlate with brain atrophy.[18] We tested the hypothesis that plasma NfL could identify asymptomatic f-FTLD mutation carriers at high risk of progression to symptomatic disease. We examined baseline plasma NfL differences related to phenotype, genotype and disease severity, and whether it predicts disease progression in two independent cohorts.

Methods

The primary research question was: Do plasma NfL concentrations identify f-FTLD mutation carriers at risk of clinical progression (Class I level of evidence)?

Standard protocol approvals, registrations, and patient consents

Participants or their caregivers provided written informed consent and the study procedures were approved by the local Institutional Review Board committees at each of the participating centers. Patients were recruited through the North American multicenter observational studies Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects (LEFFTDS, ClinicalTrials.gov NCT02372773) and Advancing Research & Treatment in Frontotemporal Lobar Degeneration (ARTFL, ClinicalTrials.gov NCT02365922),[19] and the Genetic Frontotemporal dementia Initiative (GENFI).[20]

Participants

Participants were divided into *original* (LEFFTDS/ARTFL, n = 277) and *validation* (GENFI, n = 297) cohorts. LEFFTDS/ARTFL is a North American network of 19 clinical research centers. LEFFTDS enrolled members of families with a known mutation in one of the three major FTLD genes: *C9orf72*, *GRN* and *MAPT*. ARTFL enrolled participants who met research criteria for an FTLD syndrome and asymptomatic individuals with family history of an FTLD syndrome, whether or not an FTLD-causing mutation had been identified in the family.[19] Upon evaluation, some participants with family history of FTLD were determined to have prodromal disease or mild cognitive or behavioral impairment (MBI/MCI), as defined previously.[21] GENFI involves 25 research centers across Europe and Canada, and enrolls symptomatic carriers of mutations in the three major FTLD genes with frontotemporal dementia, and those at risk of carrying a mutation because a first-degree relative is a known symptomatic carrier. Both cohorts consisted of participants with available baseline NfL concentrations, known genotype and CDR® Dementia Staging Instrument plus behavior and language domains from the National Alzheimer's Disease Coordinating Center FTLD module (CDR®+NACC-FTLD) global and sum of boxes scores.[21] Mutation non-carriers with CDR®+NACC-FTLD global score > 0 were excluded (11 in the original cohort and 22 in the validation cohort). The validation cohort data have been reported previously.[22] In the original cohort, clinically defined phenotypes included: 184 normal (66.7%), 12 mild behavioral impairment (4.3%), 16 mild cognitive impairment (5.8%), 3 amnestic

dementia (1.1%), 48 behavioral variant frontotemporal dementia (bvFTD, 17.4%), 7 frontotemporal dementia with amyotrophic lateral sclerosis (FTD/ALS, 2.5%), 4 primary progressive aphasia (PPA, non-fluent or semantic, 1.4%) and 3 corticobasal syndrome (CBS, 1.1%). Participants in the validation cohort included: 240 normal (80.8%), 36 bvFTD (12.1%), 6 FTD/ALS (2%), 3 CBS (1%) and 12 PPA (4%). Data on whether or not there was conversion from asymptomatic to MBI/MCI or full phenotype, or from MBI/MCI to full phenotype were available in 221 out of 277 subjects in the original cohort and in 159 out of 297 subjects in the validation cohort.

Clinical procedures

Participants underwent annual standardized evaluations that included neurological assessment, caregiver or companion interview, neuropsychological testing, brain MRI and biofluid collection for up to 3 years in the original cohort, and for 2 years in the validation cohort. Clinical scales included: CDR®+NACC-FTLD global and sum of boxes (sb) scores[21] and Clinical Global Impression of Severity (CGI-S),[23] which are based on semi-structured interviews and provide global measures of clinical severity; Montreal Cognitive Assessment (MoCA); Unified Parkinson's Disease Rating Scale III, Motor Section (UPDRS);[24] Schwab and England Activities of Daily Living (SEADL), for measurement of impairment in activities of daily living;[25] Functional Assessment Scale (FAS), for assessment of impairment in instrumental activities[26] and Neuropsychiatric Inventory (NPI).[27] CDR®+NACC-FTLD and Mini-Mental State Examination (MMSE) were the only severity scales available in the validation cohort. Neuropsychological testing available in both cohorts included the California Verbal Learning Test (CVLT) – Short Form, immediate and delayed recall,[28] the Benson figure recall;[29] forward and backward digit span, number of correct trials, Trail-making Test parts A and B (time to completion)[30] and phonemic and semantic fluency. In the original cohort, blood samples were centrifuged at 1500 g at 4°C for 15 minutes. Plasma was aliquoted in 1000 microliter vials and stored at -80°C at the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD). In

the validation cohort, blood samples were collected and processed as previously reported.[22] Genetic screening was conducted to identify FTLD-causing mutations in the *C9orf72*, *GRN* and *MAPT* genes, and *APOE* polymorphisms as described previously.[22, 31]

Plasma neurofilament-light chain measurement

In the original cohort, plasma NfL concentrations were measured at baseline with single molecule array technology (Simoa), using the commercially available NF-light® digital immunoassay kit (Quanterix, Lexington, MA). Plasma samples were thawed at room temperature (one cycle), mixed thoroughly and centrifuged at 14000 g for 3 minutes. The supernatant was loaded onto a Quanterix HD-1 Analyzer with a 1:4 specified dilution. Measures were completed in duplicate over a total of six batches, each one with an 8-point calibration curve tested in triplicate and two controls tested in duplicate. Plasma concentrations were interpolated from the calibration curve within the same batch and corrected for the dilution. All samples were quantifiable within the dynamic range of 0.69 to 2000 pg/mL and with an average coefficient of variation of 6.5%. Measurements were completed using the same platform in two centers: Quanterix (n = 226, February 2018) and Novartis Institutes for Biomedical Research (n = 64, July 2018). Samples from a subset of 186 participants were analyzed twice, independently by each center, with plasma NfL concentrations that were highly correlated ($r = 0.98$, $p < 0.001$). The samples analyzed by the two centers also had comparable means and standard deviations (21.8 ± 35 pg/mL, Quanterix and 20.2 ± 34 pg/mL, Novartis) and there were no differences in the median plasma NfL concentrations in two groups of age-matched asymptomatic non-carrier controls measured separately (6.9 ± 4 pg/mL Quanterix, n = 38 vs. 6.4 ± 6 pg/mL Novartis, n = 50, $p = 0.6$). The center where samples were analyzed was added as a covariate in statistical analyses. Instrument operators were blinded to clinical and genetic information. In the validation cohort, plasma NfL concentrations were measured with the multiplex Simoa Neurology 4-Plex A kit.[22]

CSF biomarker measurements

CSF biomarkers were available in 113 of the 277 participants at baseline in the original cohort only. Using fit-for-purpose immunoassays, CSF samples were analyzed for NfL, tau, phosphorylated tau_{181} (p-tau), neurogranin, and phosphorylated neurofilament heavy chain (p-NfH) at the following dilutions, 1:50, neat, 1:20, neat, and 1:4, respectively. NfL and tau were measured on the Quanterix Simoa HD-1 (catalog# 103186 and 101552, respectively), p-tau was measured using the Innotest kit (catalog # 81581), neurogranin was measured using the Euroimmun kit (item code: EQ-6551-9601-L) and p-NfH was measured on the Protein Simple Ella platform (catalog # SPCKB-PS-000519). Measurements were conducted by an independent lab, with operators blinded to clinical data (Biogen, Inc.)

Neuroimaging

Brain MRI was obtained in the original cohort as described previously,[32] within 45 days of plasma collection except for 15 patients for whom images were obtained within more than 45 days of plasma collection (median 60 days, range 50-423 days). To simplify relationships with plasma NfL and control for multiple comparisons, bilateral frontal and temporal gray matter lobar composites were created with regions of interest involved in FTLD syndromes. Frontal regions included frontal pole, lateral orbitofrontal cortex, medial orbitofrontal cortex, middle frontal gyrus, pars opercularis, pars orbitalis, pars triangularis, superior frontal gyrus, and precentral gyrus. Anterior cingulate (caudal and rostral) and insula were also included in the frontal composite, given their significant involvement in FTLD.[33] Temporal regions included banks of the superior temporal sulcus, entorhinal cortex, fusiform gyrus, middle temporal gyrus, parahippocampal cortex, superior temporal gyrus, temporal pole, and transverse temporal gyrus.

Statistical analyses

Biofluid measurements, disease status determination and statistical analyses were separately performed by different investigators. Original and validation cohort data were handled

independently. Data were visually explored with box plots. NfL data were not normally distributed. Group differences in NfL concentrations were determined with non-parametric tests. Log transformed NfL data were used as outcome in general linear models to determine between-group differences in NfL concentrations corrected for age and sex. Receiver operating characteristic (ROC) curves tested the diagnostic accuracy of plasma NfL concentrations. Combined forward and backward stepwise linear regressions controlling for age, sex, and genotype determined baseline associations between plasma NfL and clinical variables. Starting with minimal models, the stepwise criteria were such that a variable entered a model when $p < 0.05$, and it was removed when $p \geq 0.1$. For associations with gray matter volumes, total intracranial volume was an additional control variable.[32] Linear mixed models tested the ability of baseline log plasma NfL to predict change in clinical variables. All models included interaction terms of log plasma NfL with time as a discrete predictor. Models used compound symmetry covariance, random slows and intercepts, and were controlled by sex, age, genotype, clinical center and, when modeling prediction of gray matter volumes, also by total intracranial volume. Models were run with log plasma NfL as a continuous independent variable and, subsequently, as a categorical independent variable based on cutoff points derived from Youden indices estimated with ROC curves. Models were run separately for each one of the disease severity levels defined by the CDR®+NACC-FTLD global score: normal or asymptomatic (carriers and non-carriers ran independently) (0), MBI/MCI or prodromal disease (0.5) and dementia or full phenotype (≥1).[21] Model results were corrected for multiple comparisons across dependent variables for a given disease severity level using False Discovery Rate.[34] Analyses were done with SPSS Statistics software, version 26 (IBM, Armonk, NY) and GraphPad Prism, version 8.4 (GraphPad, La Jolla, CA).

Data Availability

Joint ARTFL and LEFFTDS data and biospecimens and GENFI data are available to qualified investigators for replication of the present study results or further projects.

Results

Group differences in baseline plasma NfL concentrations, original cohort

Of 277 subjects with baseline evaluations (**Table 1**), 221 (79.7%) and 148 (53.4%) also had follow-up data available for years 1 and 2, respectively. In all genotypes combined, and after correction for age and sex, amnestic dementia, bvFTD, FTD/ALS, CBS and PPA phenotypes had higher plasma NfL concentrations than asymptomatic participants (mutation carriers and non-carriers combined) and MCI (**Figure 1**).

As defined by disease severity, 65.7% of the participants (33.2% carrier and 32.5% non-carrier) were asymptomatic (CDR®+NACC-FTLD = 0), 11.9% had MBI/MCI (CDR®+NACC-FTLD = 0.5) and 22.4% had full phenotype (CDR®+NACC-FTLD≥ 1). Median baseline plasma NfL concentrations were highest in participants with full phenotype (**Figure 2**). There were no differences in NfL concentrations between asymptomatic mutation carriers and non-carriers for any genotype. Median plasma NfL concentrations tended to be higher in MBI/MCI than asymptomatic mutation carriers, but the results did not reach statistical significance (12.2 ± 10 pg/mL vs. 7.5 ± 6 pg/mL, $p = .085$, mean estimate difference 0.44, 95% CI: 0.85 – 0.99, $p = 0.016$) in all genotypes combined. In *C9orf72* carriers, NfL concentrations were higher in MBI/MCI compared to asymptomatic individuals (13.6 ± 34 pg/mL vs. 6.6 ± 5 pg/mL, $p < 0.001$, **Figure 3**), but not in *GRN* or *MAPT*. There were no genotype-related differences in NfL in asymptomatic mutation carriers or MBI/MCI. In full phenotype, NfL was higher in *GRN* (61.5 ± 54 pg/mL) than in *C9orf72* (33.9 ± 33 pg/mL, $p < 0.001$) and *MAPT* (20.5 ± 11 pg/mL, $p < 0.001$).

In all participants combined, a cut point of ≥ 13.6 pg/mL discriminated individuals with full phenotype from asymptomatic or MBI/MCI with 87.5% sensitivity, 82.7% specificity, 59.7% positive predictive value and 96.2% negative predictive value [area under the curve (AUC) 0.901, 95% CI: 0.861 – 0.942, $p < 0.001$]. Plasma NfL was a poor discriminator between asymptomatic mutation carriers and MBI/MCI (AUC 0.676, 95% CI: 0.588 – 0.724, $p < 0.001$),

but it was a better discriminator between MBI/MCI and full phenotype (0.803, 95% CI: 0.744 – 0.862, $p < 0.001$). The proportion of participants with high (≥ 13.6 pg/mL) NfL differed by severity group: 12.2% in asymptomatic mutation non-carriers, 14.1% in asymptomatic mutation carriers, 39.4% in MBI/MCI and 88.7% in full phenotype ($\chi^2 = 119.6$, $p < .001$).

Baseline correlations with clinical variables, original cohort

Baseline NfL strongly correlated with age in the overall sample ($\rho = 0.69$, 95% CI: 0.505 – 0.695, $p < 0.001$), and in asymptomatic ($\rho = 0.63$, 95% CI: 0.437 – 0.769, $p < 0.001$) and MBI/MCI individuals ($\rho = 0.71$, 95% CI: 0.364 – 0.917, $p < 0.001$), and weakly in full phenotype ($\rho = 0.23$, 95% CI: -0.109 – 0.402, $p = 0.07$). NfL concentrations were higher in women than in men (10.7 ± 13 pg/mL vs. 7.6 ± 9 pg/mL, mean estimate difference 0.75, 95% CI: 0.59 – 0.95, $p = 0.01$), even after controlling for age, disease severity and genotype ($\beta = 0.251$, 95% CI: 0.092 – 0.409 $p = 0.002$). In all participants, plasma NfL was strongly associated with all clinical, neuropsychological and gray matter volume variables at baseline. None of the relationships were affected by genotype and they remained essentially unchanged after exclusion of asymptomatic non-carriers (**eTable 1**). The strongest associations were observed with measures of disease severity, including CDR®+NACC-FTLDsb, CGI-S, SEADL and FAS. Weaker associations were observed with gray matter volumes. CSF biomarkers were available in 113 (40.7%) participants (34 asymptomatic non-carriers, 46 asymptomatic mutation carriers, 14 MBI/MCI and 19 full phenotype). Plasma NfL correlated with CSF NfL ($\rho = 0.74$, $p < 0.001$), CSF p-NfH ($\rho = 0.73$, $p < 0.001$) and CSF tau ($\rho = 0.45$, $p < 0.001$), but not with CSF neurogranin ($\rho = 0.06$, $p = 0.94$) or CSF p-tau ($\rho = 0.07$, $p = 0.46$). There were no differences in the proportion of *APOE* carriers as a function of clinical phenotype, genotype or disease severity, or differences in NfL concentrations by *APOE* genotype.

Baseline NfL, phenoconversion and disease progression, original cohort

Twenty-six mutation carriers phenoconverted after two years [15 asymptomatic (12 to MBI/MCI and 3 to full phenotype) and 11 MBI/MCI to full phenotype]. Phenoconversion occurred in 10/21 (47.6%) of asymptomatic or MBI/MCI mutation carriers with baseline NfL ≥ 13.6 pg/mL, compared to 16/84 (11.4%) of those with baseline NfL < 13.6 pg/mL (p = 0.007). Median baseline NfL concentrations were higher in asymptomatic mutation carriers who phenoconverted to either MBI/MCI or dementia over the next two years as compared to those who remained asymptomatic (11.4 ± 7 pg/mL vs. 6.7 ± 5 pg/mL, p = 0.002, **Figure 4**). Plasma NfL concentrations were also higher in asymptomatic mutation carriers whose CDR®+NACC-FTLDsb scores progressed by 1 point, even in the absence of phenoconversion (10.8 ± 8 pg/mL), compared to those whose scores remained stable (6.6 ± 3 pg/mL, p = .0017, data available from Dryad: https://doi.org/10.7272/Q6W957CZ, **eFigure 1**).

Asymptomatic mutation carriers

As a continuous variable, baseline NfL related to future decline in CDR®+NACC-FTLDsb, CGI-S and FAS (**Table 2**). For example, every baseline log NfL pg/mL in asymptomatic mutation carriers was associated with a 1.6 point increase in CDR®+NACC-FTLDsb at year 1 (95% CI: 0.75 – 2.6; p < 0.001) and a 2.5 point increase at year 2 (95% CI: 1.6 – 3.4; p < 0.001). Similar results were observed when NfL was analyzed as a categorical variable. For example, asymptomatic mutation carriers with high (≥ 13.6 pg/mL) baseline NfL had CDR®+NACC-FTLDsb scores that were 1.6 points higher at 1 year (95% CI: 1.0 – 2.2; p < 0.001) and 2.4 points higher at 2 years (95% CI: 1.8 – 3.0; p < .001) than those with low baseline NfL (**Figure 5**). High NfL also related to lower frontal and temporal brain volumes after two years. NfL did not predict change in any of the clinical scales or brain volumes in mutation non-carriers.

MBI/MCI

In mutation carriers with MBI/MCI at baseline (CDR®+NACC-FTLD = 0.5), baseline NfL was strongly associated with decline at year 2 on CDR®+NACC-FTLDsb, MoCA, SEADL, FAS,

CVLT immediate recall, Benson recall, digits forward and semantic fluency, but not in brain volumes (**Table 2**).

Full phenotype

In mutation carriers with full phenotype (CDR®+NACC-FTLD ≥ 1), baseline NfL related to decline in CDR®+NACC-FTLDsb, MoCA, SEADL phonemic fluency, and brain volume composites after two years (**Table 2**).

Validation cohort

In the validation cohort, of 297 participants with baseline evaluations, 189 (63.6%) had follow up year 1 data (available in Dryad: https://doi.org/10.7272/Q6W957CZ**, eTable 2**). Plasma NfL concentrations were higher in all symptomatic mutation carriers compared to asymptomatic participants, except for CBS (**Figure 1**). Median baseline plasma NfL concentrations were higher in participants with a full phenotype (50.6 ± 59 pg/mL) compared to asymptomatic mutation non-carriers (8.8 ± 5 pg/mL), asymptomatic mutation carriers (9.1 ± 8 pg/mL) and MBI/MCI (12.1 ± 20 pg/mL, $p < 0.001$) (**Figure 2**). A cut point of ≥ 19.8 pg/mL discriminated subjects with full phenotype from asymptomatic or MBI/MCI with 87.4% sensitivity, 84.3% specificity, 58.1% positive predictive value and 96.4% negative predictive value (AUC 0.907, 95% CI: 0.861 – 0.954, $p < 0.001$). This cut point was also a fair discriminator between MBI/MCI and full phenotype (AUC 0.805, 95% CI: 0.704 – 0.906), but not between asymptomatic mutation carriers and MBI/MCI (AUC 0.641, 95% CI: 0.530 – 0.752). The proportion of participants with high (≥ 19.8 pg/mL) NfL was different in each disease severity group (6.1% in asymptomatic mutation non-carriers, 13.9% in asymptomatic mutation carriers, 28.1% in MBI/MCI and 84.3% in full phenotype, $\chi^2 = 122.6$, $p < 0.001$). In the whole cohort or in mutation carriers only, baseline plasma NfL correlated with CDR®+NACC-FTLDsb, MMSE and all neuropsychological measures (**eTable 2**).

Twenty one mutation carriers phenoconverted after 1 year [15 asymptomatic (13 to MBI/MCI and 2 to full phenotype) and 6 MBI/MCI to full phenotype]. Plasma NfL concentrations were higher in phenoconverters than non-phenoconverters in asymptomatic mutation carriers (14.1 ± 12 pg/mL vs. 8.7 ± 6 pg/mL, $p = 0.038$) and MBI/MCI (67.3 ± 49 pg/mL vs. 9.0 ± 8 pg/mL, $p = 0.006$) (**Figure 4**). Plasma NfL concentrations were also higher in asymptomatic mutation carriers whose CDR®+NACC-FTLDsb scores progressed by 1 point, even in the absence of phenoconversion (15.3 ± 33 pg/mL), compared to those whose scores remained stable (8.9 ± 7 pg/mL, $p = 0.014$, **eFigure 1**). In asymptomatic mutation carriers, baseline NfL predicted worsening at year 1 in CDR®+NACC-FTLDsb, MMSE and Trails-making Test A. In MBI/MCI, baseline NfL predicted decline at year 1 in CDR®+NACC-FTLDsb, MMSE, Trails-making test B and phonemic fluency. In full phenotype, baseline NfL was associated with subsequent decline in MMSE and Trails-making Test A, but the relationships did not survive correction for multiple comparisons (available in Dryad: https://doi.org/10.7272/Q6W957CZ **eTable 3**).

Discussion

We analyzed the prognostic value of plasma NfL concentrations in carriers of the most common FTLD-causing mutations, *C9orf72*, *GRN* and *MAPT,* over 1-2 years of follow up, with a special emphasis on asymptomatic mutation carriers and carriers with prodromal disease (MBI/MCI). In two independent cohorts, plasma NfL concentrations were strongly related to disease severity with stepwise increases from asymptomatic (clinically normal), through MBI/MCI, to full phenotype. At baseline, plasma NfL was strongly correlated with global and functional status, neuropsychological scores and brain volume. Higher baseline NfL was associated with greater disease severity after one or two years of follow up, regardless of disease severity and genotype. Remarkably, this included asymptomatic mutation carriers, in whom plasma NfL was also associated with future clinical decline, allowing identification of individuals at high risk for phenoconversion to symptomatic status within two years. Consistent with this finding, NfL also

predicted worse clinical and neuropsychological status or more brain atrophy, regardless of disease severity and genotype. The results suggest a role for plasma NfL as a prognostic biomarker in f-FTLD.

The findings in our original and validation cohorts are consistent with previous studies of serum NfL in familial and sporadic FTLD. In familial FTLD, serum NfL is associated with disease severity, brain volume and brain atrophy.[18] In symptomatic sporadic FTLD, baseline serum NfL correlated with executive function and brain atrophy, but not with longitudinal change in neuropsychological scores,[17] which is similar to what we observed in participants with full phenotype. This study and others[18, 22, 35] found that in fully symptomatic patients, *GRN* mutation carriers had higher NfL concentrations than *C9or72* and *MAPT* mutation carriers. This does not seem to be due to differences in the number of participants by genotype or the age of symptomatic participants in each genetic group, and may reflect a faster rate of neurodegeneration in symptomatic *GRN* mutation carriers. Consistent with previous studies, we observed baseline NfL differences between symptomatic and asymptomatic FTLD-mutation carriers and between phenoconverters and non-converters.[35] Similar to those studies, we also observed a large within-group variability in NfL concentrations, regardless of clinical phenotype, disease severity, or genotype. This variability likely explains why median NfL concentrations in asymptomatic mutation carriers were not elevated, yet high concentrations were still associated with future clinical progression. In this group, NfL showed good negative predictive value, but poor positive predictive value for phenoconversion. The absolute cutoff values for discrimination between asymptomatic and symptomatic participants were similar to those reported in previous studies based on data from our validation cohort.[17, 18, 35] However, one study reported a higher cutoff (33 pg/mL)[17] that may be explained by the inclusion of older controls and sporadic cases as compared to the familial cases reported here.[36]

Unlike previous studies, we used the CDR®+NACC-FTLD to stratify patients by level of global impairment, allowing delineation of MBI/MCI, a prodromal state of mild or questionable disease between asymptomatic and full phenotype. The CDR®+NACC-FTLD is more

appropriate for FTLD patients and superior to relying on the clinical phenotype or the traditional Clinical Dementia Rating®, because the CDR®+NACC-FTLD includes measures of behavioral and language impairment.[37] We found baseline NfL concentrations in asymptomatic and MBI/MCI mutation carriers best predicted changes in global and functional scales (i.e. CDR®+NACC-FTLDsb, CGI-S and FAS). In addition, NfL predicted declines in activities of daily living, as measured by the SEADL and FAS scales and several neuropsychological tests, in MBI/MCI, but not in asymptomatic mutation carriers or full phenotype. The severity dependent differences in predictive value of baseline NfL are probably attributable to a number of factors. These include a faster rate of functional decline in MBI/MCI, differences in the duration of the MBI/MCI stage depending on the phenotype, and absence of activities of daily living impairments in asymptomatic, and a ceiling effect for deterioration in fully symptomatic individuals. Identification of MBI/MCI individuals, however, may be challenging. The sample sizes for MBI/MCI in both cohorts of this study were relatively small and the follow up durations were limited. This may explain why differences in baseline NfL concentrations in MBI/MCI participants by conversion status were not as strong, compared to differences between MBI/MCI and asymptomatic or fully symptomatic mutation carriers. These observations might also reflect a short duration in the MBI/MCI state and fluctuation in clinical status over time, with some MBI/MCI participants progressing to full phenotype and others returning to asymptomatic status. The additional follow up data that will be collected as part of the ongoing ALLFTD study[38] will improve the understanding of the clinical value of plasma NfL in prodromal f-FTLD.

Our results suggest that plasma NfL may be a promising endpoint for FTLD clinical trials. A variety of therapies that target the underlying pathological proteins encoded by the three FTLD-causing genes studied here are entering clinical trials for f-FTLD.[7] The ultimate goal for these therapies is to prevent disease onset in mutation carriers. A major challenge for testing the efficacy of such interventions is the inability to measure clinically meaningful endpoints in asymptomatic individuals who are at risk for disease. Recent US Food and Drug Administration guidance on developing therapeutics for presymptomatic or early Alzheimer's disease suggests

that therapies might be approved under an accelerated mechanism on the basis of a biomarker that is "reasonably likely to predict clinical benefit".[39] Our data show associations between plasma NfL concentrations and subsequent functional status, which are considered inherently clinically meaningful, within two years of follow up. Therefore, plasma NfL might be used as a continuous variable endpoint (difference in mean NfL concentration in placebo vs. intervention arm) or as a time to event endpoint (delay in onset of sharp rise in NfL that occurs at the transition from the asymptomatic to symptomatic phase of disease). Such an approach was previously employed for drugs to treat macular degeneration that were approved for marketing by using optical coherence tomography measurements as endpoints that are highly predictive of future declines in visual acuity.[40]

Our study has limitations. NfL is not a pathophysiology-specific biomarker of FTLD, and its elevations in a number of general conditions render it a non-specific marker of neuronal injury. Future projects should aim at identifying and deploying specific markers of disease activity and severity in FTLD, and we have previously reported the comparative diagnostic value of plasma NfL versus plasma p-tau in FTLD and Alzheimer's disease.[41] Based on work in dominantly inherited Alzheimer's,[42] longitudinal plasma NfL measurements may have better predictive ability for clinical decline than the cross sectional measures we used. Longitudinal plasma samples of participants of the LEFFTDS and ARTFL projects are being collected and future projects will examine longitudinal NfL concentrations and their relationship with disease progression. Finally, we found no influence of the *APOE* genotype on NfL concentrations or predictive ability. The analyses, however, did not examine other potential genetic risk factors such as polymorphisms within *MAPT*,[43] *TMEM106B*[44] or *EGFR*[45] that have been identified as potential modulators of FTLD risk.

Conclusions

This study adds to a large body of evidence supporting plasma NfL as a useful prognostic biomarker for syndromes associated with FTLD.[12, 14, 17, 35, 46, 47] By demonstrating the ability to

identify asymptomatic FTLD-mutation carriers at risk of progression to symptomatic status over two years, our findings provide a strong rationale for developing this biomarker as a potential inclusion criterion or endpoint for prevention studies in asymptomatic f-FTLD mutation carriers.

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Supplementary Material

eTables 1-3 and eFigure 1

Appendix 1: Authors

Name	Location	Contribution
Julio C. Rojas	University of California, San Francisco, San Francisco, CA, USA	Analyzed data, drafted manuscript
Ping Wang	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Adam M. Staffaroni	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Carolin Heller	UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Yann Cobigo	University of California, San Francisco, San Francisco, CA, USA	Imaging data collection and critical revision of the manuscript
Amy Wolf	University of California, San Francisco, San Francisco, CA, USA	Imaging data collection and critical revision of the manuscript
Sheng-Yang M. Goh	University of California, San Francisco, San Francisco, CA, USA	Imaging data collection and critical revision of the manuscript

Peter A. Ljubenkov	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Hilary W. Heuer	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Jamie C. Fong	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Joanne B. Taylor	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Eliseo Veras	Quanterix Corporation, Lexington, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
Linan Song	Quanterix Corporation, Lexington, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
Andreas Jeromin	Quanterix Corporation, Lexington, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
David Hanlon	Quanterix Corporation, Lexington, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
Lili Yu	Novartis Institutes for Biomedical Research Inc, Cambridge, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
Arvind Kinhikar	Novartis Institutes for Biomedical Research Inc, Cambridge, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
Rajeev Sivasankaran	Novartis Institutes for Biomedical Research Inc, Cambridge, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
Agnieszka Kieloch	Novartis Pharma AG, Basel, Switzerland	Fluid biomarker data collection and critical revision of the manuscript
Marie-Anne Valentin	Novartis Pharma AG, Basel, Switzerland	Fluid biomarker data collection and critical revision of the manuscript
Anna M. Karydas	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Laura L. Mitic	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Rodney Pearlman	Bluefield Project to Cure Frontotemporal Dementia, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
John Kornak	University of California,	Data analysis and critical

	San Francisco, San Francisco, CA, USA	revision of the manuscript
Joel H. Kramer	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Bruce L. Miller	University of California, San Francisco, San Francisco, CA, USA	Clinical data collection and critical revision of the manuscript
Kejal Kantarci	Mayo Clinic, Rochester, MN, USA	Clinical data collection and critical revision of the manuscript
David S. Knopman	Mayo Clinic, Rochester, MN, USA	Clinical data collection and critical revision of the manuscript
Neill Graff-Radford	Mayo Clinic, Jacksonville, FL, USA	Clinical data collection and critical revision of the manuscript
Leonard Petrucelli	Mayo Clinic, Jacksonville, FL, USA	Clinical data collection and critical revision of the manuscript
Rosa Rademakers	Mayo Clinic, Jacksonville, FL, USA	Clinical data collection and critical revision of the manuscript
David C. Irwin	University of Pennsylvania, Philadelphia, PA, USA	Clinical data collection and critical revision of the manuscript
Murray Grossman	University of Pennsylvania, Philadelphia, PA, USA	Clinical data collection and critical revision of the manuscript
Eliana Marisa Ramos	University of California, Los Angeles, Los Angeles, CA, USA	Clinical data collection and critical revision of the manuscript
Giovanni Coppola	University of California, Los Angeles, Los Angeles, CA, USA	Clinical data collection and critical revision of the manuscript
Mario F. Mendez	University of California, Los Angeles, Los Angeles, CA, USA	Clinical data collection and critical revision of the manuscript
Yvette Bordelon	University of California, Los Angeles, Los Angeles, CA, USA	Clinical data collection and critical revision of the manuscript
Bradford C. Dickerson	Harvard University/Massachusetts General Hospital, Boston, MA, USA	Clinical data collection and critical revision of the manuscript
Nupur Ghoshal	Washington University, St. Louis, MO, USA	Clinical data collection and critical revision of the manuscript
Eduard D. Huey	Columbia University, New York, NY, USA	Clinical data collection and critical revision of the manuscript
Ian R. Mackenzie	University of British Columbia, Vancouver,	Clinical data collection and critical revision of the

	British Columbia, Canada	manuscript
Brian S. Appleby	Case Western Reserve University, Cleveland, OH, USA	Clinical data collection and critical revision of the manuscript
Kimiko Domoto-Reilly	University of Washington, Seattle, WA, USA	Clinical data collection and critical revision of the manuscript
Ging-Yuek R. Hsiung	University of British Columbia, Vancouver, British Columbia, Canada	Clinical data collection and critical revision of the manuscript
Arthur W. Toga	Laboratory of Neuroimaging, University of Southern California, Los Angeles, CA, USA	Imaging data collection and critical revision of the manuscript
Sandra Weintraub	Northwestern University, Chicago, IL, USA	Clinical data collection and critical revision of the manuscript
Daniel I. Kaufer	University of North Carolina, Chapel Hill, NC, USA	Clinical data collection and critical revision of the manuscript
Diana Kerwin	Texas Health Presbyterian Hospital Dallas, Dallas, TX, USA	Clinical data collection and critical revision of the manuscript
Irene Litvan	University of California, San Diego, San Diego, CA, USA	Clinical data collection and critical revision of the manuscript
Chiadikaobi U. Onyike	Johns Hopkins Hospital, Baltimore, MD, USA	Clinical data collection and critical revision of the manuscript
Alexander Pantelyat	Johns Hopkins Hospital, Baltimore, MD, USA	Clinical data collection and critical revision of the manuscript
Erik D. Roberson	University of Alabama, Birmingham, AL, USA	Clinical data collection and critical revision of the manuscript
Maria C. Tartaglia	University of Toronto, ON, Canada	Clinical data collection and critical revision of the manuscript
Tatiana Foroud	Indiana University School of Medicine, Indianapolis, IN, USA	Clinical data collection and critical revision of the manuscript
Weiping Chen	Biogen Inc., Cambridge, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
Julie Czerkowicz	Biogen Inc., Cambridge, MA, USA	Fluid biomarker data collection and critical revision of the manuscript
Danielle L. Graham	Biogen Inc., Cambridge, MA, USA	Fluid biomarker data collection and critical

		revision of the manuscript
John C van Swieten	Erasmus Medical Centre, Rotterdam, Netherlands	Clinical data collection and critical revision of the manuscript
Barbara Borroni	University of Brescia, Brescia, Italy	Clinical data collection and critical revision of the manuscript
Raquel Sanchez-Valle	University of Barcelona, Barcelona, Spain	Clinical data collection and critical revision of the manuscript
Fermin Moreno	Donostia University Hospital, San Sebastian, Gipuzkoa, Spain	Clinical data collection and critical revision of the manuscript
Robert Laforce	Université Laval, Québec, Canada	Clinical data collection and critical revision of the manuscript
Caroline Graff	Karolinska Institutet, Solna, Sweden; Karolinska University Hospital, Solna, Sweden	Clinical data collection and critical revision of the manuscript
Matthis Synofzik	University of Tübingen, Tübingen, Germany; Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany	Clinical data collection and critical revision of the manuscript
Daniela Galimberti	IRCCS Ospedale Policlinico, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy	Clinical data collection and critical revision of the manuscript
James B. Rowe	University of Cambridge, Cambridge, UK	Clinical data collection and critical revision of the manuscript
Mario Masellis	University of Toronto, Toronto, Canada	Clinical data collection and critical revision of the manuscript
Elizabeth Finger	University of Western Ontario, London, Ontario, Canada	Clinical data collection and critical revision of the manuscript
Rik Vandenberghe	KU Leuven, Leuven, Belgium; Neurology Service, University Hospitals Leuven, Belgium	Clinical data collection and critical revision of the manuscript
Alexandre de Mendonça	University of Lisbon, Lisbon, Portugal	Clinical data collection and critical revision of the manuscript
Fabrizio Tagliavini	Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano,	Clinical data collection and critical revision of the manuscript

	Italy	
Isabel Santana	University of Coimbra, Coimbra, Portugal	Clinical data collection and critical revision of the manuscript
Simon Ducharme	McGill University, Montreal, Québec, Canada	Clinical data collection and critical revision of the manuscript
Chris R. Butler	University of Oxford, Oxford, UK	Clinical data collection and critical revision of the manuscript
Alexander Gerhard	Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK; University of Duisburg-Essen, Germany	Clinical data collection and critical revision of the manuscript
Johannes Levin	Ludwig-Maximilians-Universität München, Munich, Germany	Clinical data collection and critical revision of the manuscript
Adrian Danek	Ludwig-Maximilians-Universität München, Munich, Germany	Clinical data collection and critical revision of the manuscript
Markus Otto	University of Ulm, Ulm, Germany	Clinical data collection and critical revision of the manuscript
Sandro Sorbi	University of Florence, Florence, Italy	Clinical data collection and critical revision of the manuscript
David M. Cash	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Rhian S. Convery	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Martina Bocchetta	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Martha Foiani	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Caroline V Greaves	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Georgia Peakman	Dementia Research Centre, UCL Institute of	Clinical data collection and critical revision of the

	Neurology, Queen Square, London, UK	manuscript
Lucy Russell	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Imogen Swift	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Emily Todd	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Clinical data collection and critical revision of the manuscript
Jonathan D. Rohrer	Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK	Study concept, supervision, data collection and critical revision of the manuscript
Bradley F. Boeve	Mayo Clinic, Rochester, MN, USA	Study concept, supervision, data collection and critical revision of the manuscript
Howard J. Rosen	University of California, San Francisco, San Francisco, CA, USA	Study concept, supervision, data collection and critical revision of the manuscript
Adam L. Boxer	University of California, San Francisco, San Francisco, CA, USA	Study concept and design, overall supervision, data collection and critical revision of the manuscript

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Figure legends

Figure 1. Baseline plasma neurofilament-light chain concentrations by clinical phenotype. A) Original (LEFFTDS/ARTFL) cohort. B) Validation (GENFI) cohort. The phenotypes are based on clinical diagnosis and did not rely on severity scales. Only the original cohort included clinically-diagnosed prodromal disease (MBI or MCI). The horizontal bars represent median values. Upper and lower quartiles are delimitated by the boxes. Lowest and highest values are indicated by whiskers. bvFTD = behavioral variant frontotemporal dementia, CBS = corticobasal syndrome, FTD/ALS = frontotemporal dementia with amyotrophic lateral sclerosis, MBI = mild behavioral impairment, MCI = mild cognitive impairment, PPA = primary progressive aphasia (non-fluent or semantic). * = compared to normal; ** = compared to normal and MCI, $p < .05$.

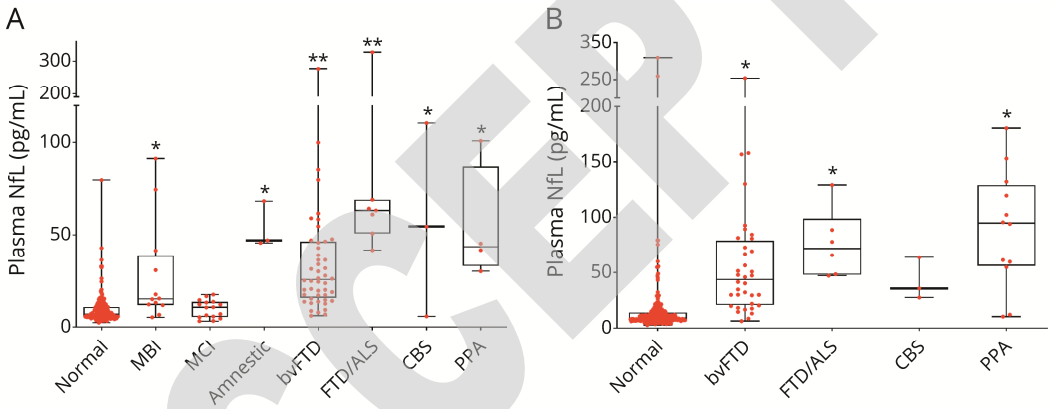

Figure 2. Baseline plasma neurofilament-light chain concentrations by disease severity and diagnostic performance. A-C) Original (LEFFTDS/ARTFL) cohort. D-F) Validation (GENFI) cohort. Severity was determined by the CDR® Dementia Staging Instrument plus Behavior and Language domains from the National Alzheimer's Disease Coordinating Center Frontotemporal Lobar Degeneration module (CDR®+NACC-FTLD). **(A, D)** The boxplots show plasma NfL concentrations in asymptomatic carriers (i.e. CDR®+NACC-FTLD = 0), mild behavioral or cognitive impairment (MBI/MCI, CDR®+NACC-FTLD = 0.5) and patients with full phenotypes (CDR®+NACC-FTLD ≥ 1). The horizontal bars represent median values. Upper and lower quartiles are delimitated by the boxes. Lowest and highest values are indicated by whiskers. **(B, E)** The receiver operating characteristic (ROC) curves show that plasma NfL was a good discriminator between subjects with full phenotype and those either asymptomatic or with MBI/MCI. **(C, F)** The proportion of patients with low or high plasma NfL concentrations, determined by the ROC curve, is presented for each disease severity. AUC = area under the curve

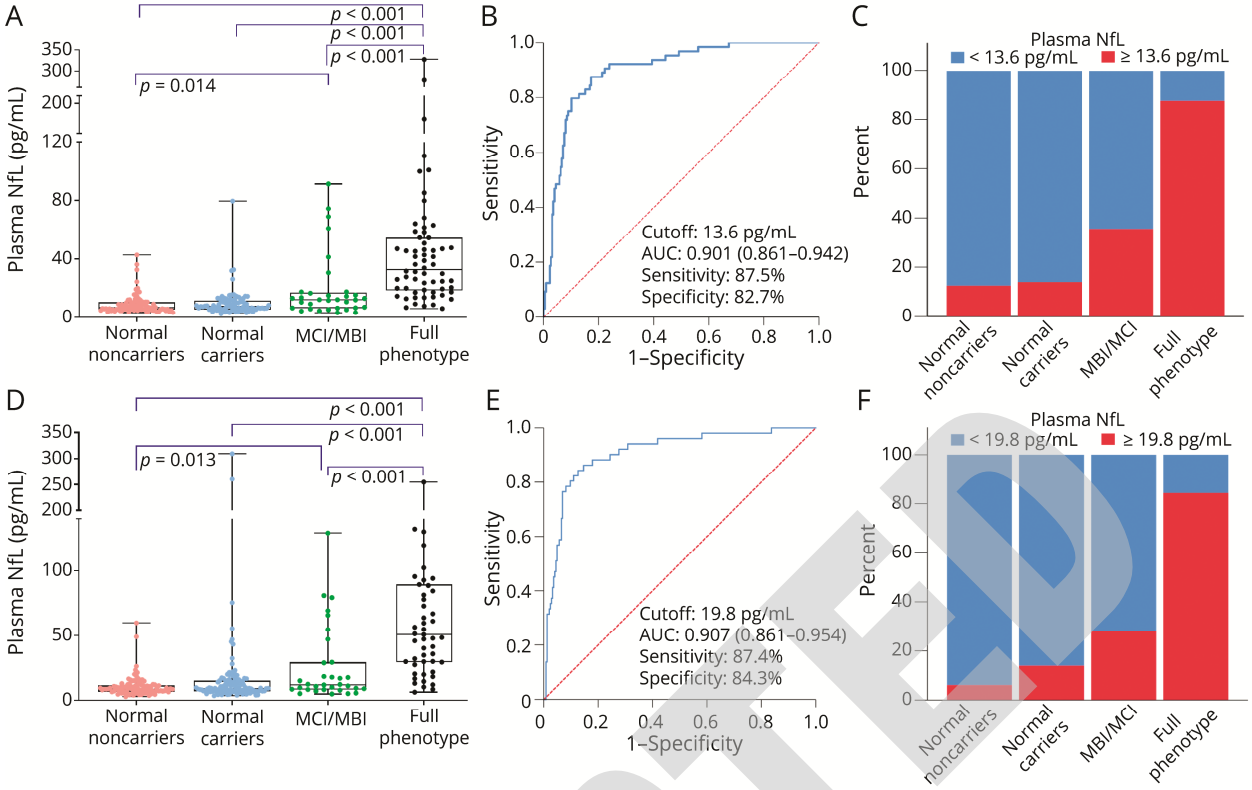

Figure 3. Plasma neurofilament light chain concentrations by disease severity in each genotype group. (A-C) Original cohort. **(D-F)** Validation cohort. MBI/MCI = Mild behavioral or cognitive impairment (CDR®+NACC-FTLD = 0.5).

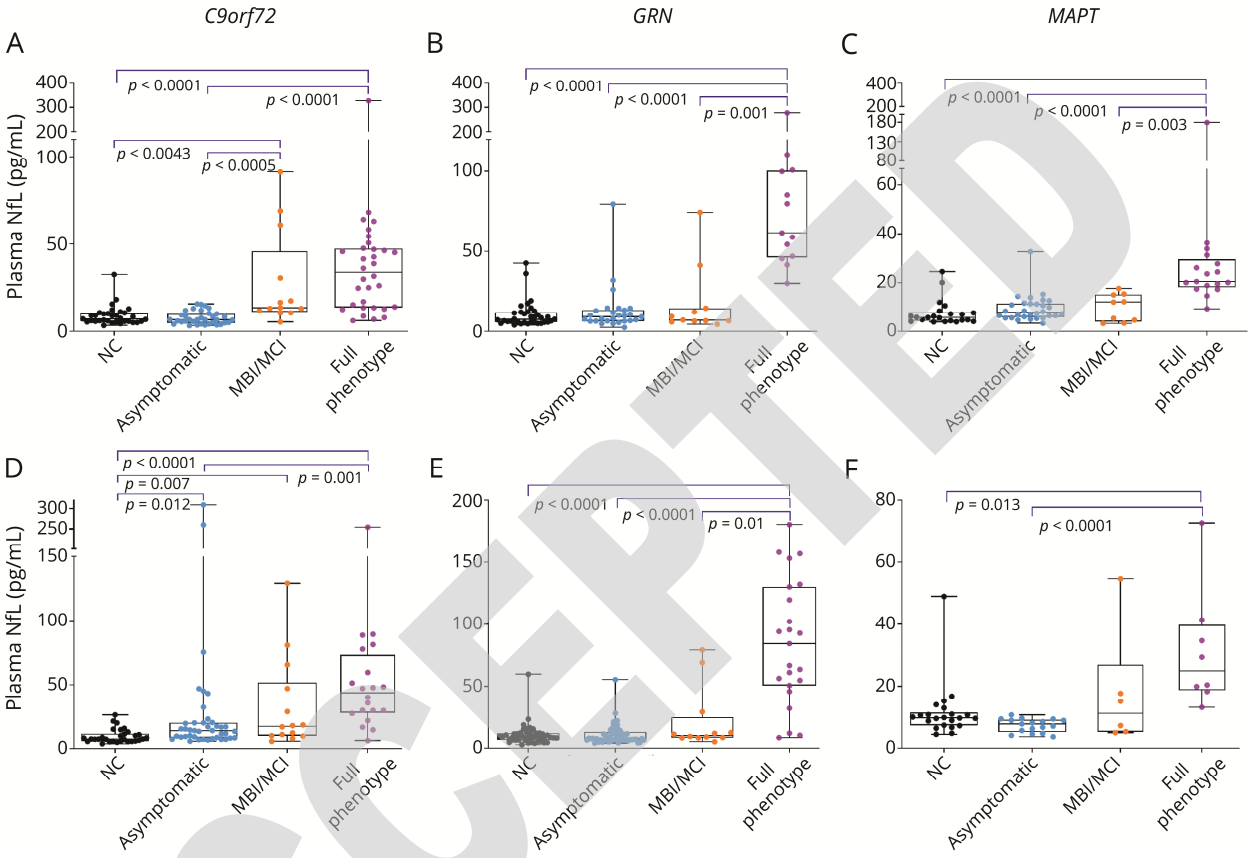

Figure 4. Baseline plasma neurofilament-light chain concentrations according to conversion status by follow up. Severity was determined with the CDR® Dementia Staging Instrument plus Behavior and Language domains from the National Alzheimer's Disease Coordinating Center Frontotemporal Lobar Degeneration module (CDR®+NACC-FTLD). **(A-C)** Original (LEFFTDS/ARTFL) cohort. **(D-F)** Validation (GENFI) cohort. **(A, D)** Median baseline NfL concentrations were higher in asymptomatic mutation carriers (CDR®+NACC-FTLD = 0) who progressed to either mild behavioral or cognitive impairment (MBI/MCI, CDR®+NACC-FTLD = 0.5) or full phenotype (CDR®+NACC-FTLD ≥ 1) upon follow up. **(B, E)** A similar trend was observed in subjects who had MBI/MCI at baseline, and when all subjects (asymptomatic mutation carriers and MBI/MCI) were combined **(C, F)**. The horizontal bars represent median values. Upper and lower quartiles are delimitated by the boxes. Lowest and highest values are indicated by whiskers. Circles = asymptomatic, Triangles = MBI/MCI. Blue = *C9orf72* mutation carriers, Yellow = *GRN* mutation carriers, Red = *MAPT* mutation carriers.

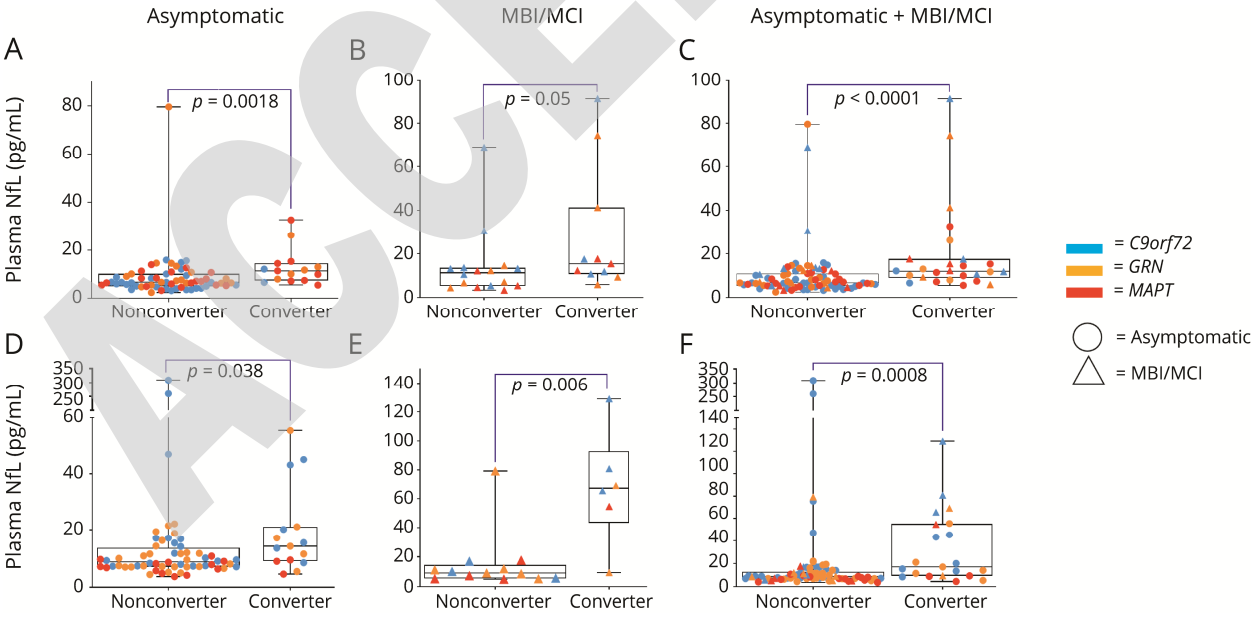

Figure 5. Prediction of clinical progression by plasma neurofilament-light chain in familial frontotemporal lobar degeneration. (A-C) Original (LEFFTDS/ARTFL) cohort. **(D-F)** Validation (GENFI) cohort. The figure shows the results of models using data from all genotypes in each severity group. In the original cohort, patients with high (red, ≥ 13.6 pg/mL) baseline plasma NfL showed worse clinical scores at 2 years, compared to patients with low (blue, < 13.6 pg/mL) NfL, which was supported by NfL level by time interaction. This differential predictive effect by NfL level was observed regardless of disease severity, including asymptomatic carriers. Similar results were observed in the validation cohort with a cut point value of 19.8 pg/mL. CDR®+NACC-FTLDsb = CDR® Dementia Staging Instrument plus Behavior and Language domains from the National Alzheimer's Disease Coordinating Center Frontotemporal Lobar Degeneration module sum of boxes score. * = between-group contrast at that time point, $p < .05$.

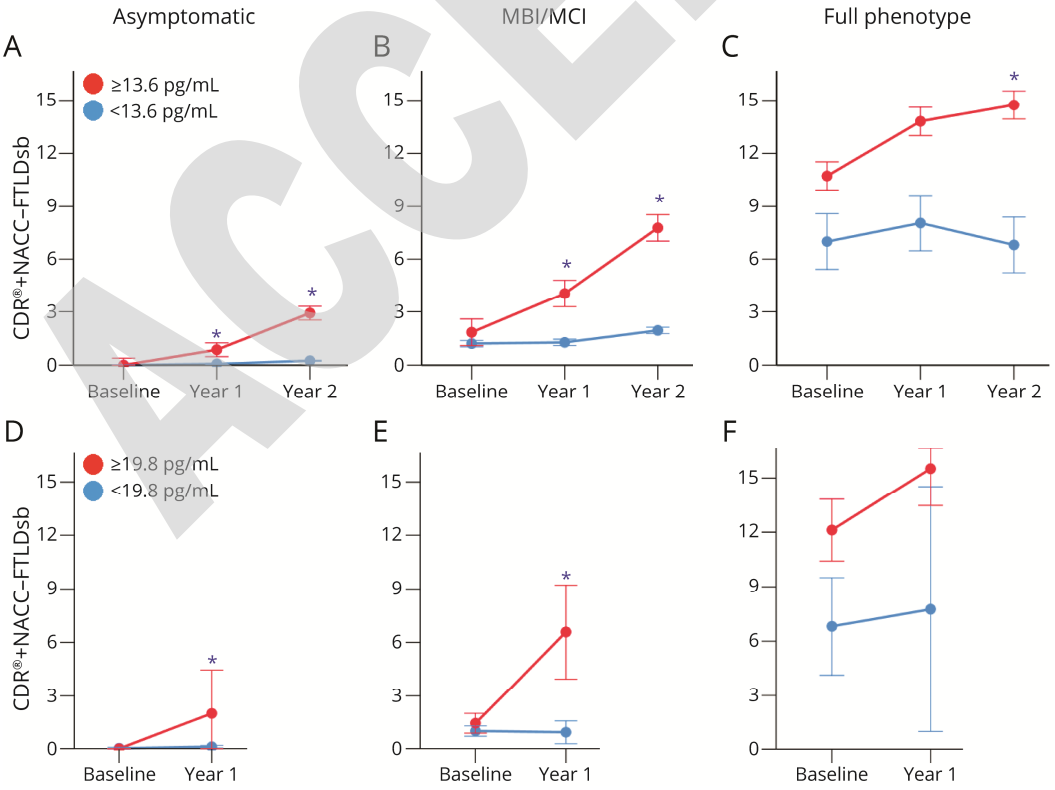

Table 1. Baseline demographic characteristics by disease severity, original cohort[a,e]

	Asymptomatic non-carrier	Asymptomatic carrier	MCI/MBI	Full phenotype
	(n = 90)	(n = 92)	(n = 33)	(n = 62)
Age: median (IQR, range)	50 (19, 24-76)	44 (21, 19-71)	54 (13, 29-80)[b]	61.5 (18, 33-74)[b,c]
Sex: male/female	32/58	43/49	18/15	24/38
Plasma NfL, pg/mL	6.4 (5)	7.1 (5)	12.2 (12)[b,c]	24.1 (21)[b,c,d]
Genotype				
Non-carriers: n (%)	90 (100)	0 (0)	0 (0)	0 (0)
NfL, pg/mL	6.7 (5)	-	-	-
C9orf72: n (%)	0 (0)	35 (43.8)	13 (16.2)	32 (40)
NfL, pg/mL	-	6.6 (5)	13.6 (34)[b]	33.9 (33)[b]
GRN: n (%)	0 (0)	27 (52.9)	11 (21.6)	13 (25.5)
NfL, pg/mL	-	9.1 (7)	7.1 (8)	61.5 (54)[b,d]
MAPT: n (%)	0 (0)	30 (53.6)	9 (16.1)	17 (30.4)
NfL, pg/mL	-	7.8 (5)	12.1 (11)	20.5 (11)[b,d]
CDR®+NACC-FTLDsb	0 (0)	0 (0)	1.5 (2)[b,c]	7.2 (5)[b,c,d]
MoCA	28 (3)	28 (3)	25 (4)[b,c]	20.5 (5)[b,c,d]
UPDRS	0 (0)	0 (0)	0 (4)[b,c]	3 (7)[b,c]
CGI-S	1 (0)	1 (0)	2 (1)[b,c]	3 (1)[b,c,d]
SEADL	100 (0)	100 (0)	90 (10)[b,c]	65 (25)[b,c,d]
FAS	0 (0)	0 (0)	0 (2)	11 (17)[b,c,d]
NPI	0 (1)	0 (2)	6 (9)[b,c]	6.5 (9)[b,c]
CVLTi	9 (3)	8 (2)	7 (3)	3.5 (6)[b,c,d]
CVLTd	8 (3)	7 (3)	6 (6)	4 (6)[b,c,d]
Benson delayed recall	13 (4)	13 (3)	12 (4)	8.5 (6)[b,c,d]
Digits forward	7 (2)	7 (2)	6 (2)	5.5 (2)[b,c,d]

Digits backward	6 (1)	5 (1)	5 (2)	4 (1)[b,c]
Trails A (sec)	22 (10)	21 (8)	25 (14)[c]	45 (17)[d]
Trails B (sec)	49 (29)	58 (28)	59 (77)[b,c]	92.5 (105)[b,c,d]
Phonemic fluency	15 (7)	15 (7)	13 (8)[b]	6 (8)[b,c,d]
Semantic fluency	23 (8)	23 (8)	21 (8)	13 (6)[b,c,d]
TIV,[f] mm^3 x 10^6	1.4 (0.1)	1.4 (0.2)	1.4 (0.1)	1.3 (0.2)
Left frontal,[f] mm^3 x 10^4	4.7 (0.5)	4.6 (0.7)	4.3 (0.9)[c]	3.7 (1.2)[b,c,d]
Right frontal,[f] mm^3 x 10^4	4.7 (0.6)	4.6 (0.7)	4.2 (1.0)[c]	3.7 (1.2)[b,c,d]
Left temporal,[f] mm^3 x 10^4	2.7 (0.3)	2.7 (0.3)	2.7 (0.8)	2.1 (0.6)[b,c,d]
Right temporal,[f] mm^3 x 10^4	2.6 (03)	2.6 (0.3)	2.6 (0.7)[c]	2.1 (0.6)[b,c,d]
CSF NfL, pg/mL	313 (359)	331.5 (375)	615.5 (834)	1659.7 (2099)[b,c]
CSF tau, pg/mL	121.2 (87)	146.3 (102)	136.8 (91)	206.3 (153)[c]
CSF p-tau, pg/mL	37.4 (20)	39.9 (16)	34.5 (12)	31.9 (23)
CSF neurogranin, pg/mL	312.8 (156)	364.7 (184)	311.7 (252)	278.6 (148)
CSF p-NfH, pg/mL	662.9 (392)	485.9 (571)	768.3 (585)	1252.1 (1368)[b,c]

CVLTd = California Verbal Learning Test, Short Form – delayed recall (number of words); CVLTi = California Verbal Learning Test, Short Form – immediate recall (number of words); CGI-S = Clinical Global Impression of Severity; FAS = Functional Assessment Scale; CDR®+NACC-FTLDsb = CDR® Dementia Staging Instrument plus Behavior and Language domains from the National Alzheimer's Disease Coordinating Center Frontotemporal Lobar Degeneration module sum of boxes score; MBI/MCI = mild behavioral impairment or mild cognitive impairment; MoCA = Montreal Cognitive Assessment; NfL = plasma neurofilament-light chain (uncorrected); NPI = Neuropsychiatric Inventory; p-NfH = phosphorylated neurofilament heavy chain; SEADL = Schwab and England Activities of Daily Living score; TIV = total intracranial volume; UPDRS = Unified Parkinson's Disease Rating Scale, motor section

a = Disease severity determined by the CDR®+NACC-FTLD, 0 = asymptomatic, 0.5 = mild cognitive or behavioral impairment, 1 ≥ full phenotype/dementia

b = $p < 0.05$, compared to asymptomatic carrier

c = $p < 0.05$, compared to asymptomatic non-carrier

d = $p < 0.05$, compared to mild cognitive or behavioral impairment

e = unless indicated otherwise, values are expressed as median (interquartile range). Other units of measure are: Benson delayed recall: points, phonemic and semantic fluency: words/min, digits forward and backward: number of digits in the largest string correctly recalled

f = volumes are expressed as mean (standard deviation)

Table 2. Prediction of disease progression at 2 years by plasma neurofilament light chain in frontotemporal lobar degeneration-causing mutation carriers, original cohort

	NfL (as a continuous variable) x time					
	Asymptomatic		**MBI/MCI**		**Full phenotype**	
	Estimate[a]	*p value*	*Estimate*[a]	*p value*	*Estimate*[a]	*p value*
CDR®+NACC-FTLDsb	2.5 (1.6 – 3.4)	**< 0.001**	6.4 (3.5 – 9.4)	**< 0.001**	6.9 (2.6 – 11.2)	**0.002**
MoCA	-2.3 (-0.01 – -4.5)	0.049*	-14.7(-21.3 – -8.1)	**< 0.001**	-13.2 (-21.3 – -5.2)	**0.002**
UPDRS	0.4 (-1.0 – 1.9)	0.56	7.1 (-0.3 – 14.7)	0.06	4.4 (-16.5 – 25.5)	0.6
CGI-S	1.2 (0.7 – 1.7)	**< 0.001**	1.2 (0.1 – 2.5)	0.07	1.7 (0.4 – 3.0)	**0.01**
SEADL	-2.8 (-15.2 – 9.4)	0.6	-38.8 (-65 – 12.7)	**0.004**	-21.0 (-51.5 – 9.4)	0.1
FAS	5.0 (2.7 – 7.3)	**< 0.001**	12.2 (5.8 – 18.5)	**< .001**	-0.8 (-14.6 – 16.2)	0.9
NPI	0.8 (-1.8 – 3.5)	0.5	-1.3 (-6.5 – 3.7)	0.5	-3.0(-14.1 – 7.5)	0.5
CVLTi	-1.7 (-3.5 –0.1)	0.07	-3.8 (-6.6 – -1.0)	**0.009**	-2.2 (-6.1 – 1.6)	0.2
CVLTd	-1.4 (-3.3 – -0.3)	0.1	-2.6 (-5.8 – 0.4)	0.09	-2.0 (-5.8 – 1.7)	0.2
Benson recall	-0.4 (-1.6 – 0.8)	0.4	-5.7 (-8.6 – -2.9)	< **0.001**	-2.3 (-9.6 – 4.9)	0.5
Digits forward	-1.0 (-2.1 – 0.1)	0.09	-2.4 (-4.1 – -0.7)	**0.005**	-1.4 (-4.6 – 1.7)	0.3
Digits backward	-1.0 (-2.3 – 0.1)	0.07	-0.9 (-2.3 – 0.4)	0.1	-1.6 (-4.2 – 0.9)	0.2
Trails A	3.7 (-11.2 – 18.7)	0.6	1.3 (-8.5 – 11.1)	0.7	-8.8 (-21.5 – 3.7)	0.16
Trails B	31.6 (-78 – 15)	0.18	4.9 (-43 – 53)	0.8	41 (-25 – 107)	0.2
Phonemic	-0.8(-4.7 – -3.0)	0.002	-1.9 (-7.0 – 3.1)	0.4	-2.3 (-2.1 – 6.8)	0.3

fluency						
Semantic fluency	-2.4(-7.0 – 2.0)	0.2	-8.2 (-14.4 – -2.1)	**0.009**	-9.6 (-16.5 – -2.7)	**0.007**
Left frontal	-3786 (-5848 – -1723)	**< .001**	-979 (-4933 – 2974)	0.5	-11349 (-19842 – -2856)	**0.012**
Right frontal	-2460 (-4422 – -498)	**0.01**	-949 (-4866 – 2967)	0.4	-2159 (-12400 – 8081)	0.6
Left temporal	-1797 (-3104 – -491)	**0.008**	-237 (-2377 – 1903)	0.8	-7874 (-13555 – -2194)	**0.01**
Right temporal	-1468 (-2419 – -516)	**0.003**	92 (-1715 – 1900)	0.9	0.1 (-6748 – 6748)	1.0

Estimates, 95% confidence intervals and *p* values are presented for the interaction of NfL with time as predictors or each of the clinical variables. a = The estimates represent the predicted change in absolute values in each scale, neuropsychological test or composite volume per increase in one log concentration unit in plasma neurofilament light chain at each time point (fixed effect). Significant associations appear in bold. * = did not survive correction for multiple comparisons within that severity level

CVLTd = California Verbal Learning Test, Short Form – delayed recall; CVLTi = California Verbal Learning Test, Short Form – immediate recall; CGI-S = Clinical Global Impression of Severity; FAS = Functional Assessment Scale; CDR®+NACC-FTLDsb = CDR® Dementia Staging Instrument plus Behavior and Language domains from the National Alzheimer's Disease Coordinating Center Frontotemporal Lobar Degeneration module sum of boxes score; SD = standard deviation; IQR = interquartile range; MBI/MCI = mild behavioral/cognitive impairment; MoCA = Montreal Cognitive Assessment; NfL = plasma neurofilament-light chain; NPI = Neuropsychiatric Inventory; SEADL = Schwab and England Activities of Daily Living score; UPDRS = Unified Parkinson's Disease Rating Scale, motor section

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