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Fluid Biomarkers in Individuals at Risk for Genetic Prion Disease up to Disease Conversion

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Abstract

Objectives

To longitudinally characterize disease-relevant CSF and plasma biomarkers in individuals at risk for genetic prion disease up to disease conversion.

Methods

This single-center longitudinal cohort study has followed known carriers of *PRNP* pathogenic variants at risk for prion disease, individuals with a close relative who died of genetic prion disease but who have not undergone predictive genetic testing, and controls. All participants were asymptomatic at first visit and returned roughly annually. We determined *PRNP* genotypes, measured NfL and GFAP in plasma, and RT-QuIC, total PrP, NfL, T-tau, and beta-synuclein in CSF.

Results

Among 41 carriers and 21 controls enrolled, 28 (68%) and 15 (71%) were female, and mean ages were 47.5 and 46.1. At baseline, all individuals were asymptomatic. We observed RT-QuIC seeding activity in the CSF of 3 asymptomatic E200K carriers who subsequently converted to symptomatic and died of prion disease. 1 P102L carrier remained RT-QuIC negative through symptom conversion. No other individuals developed symptoms. The prodromal window from detection of RT-QuIC positivity to disease onset was 1 year long in an E200K individual homozygous (V/V) at PRNP codon 129 and 2.5 and 3.1 years in 2 codon 129 heterozygotes (M/V). Changes in neurodegenerative and neuroinflammatory markers were variably observed prior to onset, with increases observed for plasma NfL in 4/4 converters, and plasma GFAP, CSF NfL, CSF T-tau, and CSF beta-synuclein each in 2/4 converters, although values relative to age and fold changes relative to individual baseline were not remarkable for any of these markers. CSF PrP was longitudinally stable with mean coefficient of variation 9.0% across all individuals over up to 6 years, including data from converting individuals at RT-QuIC-positive timepoints.

Discussion

CSF prion seeding activity may represent the earliest detectable prodromal sign in E200K carriers. Neuronal damage and neuroinflammation markers show limited sensitivity in the prodromal phase. CSF PrP levels remain stable even in the presence of RT-QuIC seeding activity.

Clinical Trials Registration

ClinicalTrials.gov NCT05124392 posted 2017-12-01, updated 2023-01-27.

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Introduction

Prion disease exhibits striking biomarker signatures at the symptomatic stage,[1-4] but data about presymptomatic changes are limited (Supplementary Background, eAppendix 1). Neurodegeneration and neuroinflammation markers may rise 2 years before onset in slowly progressive subtypes such as P102L, but only months before onset in rapidly progressive subtypes D178N and E200K,[3,5] mirroring disease duration.[6] Prion seeding activity has been detected by RT-QuIC in CSF before onset in E200K individuals, but the prognostic value is unknown. Here, we report fluid biomarker trajectories associated with 4 disease onsets over 6 years in a longitudinal natural history of individuals at risk for genetic prion disease.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Participants provided written consent. The study was approved by the MGB Institutional Review Board (2017P000214). Assay validation used MIND Tissue Bank (2015P000221) samples. This study is registered with ClinicalTrials.gov (NCT05124392).

Study Participants

This previously described[7] cohort study at Massachusetts General Hospital includes asymptomatic individuals with or without pathogenic *PRNP* variants (Table 1; eFigure 1; eMethods), invited to contribute blood and CSF approximately annually. Data presented here include data previously reported.[7,8] We performed *PRNP* genotyping on all individuals including those who did not know their own genetic status; steps taken to prevent self-identification are described in eMethods.

Biomarker Assays

Biomarker assays used were RT-QuIC (IQ-CSF protocol),[9] PrP ELISA[8] (eFigure 2), Simoa (Quanterix) GFAP, and Ella (Bio-Techne) NfL, T-tau (eFigure 3), and β-syn (eFigure 4), see eMethods.

Statistical Analysis

Biomarker relationships with age and genotype were assessed by log-linear regression; curve fits shown in figures are the separate best fits for pathogenic variant carriers and for controls, while p values are for the effect of carrier status in a combined model: lm(log(value) ~ age + carrier). For details of RT-QuIC data analysis, see eMethods. p values < 0.05 were considered nominally significant. Analyses were conducted in R 4.2.0.

Data Availability

Full biomarker values for all participants will be made available to qualified investigators with ethical approval and a data use agreement upon request. Source code, summary statistics for all participants, and individual biomarker values for converting participants are freely available at github.com/ericminikel/mgh_prnp_freeze2.

Results

Sixty-two participants completed at least 1 study visit. 41 harbored pathogenic *PRNP* variants ("carriers"), and 21 were negative ("controls""). Groups were well-matched, and distribution of *PRNP* genotypes was consistent with pathogenic variant prevalence[10] (Table 1). We collected 155 CSF samples and 160 plasma samples. From July 2017 to February 2023, 4 carriers converted to active disease (N = 3 E200K, N = 1 P102L). We performed fluid biomarker analyses on samples from both converters (eTable 1) and nonconverting carriers and controls (eTable 2).

Testing of longitudinal CSF samples by RT-QuIC identified 6 positive samples (Figure 1A), all of which belonged to the 3 E200K individuals who had developed disease and died. Each CSF sample from these individuals was re-tested by endpoint dilution[5,9] to determine the prion titer (prion seeds per μL). Of these 3 E200K individuals, 2 *PRNP* codon 129 heterozygotes (each cis-129M, trans-129V) were already RT-QuIC positive at first lumbar puncture (2.5 and 3.1 years before onset) and prion titer in CSF did not appreciably rise thereafter (Figure 1B). One homozygote (V/V) was negative at the first 2 visits, became RT-QuIC positive on study and subsequently became symptomatic 1 year later.

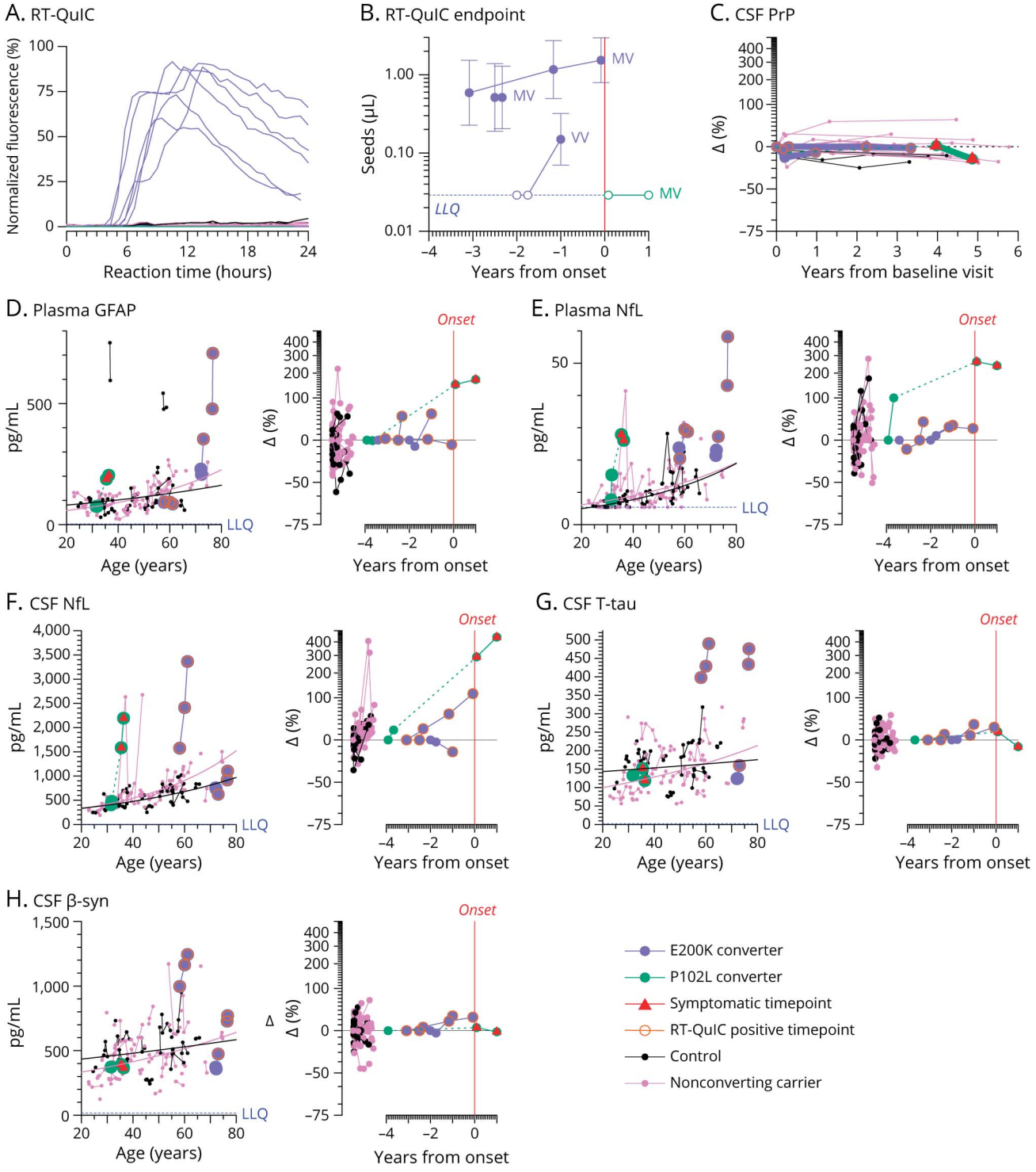
CSF total PrP levels varied between individuals and were lower in carriers (eFigure 2, eTable 3) but were longitudinally stable

Table 1 Demographic Characteristics of the Cohort

Group	N	Sex	Age (y)	Follow-up (y)	Total visits	CSF samples	Plasma samples	Pathogenic variants
Pathogenic variant carrier	41	13M/28F	47.5 ± 14.0	2.0 ± 1.9	126	104	109	6 P102L 7 D178N 22 E200K 6 other
Control	21	6M/15F	46.1 ± 13.3	1.4 ± 1.5	57	51	51	21 none

"Age" represents age last seen, follow-up is years from first visit to last visit, and both are represented by mean ± SD.

Figure 1 Fluid Biomarker Changes in the Cohort



(A) RT-QuIC kinetic curves for N = 149 CSF samples tested (98 from carriers and 51 from controls), showing 6 positive CSF samples (each with 4/4 replicates positive). (B) RT-QuIC endpoint titration of N = 10 CSF samples from 4 unique individuals who developed disease, including the 6 positive CSF samples from 3 E200K converters, with codon 129 genotypes of converters indicated. (C) CSF PrP concentrations represented as changes (Δ) relative to individual baseline, shown for the 4 converters plus all individuals with at least 3 years between first and last available CSF sample. N = 76 CSF samples from 19 unique individuals, see eTable 4. (D–H) Biomarkers plasma GFAP (N = 158 samples from 61 unique individuals) (D), plasma NfL (N = 160 samples from 62 unique individuals) (E), CSF NfL (N = 155 samples from 60 unique individuals) (F), CSF T-tau (N = 151 samples from 60 unique individuals) (G), and CSF β-syn (N = 150 samples from 60 unique individuals) (H) are represented by 2 views each. Left: individual age vs absolute concentration in pg/mL, with sequential samples from the same individual connected by thin lines, while thicker lines represent the separate log-linear best fit curves for controls and for nonconverting carriers. Right: years from disease onset vs change (Δ) relative to individual baseline in converters, with the same for controls and for nonconverting carriers shown on a separate x-axis. Dashed lines connect timepoints before and after symptom onset. For further breakdown and statistics, see eTable 5.

in each individual out to 6 years (Figure 1C, eTable 4), including samples taken after RT-QuIC positivity.

Plasma GFAP, a marker of reactive astrogliosis, was high relative to age in 2/4 converters, but change from individual baseline was unremarkable compared to controls and nonconverters (Figure 1D). Plasma NfL appeared high and increased in all 4 converters, but not outside the range of nonconverters and controls (Figure 1E, eTable 5). CSF NfL, CSF T-tau, and CSF β-syn were each elevated in 2/4 converters and normal in 2/4 (Figure 1, F–H, eTable 5); different converting individuals were high for different markers.

Discussion

Here we describe fluid biomarker profiles in a longitudinal cohort of carriers of pathogenic *PRNP* variants, including 4 individuals who converted to active disease. As before,[3,5,7] at any given time, cross-sectionally, most carriers of prion disease-causing variants lacked any detectable molecular sign of the disease. Our data support the hypothesis that CSF prion seeding activity assayed by RT-QuIC may represent the first detectable change in E200K carriers. We did not detect seeding activity in the CSF of a P102L converter, consistent with RT-QuIC's lower sensitivity in the context of disease subtypes hypothesized to exhibit lower intrinsic seeding capacity.[1] We observed longer prodromal positivity in 2 codon 129 M/V heterozygotes than in 1 V/V homozygote; if replicated in larger cohorts, this difference would mirror the longer disease duration after onset in heterozygotes.[11]

Soluble PrP in CSF is reduced in symptomatic prion disease patients, presumably as a result of a disease sink process,[12] and yet pharmacologic lowering of CSF PrP may be important as a drug activity biomarker for trials of PrP-lowering drugs, and has been proposed as a surrogate endpoint in prevention trials.[12] Our data suggest CSF PrP does not decline prior to symptom onset, even in the presence of RT-QuIC positivity, suggesting its use in asymptomatic individuals will not be confounded.

Neuronal damage and neuroinflammation markers rise with age and may vary between individuals. Neither when normalized to age nor to individual baseline did any of these markers consistently provide distinctive signal in all 4 of our converting individuals relative to nonconverters and controls. Despite the excellent diagnostic utility of β-syn in discriminating prion disease from other rapidly progressive dementias,[2] it was not more consistently elevated than CSF T-tau or CSF NfL in individuals proximate to conversion. While these markers may be useful as an adjunct, none is likely to provide the prognostic specificity of RT-QuIC. RT-QuIC, meanwhile, may offer just 1 year of advance signal in some E200K cases, and currently faces limited sensitivity to other subtypes. Assay improvement, biomarker discovery, and continued sample accrual will be vital to identifying additional prognostic markers, particularly for non-E200K subtypes. At any given time, most carriers appear nonprodromal; thus, in this rare disease, prodromal individuals are unlikely to be identified in sufficient numbers to power clinical trials. Primary prevention trials with inclusion based on genotype and CSF PrP as primary endpoint are one possibility,[12] which would honor the outsize benefit of early treatment observed in animal models.[13] Nonetheless, treatment of prodromal individuals could feature as a supportive arm and/or randomization off-ramp for carriers who develop a prodromal signature during a trial and enhancing our ability to identify prodromal states should be a research priority.

Our study has limitations. Four symptom onsets is a small absolute number from which to draw conclusions. Reflecting study enrollment and overall genotypic prevalence, our observed onsets are skewed toward E200K. Some annual visits were missed due to COVID-19. We did not collect emerging sample types such as nasal brushings[14] or tears,[15] and we did not perform MRI or ^{18}FDG-PET. We used only standard RT-QuIC conditions[9] and did not explore alternative recombinant PrP substrates such as bank vole PrP[5] or E200K PrP,[15] which might enhance sensitivity in certain genetic subtypes. Additional presymptomatic natural history work across multiple sites will be required to build confidence in our observations.

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Disclosure

S.M. Vallabh has received speaking fees from Ultragenyx, Illumina, Biogen, Eli Lilly, consulting fees from Invitae and Alnylam, and research support from Ionis, Gate, Sangamo. E.V. Minikel has received speaking fees from Eli Lilly, consulting fees from Deerfield and Alnylam, and research support from Ionis, Gate, Sangamo, Eli Lilly. S.E. Arnold has received speaking fees from Abbvie, Biogen, EIP Pharma, Roche, and Sironax, consulting fees from Athira, Biogen, Cassava, Cognito, Cortexyme, Sironax, and vTv, and research support from Abbvie, Amylyx, EIP Pharma, and Merck. The other authors report no relevant disclosures. Go to Neurology.org/N for full disclosures.

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SUPPLEMENT

Biomarker changes preceding symptom onset in genetic prion disease

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eAppendix 1. Supplementary Background

Prion disease is a rapidly progressive neurodegenerative disease caused by templated misfolding of the prion protein (PrP), arising either sporadically (85%), or by genetic (15%) or acquired means (<1%)[1]. Prion disease often features striking biomarker signatures[2–5], but limited data exist on pre-symptomatic changes, a challenge when considering early recognition and preventive treatment. It is currently impossible to determine which individuals in the population will develop sporadic prion disease, so pre-symptomatic reports in this population are limited to serendipitous case reports, which generally suggest a brief window of prodromal change, with cortical diffusion-weighted signal abnormalities indicative of prion disease appearing on magnetic resonance imaging (MRI) only 1-14 months before onset[6–9]. In contrast, highly penetrant *PRNP* variants[10] offer an opportunity to prospectively follow individuals at >90% disease risk prior to onset, though clinical presentation and utility of available biomarkers both differ by *PRNP* variant. E200K is rapid (median survival 6 months[11] from first symptom), typically presents as Creutzfeldt-Jakob disease (CJD), and has imaging and biomarker signatures similar to sporadic CJD[4,12,13]. Both D178N, which is slightly slower (median survival 12 months[11]) and presents as either CJD or fatal familial insomnia (FFI), and P102L, which is much slower (median survival 5 years[11]) and usually presents as Gerstmann-Straussler-Scheinker (GSS) disease, exhibit only subtle biomarker signatures even at the symptomatic stage. For instance, real-time quaking induced conversion (RT-QuIC), an *in vitro* assay that tests the ability of prion "seeds" in CSF to template the misfolding of bacterially expressed recombinant prion protein (PrP)[14], is highly sensitive and specific in symptomatic patients with sporadic CJD and E200K disease but is usually negative for symptomatic D178N and P102L individuals[4,12,15,16]. Dramatic rises in neurofilament light (NfL), total tau (T-tau), and β-synuclein (β-syn) in both CSF and plasma, and rise in glial fibrillary acidic protein (GFAP) in plasma only[4,2,3,17,5,18,19], are observed in sporadic CJD and E200K, but only smaller increases in NfL and T-tau have been reported for D178N and P102L[4,20,21].

Several longitudinal studies worldwide have followed individuals at genetic risk, seeking to identify imaging and fluid biomarker changes before onset[5,13,18,22–25]. Prion seeds have been detected by RT-QuIC in the CSF of a handful of pre-symptomatic E200K individuals[18,25], and in the lacrimal fluid of pre-symptomatic individuals with multiple different variants[26], but its prognostic value has remained unclear. Based on animal models, prion titer might be expected to rise early in the disease process and then plateau before symptom onset[27,28], but no longitudinal rise in prion titer in CSF has yet been detected[18]. Rises in plasma neurofilament light (NfL) and glial fibrillary acidic protein (GFAP) have also been reported preceding symptom onset, though only by months in individuals with rapidly progressive variants[5,18], and the temporal relation of these changes to the beginning of detectable prion seeding has not been determined. CSF β-syn was recently reported to possess strong diagnostic utility in prion disease[3], perhaps owing to its specificity for rapid synaptic loss[29,30], but its behavior before symptom onset has not been examined. Genotype at the common *PRNP* polymorphism M129V is a key determinant of both disease duration[11] and biomarker utility[4] in symptomatic genetic prion disease, but its impact on prognostic value of biomarkers at the pre-symptomatic stage is unknown.

Supplementary Methods

Study participants. This previously described[25] cohort study at Massachusetts General Hospital includes asymptomatic individuals with pathogenic *PRNP* variants; individuals at risk for same due to a first or second degree relative who died of genetic prion disease; and controls. Individuals with contraindication to lumbar puncture were excluded. Participants were recruited through Mass General Brigham (MGB) Rally, Prion Alliance, CJD Foundation, and PrionRegistry.org. An enrollment flowchart is provided in Figure S1. Each visit included CSF and plasma collection, a medical history and physical, and a battery of cognitive, psychiatric, and motor tests and inventories. Individuals were invited to complete a baseline visit, a short-term repeat 2-4 months later (pre-2020), and approximately yearly visits thereafter.

Genotypes and non-disclosure. Genotypes for all participants were determined by deep short-read sequencing and further confirmed by Sanger sequencing and gel sizing (see Supplementary Methods). Our study does not disclose *PRNP* genotypes nor biomarker values to participants. We have therefore taken measures to mitigate the risk of self-identification. First, some participants know which pathogenic variant runs in their family, such that the presence of that variant in our cohort could reveal their genotype. To mitigate this risk, rarer *PRNP* variants are grouped as "Other", with only the 3 most common variants broken out individually. Note that because we do not disclose genotypes to participants, it is impossible to exclude on the basis of the specific *PRNP* variant identified, thus, the "Other" group includes both high and low penetrance[10] variants. Second, even for more common pathogenic variants, the combination of age and the number and spacing of visits completed could also uniquely identify some individuals. To mitigate this risk, for controls and non-converting carriers in data visualizations, ages were obfuscated by addition of a normally distributed random variable with mean of 0 and standard deviation of ±3 years, and visit spacing intervals were obfuscated by multiplication by a normally distributed random variable with mean 1 and standard deviation ±25%, capped at a maximum increase of +25% to avoid visually exaggerating the study's duration. True ages and true visit intervals for all participants are used in all descriptive statistics and statistical models and true ages and true visit intervals are shown in plots for the individuals who converted to active disease.

Genotyping. Whole blood was frozen hemolyzed and genomic DNA was extracted. All samples were genotyped by two orthogonal methods. DNA was submitted for targeted capture using a custom set of probes (Twist Biosciences) directed against ~150 kb of genomic sequence in and surrounding *PRNP*, then enriched DNA was subjected to deep short-read sequencing (Illumina) at the Broad Institute's Genomics Platform. Data were aligned to the hg38 reference genome and processed using Dragen 3.7.8 to yield multi-sample VCF files. In parallel, DNA also underwent a previously described[31] protocol implemented by Genewiz, combining Sanger sequencing to detect SNPs and short indels with gel sizing to detect octapeptide repeat insertions (OPRI). Briefly, the primers utilized are: Int5: 5′-TgCATgTTTTCACgATAgTAACgg-3′, DG2: 5′-gCAgTCATTATggCgAACCTTggCTg-3′, and 3′Sal: 5′-gTACTgAggATCCTCCTCATCCCACTATCAggAAgA-3′. The prodcut of the DG2/3′Sal reaction is subjected to Sanger sequencing; the product of the DG2/Int5 reaction is run on a 2% agarose gel (the wild-type product is 464 bp). Genotypes obtained by the two different methods were in agreement for all samples. Determination of haplotypes was accomplished by molecular phasing of codon 129 to pathogenic variants by paired-end Illumina sequencing reads using a custom Python 3 script run on Terra (Terra.bio); source code is available in the study's online GitHub repository. Our study includes individuals who are at risk for inheriting a *PRNP* mutation but have not undergone predictive testing; genotypes were determined for research purposes only and were not disclosed to participants.

Sample processing. Blood was collected in purple top K+ EDTA tubes, inverted gently, and centrifuged at 1,500 *g* for 10 minutes to retrieve plasma, aliquoted, and frozen at -80°C. 20 mL of CSF was collected via gentle aspiration lumbar puncture using a 24G atraumatic Sprotte needle into 4x 5 mL syringes. Because PrP

in CSF is highly sensitive to polypropylene adsorption, we followed the protocol described in Vallabh 2019 Figure S8, where 2 of the 4 collected 5 mL aliquots were ejected into tubes pre-loaded with the zwitterionic detergent CHAPS (3% wt/vol stock solution at 1% volume to yield a final 0.03% CHAPS concentration). All CSF were centrifuged at 2,000 *g* for 10 minutes to remove cells, and then aliquoted and frozen at -80°C. In instances where the LP yielded only a limited volume of CSF, CHAPS aliquots were prioritized. CHAPS aliquots were used for PrP and NfL quantification. Neat aliquots were used for RT-QuIC. For T-tau and beta-synuclein, neat aliquots were used where available, while CHAPS aliquots were used when these were the only available samples; an assessment of the effect of 0.03% CHAPS on these assays is provided in Figures S3-S4. To minimize bias, technicians processing samples and performing biomarker assays were blinded to genotype. Because not all samples yielded volumes sufficient for all biomarker assays, and the exact N of samples run for each assay is provided in the legend of Figure 1 and in Tables S4 and S5 below.

RT-QuIC. Real-time quaking-induced conversion (RT-QuIC) was performed according to the protocol of Orru et al 2015, widely referred to as the IQ-CSF protocol[14]. The substrate was recombinant N-terminally truncated Syrian hamster PrP (SHaPrP90-230) expressed in *E. coli* and produced in-house according to a published protocol[32,33] and filtered by centrifugation at 3,214 *g* through a 100 kDa filter (PALL OD100C33). Final concentration in the reaction was 300 mM NaCl (Broad Institute SQM), 10 mM sodium phosphate pH 7.4 (Molecular Toxicology; Thermo C790B91), 1 mM EDTA (Broad Institute SQM), 10 µM thioflavin T (Sigma T3516-5G), 0.002% sodium dodecyl sulfate (SDS) (Invitrogen 15553-035), and 0.1 mg/mL recombinant PrP, all diluted into distilled water (InvitroGen UltraPure 10977-015). 80 µL of a 1.25x concentrated master mix was loaded into each well of a 96-well plate (Nunc; Thermo 265301) and then 20 µL of CSF was added. Plates were sealed with adhesive film (VWR 37000-548). The assay was run at 55°C for 24 hours on a BMG FLUOStar OPTIMA platereader with alternating cycles of 1 minute rest and 1 minute 800 rpm shaking, with thioflavin T fluorescence measurements obtained via bottom read at 45-minute intervals with 450 nm excitation and 480 nm emission. Fluorescence kinetic curves were normalized so that 0% represents the baseline fluorescence value at first reading and 100% represents the instrument's maximum value of 65,000 fluorescence units. We committed to the pre-specified criteria of Orru et al[14]: a CSF sample was called positive if at least 50% of technical replicates (e.g. 2/4) yielded at least 10% normalized signal within 24 hours. In practice, when screening undiluted CSF, all our positive samples were positive in 4/4 replicates while all negatives were positive in 0/4 replicates. For initial screening of neat samples, 20 µL of CSF was always used. For endpoint titration of positive samples, 3-fold serial dilutions of CSF were run by adding 20, 6.7, 2.2, or 0.7 µL of CSF and then 0, 13.3, 17.8, or 19.3 µL of distilled water (InvitroGen UltraPure 10977-015). Titers were determined by Spearman-Karber analysis[34]; the source code is available in this study's online GitHub repository.

PrP ELISA. PrP enzyme-linked immunosorbent assay (ELISA) was performed according to an in-house protocol previously published and described in detail[35]. The assay uses antibodies EP1802Y (Abcam ab52604) for capture and 8H4 (Abcam ab61409), biotinylated in-house, for detection. The standard curve is recombinant full-length mouse PrP (MoPrP23-231) produced in house, plated at concentrations from 0.05 ng/mL to 5 ng/mL. CSF was run at a dilution factor of 80, at which the lower limit of quantification (LLOQ) is 4 ng/mL. For longitudinal analysis (Figure 1C, Table S4), each individual was normalized to their own baseline. For comparison across mutations (Figure S2, Table S3), all individuals were normalized to the mean value in mutation-negative subjects, which was 70.6 ng/mL.

GFAP. Plasma GFAP was quantified using Simoa (Quanterix) according to manufacturer instructions at a dilution factor of 4, yielding an LLOQ of 2.744 pg/mL. Samples were run in technical duplicate with a mean CV of 6.0%.

NfL. Plasma and CSF NfL were quantified using Ella by ProteinSimple (Bio-Techne) at a dilution factor of 2 yielding an LLOQ of 5.4 pg/mL. CSF aliquots containing CHAPS were used. For all Ella assays, samples were plated onto cartridges in singlicate; each sample is then run in technical triplicate with three glass nanoreactors (GNRs).

T-tau. CSF T-Tau was analyzed both by ELISA (Fujirebio) and by Ella (Bio-Techne). For Ella, samples were run at a dilution factor of 2 (except for N=6 samples run at a dilution factor of 3 due to limited volume), with an LLOQ of 1.68 pg/mL. For ELISA, samples were run at a dilution factor of 4, with an LLOQ of 39.5 pg/mL. Ella results are reported in Figure 1G, while a comparison of the two assays is given in Figure S3.

Beta-synuclein. CSF beta-synuclein was analyzed by Ella (Bio-Techne) at a dilution factor of 2 for CSF (LLOQ: 15.9 pg/mL) and either 4 or 8 for plasma depending on available sample volume (LLOQ: 31.8 pg/mL or 63.7 pg/mL respectively). As shown in Figure S4, all plasma samples from study participants were at LLOQ.

Supplementary Figures

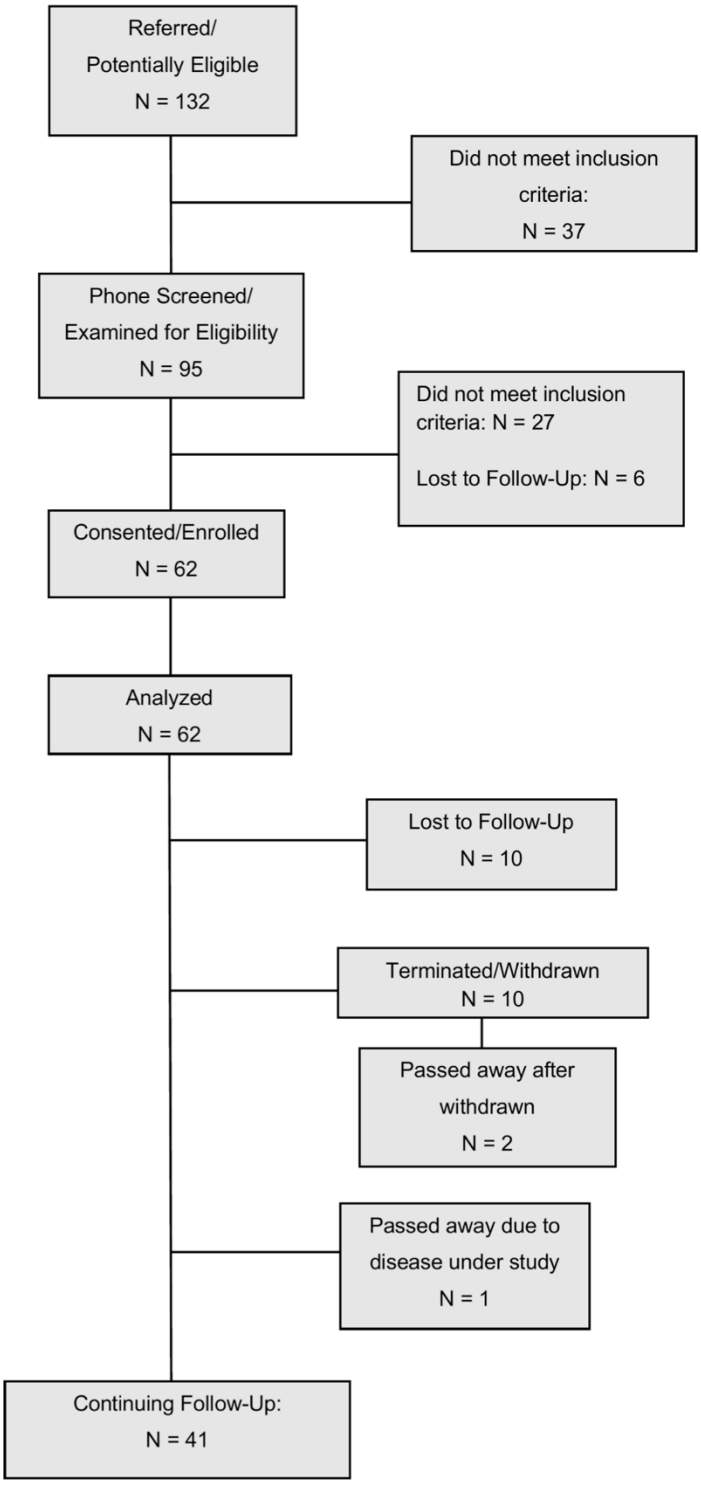

Figure S1. Flow chart of participant recruitment.

At launch in July 2017, the study was open to known mutation carriers, those at risk, and known controls. From November 2021 new enrollment restricted to only known carriers, but already-enrolled individuals were invited to continue to participate regardless.

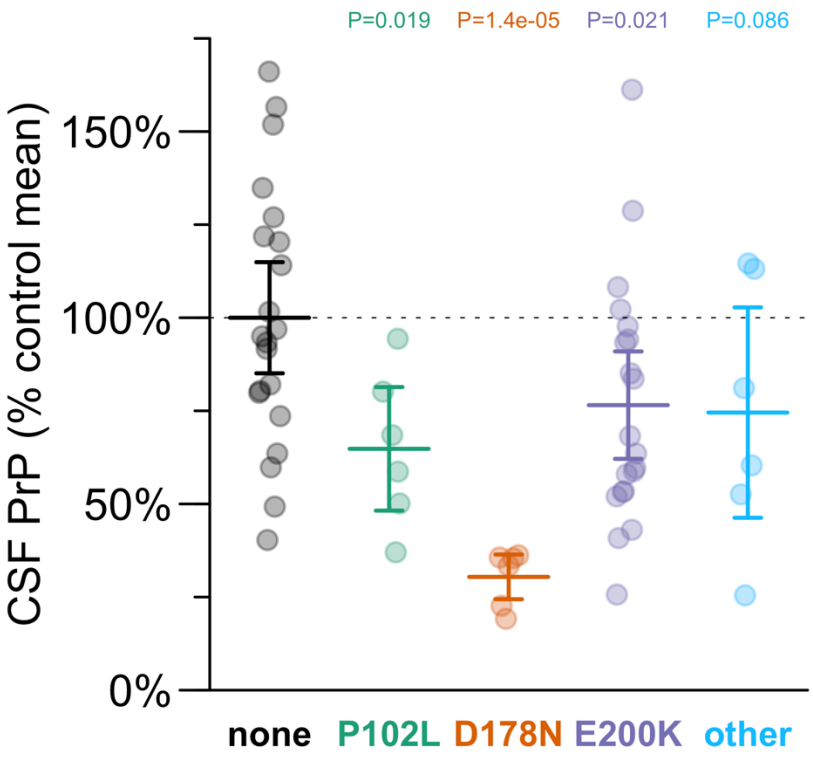

Figure S2. CSF PrP concentration by PRNP mutation.

Each point represents the mean of all available CSF samples for one study participant. Data are normalized to the mean of the mutation-negative controls ("none"). P values are for differences from the control group in a linear model (lm in R, equivalent to Type I ANOVA).

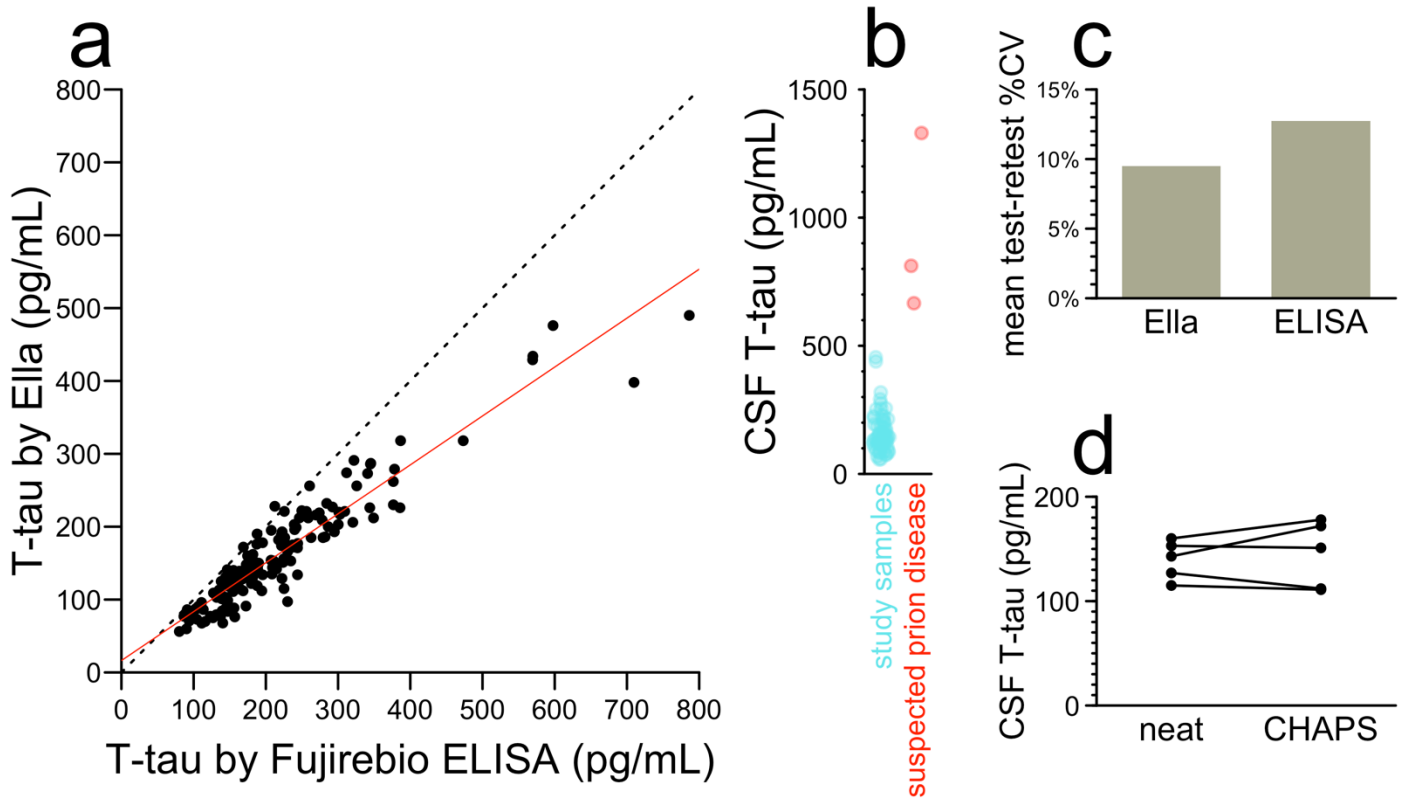

Figure S3. Quality control analyses on the Ella T-tau assay.

A) *Comparison of CSF T-tau concentrations in pg/mL for N=151 CSF samples determined by Fujirebio ELISA (x axis) versus Ella (y axis). The red line shows the best fit linear regression which is ella = 16.3 pg/mL + 67% × elisa. The Pearson's correlation is r = 0.94, P = 5.3e-70.* ***B)*** *Comparison of mean CSF T-tau values per individual by Ella in study participants vs. 3 symptomatic patients with suspected prion disease.* ***C)*** *Mean test-retest CV for longitudinal LPs from the same individual: Ella 9.5%, ELISA 12.7%.* ***D)*** *Paired analysis of N=5 CSF samples analyzed by T-tau Ella both with and without the addition of 0.03% CHAPS. Mean value with CHAPS is 3% higher, P = 0.55 by paired T test.*

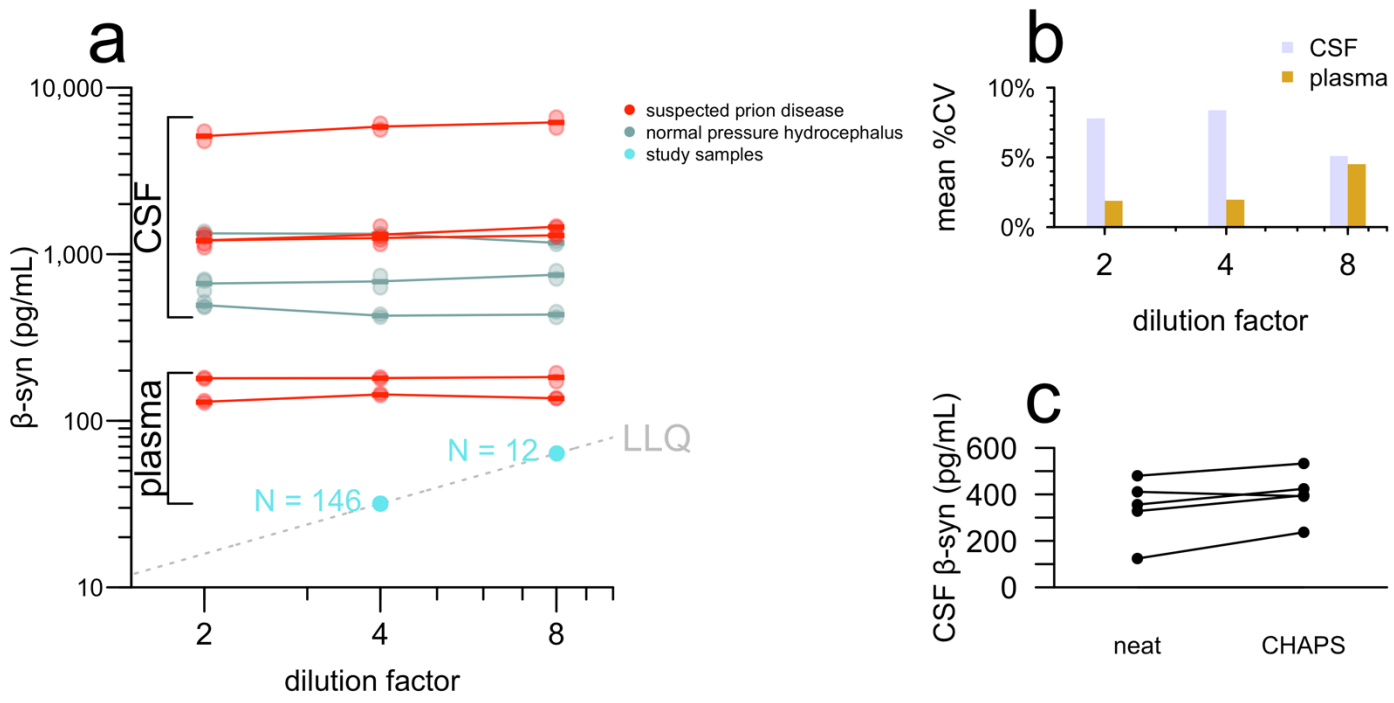

Figure S4. Quality control analyses on the Ella beta-synuclein assay.

A) *Parallelism (also called dilution linearity) tested on 6 CSF samples (3 suspected prion disease and 3 normal pressure hydrocephalus) and 2 plasma samples (2 suspected prion disease). Suspected prion disease patients are symptomatic individuals seen clinically at Massachusetts General Hospital outside of our study. All plasma samples from participants in our study were at the lower limit of quantification (LLQ).* ***B)*** *Mean coefficient of variation among 2 technical replicates per sample for the samples shown in (A). Note that this refers to plating the same sample twice, in separate wells, on the Ella cartridge; the measurement in each well is in turn the average of 3 replicate measurements.* ***C)*** *Comparison of 5 CSF samples from study participants analyzed both with and without the addition of 0.03% CHAPS, a detergent shown to reduce loss of PrP to plastic. Mean 27% higher reading in CHAPS samples, P = 0.057, paired T-test.*

Supplementary Tables

Supplementary tables are also available in the attached Excel spreadsheet and as tab-separated text files in the study's online GitHub repository.

Table S1. All biomarker values from all visits by individuals who developed active disease.

Genotype shows the pathogenic variant and codon 129. The 129MV individuals in this table are all cis-129M, trans-129V. Months from onset is negative for visits prior to symptom onset and positive for visits after symptom onset. Blank cells indicate assays not done because samples not collected (unsuccessful LP or virtual visit) or due to limited sample volume. MoCA[36] and MRC Scale[37] have been described elsewhere.

Individual	Genotype	Age of onset bin	Visit number	Months from onset	CSF RT-QuIC	CSF RT-QuIC replicates	CSF T-tau (pg/mL)	CSF NfL (pg/mL)	CSF β-syn (pg/mL)	CSF PrP (ng/mL)	Plasma NfL (pg/mL)	Plasma GFAP (pg/mL)	MoCA score	MRC Scale
A	E200K MV	75-79	1	-30	+	4/4	434	918	728	73.0	43.1	478.0	27	20
			2	-28	+	4/4	476	1103	771	69.8	58.2	707.7	25	20
B	P102L MV	35-39	1	-47				406	373	37.5	7.7	75.4	26	20
			2	-44			133	478		33.9	15.4	74.8	27	20
			3	1	-	0/4	152	1584	392	38.1	27.9	187.9	27	20
			4	12	-	0/4	119	2194	366	30.6	26.1	204.0	28	19
C	E200K VV	65-69	1	-24	-	0/4	124	757	372	32.8	21.4	229.6	29	20
			2	-21	-	0/4	125	732	358	27.6	23.1	207.2	28	20
			3	-12	+	4/4	160	623	474	29.9	27.3	353.7	24	20
D	E200K MV	60-64	1	-41							23.8	91.8	26	20
			2	-37	+	4/4	398	1575	997	66.4	20.5	94.1	26	20
			3	-16									26	20
			4	-14	+	4/4	429	2413	1164	66.2	29.5	93.1	27	20
			5	-1	+	4/4	490	3365	1244	64.8	28.8	85.3	29	20

Table S2. Means, standard deviations, and ranges of biomarker values from all visits by participants who did not develop active disease, by mutation status.

These summary statistics exclude all visits from the 4 participants who converted to active disease. In each cell, the top row shows mean±SD, while the bottom row shows range (min-max). CSF RT-QuIC positive shows the number of CSF samples that yielded an overall positive call. Each RT-QuIC reaction was run in quadruplicate; in this study, every positive sample was positive in all 4/4 replicates, while every negative sample was positive in 0/4 replicates.

Group	N visits	CSF RT-QuIC positive	CSF T-tau (pg/mL)	CSF NfL (pg/mL)	CSF β-syn (pg/mL)	CSF PrP (ng/mL)	Plasma NfL (pg/mL)	Plasma GFAP (pg/mL)	MoCA	MRC
Mutation-negative control	57	0/51	164±49 (75-318)	573±208 (242-1018)	516±144 (243-952)	71±24 (27-120)	9.7±4.6 (5.4-28.2)	145.9±150.1 (30.6-751.1)	28±3 (14-30)	20±0 (17-20)
Non-converting mutation carrier	112	0/88	149±65 (56-318)	685±404 (192-2677)	472±201 (124-1171)	45±24 (10-126)	11.1±6.6 (5.4-41.4)	112.8±53.3 (22.3-267.5)	28±2 (22-30)	20±0 (19-20)

Table S3. Mean CSF PrP concentration (ng/mL) by mutation.

These are the numeric values for the data shown in Figure S2. Results were grouped first by individual to determine mean CSF PrP concentration across longitudinal CSF samples, then grouped by mutation to determine mean and SD across individuals. N is the number of individuals in each group.

Mutation	N	Mean	SD	Normalized mean	Linear regression P value
none	21	69.9	24.4	100.0%	—
P102L	6	45.3	14.5	64.8%	1.92e-02
D178N	6	21.3	5.2	30.4%	1.42e-05
E200K	20	53.5	23.0	76.5%	2.06e-02
other	6	52.1	24.7	74.5%	8.64e-02

Table S4. Long-term test-retest reliability of CSF PrP.

Summary of data from Figure 1C. Test-retest mean CV summarized for converters and all non-converting study participants with ≥3 years of longitudinal CSF data.

Group	N individuals	N samples total	Mean CV
non-converting carrier	12	52	9.71%
control	3	12	10.20%
converting carrier	4	12	5.78%
total	19	76	8.96%

Table S5. Descriptive statistics and log-linear model fits on CSF and plasma biomarkers.

						Overall model lm(log(value) ~ age + carrier, data = all)				
Biomarker	**N individuals**	**N samples**	**Carrier samples**	**Control samples**	**Test-retest mean CV without converters**	**Y intercept**	**Annual increase**	**Annual increase P value**	**Carrier difference**	**Carrier difference P value**
CSF NfL	60	155	104	51	16.3%	183.69	2.3%	8.1e-20	0.0028	0.0028
CSF T-tau	60	151	100	51	9.6%	101.29	0.9%	2.8e-04	0.0734	0.0734
CSF β-syn	60	150	99	51	10.8%	329.05	0.9%	3.8e-04	0.0819	0.0819
plasma NfL	62	160	109	51	19.6%	3.51	2.0%	9.3e-14	0.0558	0.0558
plasma GFAP	61	158	107	51	18.4%	45.78	1.9%	6.3e-08	0.6014	0.6014

	Model of non-converting carriers only lm(log(value) ~ age, data = NCcarriers)			**Model of controls only lm(log(value) ~ age, data = controls)**		
Biomarker	**Y intercept**	**Annual increase**	**Annual increase P value**	**Y intercept**	**Annual increase**	**Annual increase P value**
CSF NfL	193.9	2.6%	2.7e-15	233.9	1.8%	5.4e-06
CSF T-tau	77.5	1.3%	2.8e-04	133.9	0.3%	0.35
CSF β-syn	267.5	1.1%	0.0014	393.6	0.5%	0.13
plasma NfL	4.2	1.9%	1.9e-07	3.2	2.3%	1.0e-09
plasma GFAP	37.1	2.3%	6.2e-10	64.1	1.2%	0.11

STROBE checklist

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Location
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1 line 20
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 1 line 15-43
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 1 line 48-54, supplement page 2 line 30-65
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 1 line 17-18
Methods			
Study design	4	Present key elements of study design early in the paper	Page 2 line 62
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 2 line 62, Supplement page 3 line 70
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Supplement page 3 line 70, Figure S1
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Table 1, Table S5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Supplement p. 3-4 lines 128 - 176
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Supplement p. 3-4 lines 128 - 176
Bias	9	Describe any efforts to address potential sources of bias	Supplement p. 4 line 124
Study size	10	Explain how the study size was arrived at	p. 2 line 87
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	p. 2 line 72-76, supplement p. 3 line 79-94
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	p. 2 line 72-76, supplement p. 3 line 79-94

		(*b*) Describe any methods used to examine subgroups and interactions	p. 2 line 72-76, supplement p. 3 line 79-94
		(*c*) Explain how missing data were addressed	Supplement p. 4 line 125-126
		(*d*) If applicable, explain how loss to follow-up was addressed	Figure S1
		(e) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure S1
		(b) Give reasons for non-participation at each stage	Figure S1
		(c) Consider use of a flow diagram	Figure S1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Tables S2, S3, S5
		(c) Summarise follow-up time (eg, average and total amount)	Table 1
Outcome data	15*	Report numbers of outcome events or summary measures over time	p. 2-3 line 87-110
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table S1, Figure 1
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	p. 2 line 87 - p. 3 line 110

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p. 4 line 149-155
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	p. 3-4 line 117 - 147
Generalisability	21	Discuss the generalisability (external validity) of the study results	p. 3-4 line 133 - 155
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	p. 5 line 164

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