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Cerebrospinal Fluid Total Prion Protein in the Spectrum of Prion Diseases

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Abstract

Cerebrospinal fluid (CSF) total prion protein (t-PrP) is decreased in sporadic Creutzfeldt-Jakob disease (sCJD). However, data on the comparative signatures of t-PrP across the spectrum of prion diseases, longitudinal changes during disease progression, and levels in pre-clinical cases are scarce. T-PrP was quantified in neurological diseases (ND, $n = 147$) and in prion diseases from different aetiologies including sporadic (sCJD, $n = 193$), iatrogenic (iCJD, $n = 12$) and genetic ($n = 209$) forms. T-PrP was also measured in serial lumbar punctures obtained from sCJD cases at different symptomatic disease stages, and in asymptomatic prion protein gene (*PRNP*) mutation carriers. Compared to ND, t-PrP concentrations were significantly decreased in sCJD, iCJD and in genetic prion diseases associated with the three most common mutations E200K, V210I (associated with genetic CJD) and D178N-129M (associated with fatal familial insomnia). In contrast, t-PrP concentrations in P102L mutants (associated with the Gerstmann-Sträussler-Scheinker syndrome) remained unaltered. In serial lumbar punctures obtained at different disease stages of sCJD patients, t-PrP concentrations inversely correlated with disease progression. Decreased mean t-PrP values were detected in asymptomatic D178-129M mutant carriers, but not in E200K and P102L carriers. The presence of low CSF t-PrP is common to all types of prion diseases regardless of their aetiology albeit with mutation-specific exceptions in a minority of genetic cases. In some genetic prion disease, decreased levels are already detected at pre-clinical stages and diminish in parallel with disease progression. Our data indicate that CSF t-PrP concentrations may have a role as a pre-clinical or early symptomatic diagnostic biomarker in prion diseases as well as in the evaluation of therapeutic interventions.

Keywords Cerebrospinal fluid · Prion protein · Sporadic Creutzfeldt-Jakob disease · Genetic prion disease · Iatrogenic prion disease

Introduction

Transmissible spongiform encephalopathies are fatal neurodegenerative disorders characterised by rapid progression and microvacuolation in the grey matter of the brain. They are also known as prion diseases relating to the causative agent, which is the abnormally folded isoform the prion protein scrapie (PrPsc) that creates insoluble aggregates and accumulate in the brain [1]. Prion diseases affecting humans include sporadic Creutzfeldt-Jakob disease (sCJD), genetic prion diseases (gPD) and acquired forms, such as iatrogenic CJD (iCJD) or variant CJD (vCJD). sCJD is the most prevalent form accounting for about 85–90% of total cases, followed by hereditary forms (~ 10%). By contrast, acquired forms only account for at most 2–5% of prion disease cases [2, 3]. Despite sharing a common pathological agent, prion diseases present a broad heterogeneity in clinical symptoms and

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disease manifestations. A cause of variability is the polymorphism at codon 129 of the PrP encoding gene, *PRNP*, which can be methionine (M129) or valine (V129) [4]. In combination with biochemically characterised conformational variants of PrPsc, sCJD can be stratified into at least six molecular subtypes, with MM1/MV1 and VV2 the most prevalent ones (> 80% of total sCJD cases [5]). gPD are associated with specific mutations in the *PRNP* and include familial/genetic Creutzfeldt-Jakob disease (gCJD), Gerstmann-Sträussler-Scheinker syndrome (GSS-S) and familial fatal insomnia (FFI). Several *PRNP* mutations cause gCJD and GSS-S (reviewed elsewhere [6]), with E200K and V210I the most prevalent mutations in gCJD and P102L in GSS-S. In contrast, FFI is associated with a unique haplotype, D178N-129M (D178N-M) [6, 7]. Acquired human prion diseases develop from exogenous prions and are transmitted from infected humans (iCJD) or cattle (vCJD). iCJD is caused by human-to-human transmission during medical treatments, with dura matter grafts and administration of human growth hormone the main causes of cross-contamination through surgical and medical procedures [8].

Clinical diagnosis of prion diseases is supported by cerebrospinal fluid (CSF) biomarkers. The CSF profile of prion disease patients is characterised by elevated concentrations of surrogate protein markers of the pathology such as 14-3-3, tau and alpha-synuclein [9–11], as well as by the presence of prion seeding activity [12]. By contrast, the CSF total PrP (t-PrP) concentrations are decreased in sCJD. The specificity of this decrease remains unclear as some authors reported reduced CSF t-PrP in various neurodegenerative diseases, such as Alzheimer's disease (AD), dementia with Lewy bodies and Parkinson's disease [13], while others suggested that t-PrP is specifically reduced in sCJD compared to AD, supporting its use in the differential diagnostic context [14, 15]. Among sCJD molecular subtypes, no difference was found in CSF t-PrP levels between MM1 and VV2 cases [16] and scant data exist for acquired cases. When CSF t-PrP was investigated by western blot, no variation appeared in the truncation or glycosylation state of the protein between different sCJD subtypes or in comparison with hereditary prion diseases [17–19]. To date, no further quantification of CSF t-PrP has been carried out in genetic prion diseases, including a significant number of mutation types. In addition, few data are available about the relationship of CSF t-PrP with demographic parameters, as are data on longitudinal t-PrP CSF levels in prion diseases. A preliminary exploration showed a decrease in t-PrP levels with disease progression although quantification was lacking and only six sCJD cases were studied [18].

While age does not influence t-PrP concentration in diseased individuals, low levels are associated with advanced disease stages [13]. Correlation analysis with other CSF biomarkers indicated a direct correlation between t-PrP and amyloid-beta 42 peptide in sCJD [13].

The goal of the present study was to provide complete CSF t-PrP signatures across the broad spectrum of prion diseases. Thus, we quantified the CSF t-PrP in a large number of cases that include sCJD, iCJD, various forms of gPD, and diverse neurological diseases composing our control group. In addition, we also investigated longitudinal CSF t-PrP alterations in serial lumbar punctures from sCJD cases and asymptomatic *PRNP* mutant carriers.

Methods

Patients

The study included 561 CSF samples. Samples from patients with non-primarily neurodegenerative neurological diseases (ND, $n = 147$) and sporadic Creutzfeldt-Jakob disease (sCJD, $n = 193$) were collected at the Clinical Dementia Center and the National Reference Center for CJD Surveillance at the University Medical Center of Göttingen (Germany). The ND group was composed of cases diagnosed with neurological conditions not associated with neurodegenerative pathology including the following diagnostic groups: psychosis, paranoid psychosis, bipolar disorder, schizophrenia, ischemic stroke, multiple cerebral infarcts, epilepsy, meningitis, alcohol abuse, vertigo, acute or chronic headache, pain syndromes, acute hypoxia, polyneuropathy, cerebral lymphoma, astrocytoma and paraneoplasia. ND cases were diagnosed according to acknowledged standard neurologic clinical and para-clinical findings based on the ICD 10 definitions. The presence of neurodegenerative diseases in the ND group was excluded by follow-up evaluations. All patients with sCJD were classified as probable or definite cases according to diagnostic consensus criteria [20, 21].

Iatrogenic ($n = 12$) and genetic ($n = 209$) prion diseases were collected in the following CJD reference centres: (1) Clinical Dementia Center and the National Reference Center for CJD Surveillance at the University Medical Center, Göttingen, Germany, (2) Neurochemistry Laboratory, Neurology Department of Coimbra University Hospital, Coimbra, Portugal, (3) Alzheimer's Disease and Other Cognitive Disorders Unit, Hospital Clínic, Barcelona, Spain, (4) National Centre of Microbiology-Carlos III Institute of Health, Madrid, Spain, (5) Istituto Superiore di Sanità, Rome, Italy, (6) Slovak Medical University, Bratislava, Slovakia, (7) Australian National CJD Registry, The Florey Department of Neuroscience and Mental Health, Melbourne, Australia, and (8) Department of Neurology, Memory and Aging Center, University of California, San Francisco (UCSF), USA. The diagnoses of genetic prion diseases were carried out according to surveillance criteria after prion protein gene (*PRNP*) analysis [22] and World Health Organization (WHO) criteria [23]. Iatrogenic CJD was diagnosed according to established WHO criteria [23]. Eleven iatrogenic cases were associated with dura matter grafts and one with corneal transplantation.

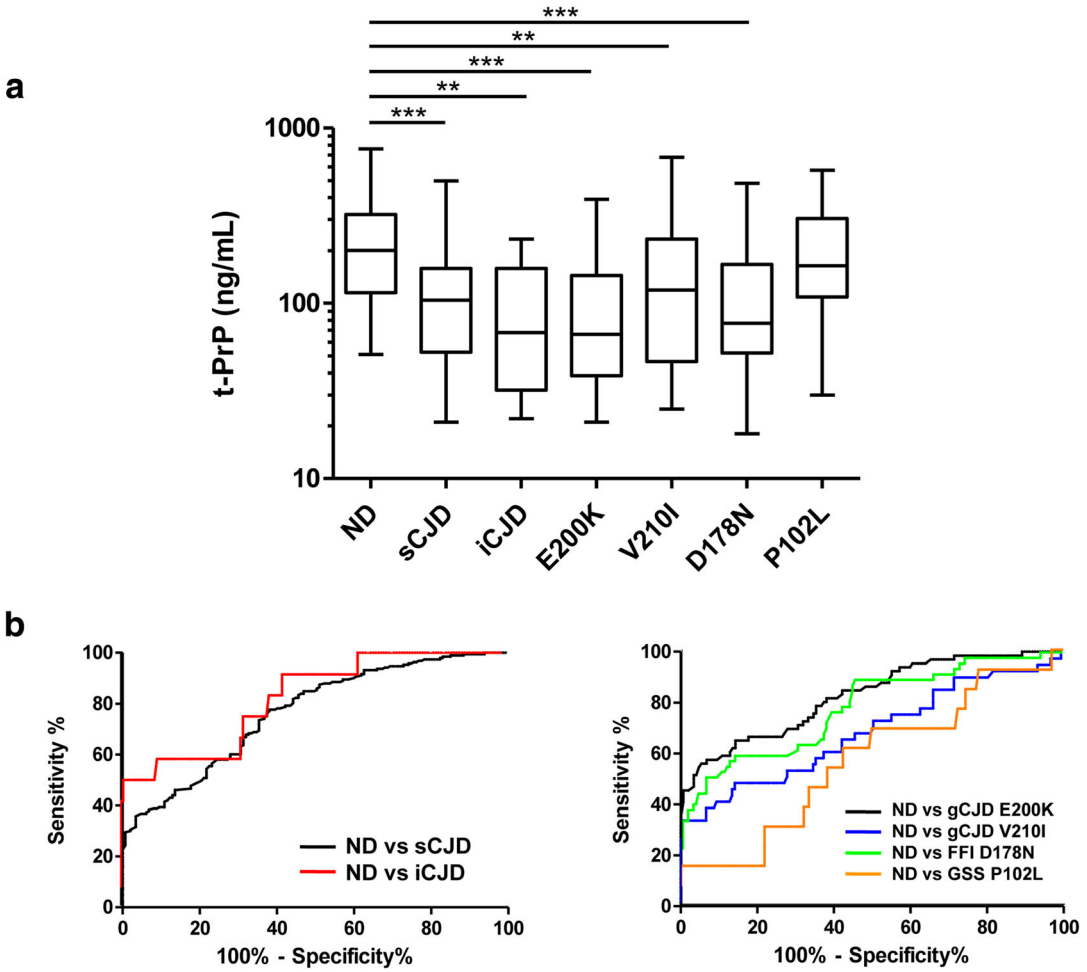
Table 1 Demographic and biomarkers data from our study population. Number of cases (*n*), age in years (mean values ± standard deviation), sex (female (f)/males (m)), codon 129 *PRNP* genotype and t-PrP concentrations (mean values (mean) ± standard deviation (SD) in ng/mL), and 95% CI (in ng/mL) are indicated. Mutation in the *PRNP* gene is indicated for genetic prion diseases (gPD). *gCJD* genetic Creutzfeldt-Jakob disease, *GSS-S* Gerstmann-Sträussler-Scheinker syndrome, *FFI* fatal familial insomnia, *M* methionine, *V* valine, *NA* not available. Other mutations/variants refer to cases diagnosed as prion disease with changes on the *PRNP* gene without clear evidence of being disease causative mutations

	Number of cases	Age	Sex	PRNP 129				t-PrP	
		Mean ± SD (years)	f/m	MM	MV	VV	NA	Mean ± SD (ng/mL)	95% CI (ng/mL)
Control									
ND	147	65 ± 11	78/69	0	0	0	147	230 ± 140	207–253
Sporadic prion disease									
sCJD	193	65 ± 10	114/79	74	37	45	37	120 ± 83	108–132
Aquired prion disease									
iCJD	12	48 ± 10	5/7	9	2	0	1	95 ± 71	50–140
Genetic prion disease									
Pathogenic variants associated to gCJD									
D178N-V	1	48	1/0	0	0	1	0	29	0
V180I	1	77	0/1	1	0	0	0	300	0
T188A	1	82	1/0	1	0	0	0	142	0
T188K	1	57	1/0	0	1	0	0	173	0
K194E	1	71	0/1	0	1	0	0	232	0
E196K	3	69 ± 4	2/1	1	1	1	0	69 ± 4	59–79
E200K	66	61 ± 11	39/27	45	18	2	1	98 ± 77	78–117
R208H	4	62 ± 7	2/2	3	1	0	0	243 ± 146	10–475
V210I	41	64 ± 10	24/17	31	8	2	0	161 ± 147	114–207
P238S	1	68	1/0	0	1	0	0	52	0
Pathogenic variants associated to GSS-S									
P102L	13	53 ± 11	8/4*	11	1	1	0	200 ± 148	111–289
P105T	3	36 ± 17	2/1	0	3	0	0	225 ± 168	0–642
G114V	1	20	0/1	0	1	0	0	114	0
A117V	1	47	1/0	0	0	1	0	95	0
A133V	1	62	1/0	1	0	0	0	68	0
V176G	1	61	1/0	0	0	1	0	31	0
F198S	1	51	0/1	0	1	0	0	47	0
Q217R	1	59	0/1	1	0	0	0	165	0
Y218N	1	NA	1/0	0	1	0	0	254	0
Pathogenic variants associated to FFI									
D178N-M	47	50 ± 10	16/31	31	16	0	0	119 ± 94	91–146
Insert mutations									
OPRI	14	63 ± 9	8/6	5	4	5	0	105 ± 113	40–171
Nonsense mutations									
Q160X	1	27	0/1	1	0	0	0	30	0
Other mutations/variants									
Q52P	1	80	1/0	0	1	0	0	141	0
N173K	1	73	0/1	0	1	0	0	203	0
Q212H	1	63	1/0	1	0	0	0	37	0
I215V	1	77	0/1	1	0	0	0	81	0

*Sex unknown in one case

CSF Tests

CSF t-PrP was centrally quantified (Clinical Dementia Center-Göttingen) using a commercially available enzyme-linked immunosorbent assay (ELISA) specific for human prion protein (Analytik Jena AG). Inter- and intra-assay coefficients of variation in our study were below 18 and 12%, respectively. CSF neurofilament-light (NFL) was quantified using a commercially available ELISA (Uman-Diagnostics). The analysts were masked to clinical data.



	sCJD	iCJD	gCJD E200K	gCJD V210I	FFI D178N-M	GSS P102L
Number of cases	193	12	66	41	47	13
AUC (95%CI)	0.76 (0.72-0.81)	0.82 (0.70-0.94)	0.83 (0.77-0.89)	0.69 (0.58-0.79)	0.78 (0.70-0.86)	0.57 (0.41-0.73)

Fig. 1 Analysis of CSF t-PrP concentrations in sporadic, iatrogenic and genetic prion diseases. **a** Whisker and boxplots of CSF t-PrP concentrations in non-primarily neurodegenerative neurological diseases (ND), sporadic Creutzfeldt-Jakob disease (sCJD), iatrogenic Creutzfeldt-Jakob disease (iCJD) and genetic prion diseases (gPD) associated with mutations in the *PRNP* gene E200K, V210I, D178N-M and P102L. Boxes indicate 25th to 75th percentiles and whiskers minimum to maximum values. Statistical significance was set at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. **b** Receiver operating characteristic curves for sCJD, iCJD and gPDs associated with mutations E200K, V210I, D178N-M and P102L versus the ND group are shown. Area under the curve (AUC) and 95% confidence interval (CI) are shown for t-PrP analysis

Genetic Tests

For detection of a prion disease-associated mutation and assessment of codon 129 polymorphism in *PRNP*, genetic testing was performed as described before [24].

Statistical Analysis

For two group comparisons of biomarker levels, non-parametric Mann-Whitney *U* tests were used. For comparisons between multiple groups, Kruskal-Wallis tests followed by Dunn's post hoc tests were applied. To assess the diagnostic accuracy of t-PrP, receiver operating characteristic (ROC) curve analyses were carried out and areas under the curve (AUC) with 95% confidence intervals (95% CI) were calculated. Spearman rank correlation coefficients were used to assess associations between continuous biomarker levels. Bootstrap one-tail tests for paired ROC curves based on the pROC-R package with boot replicates = 10,000 were used to assess differences in the diagnostic accuracy between biomarkers [25]. Longitudinal biomarker data were assessed using a multi-level mixed linear model. For the analysis of differences on t-PrP concentrations

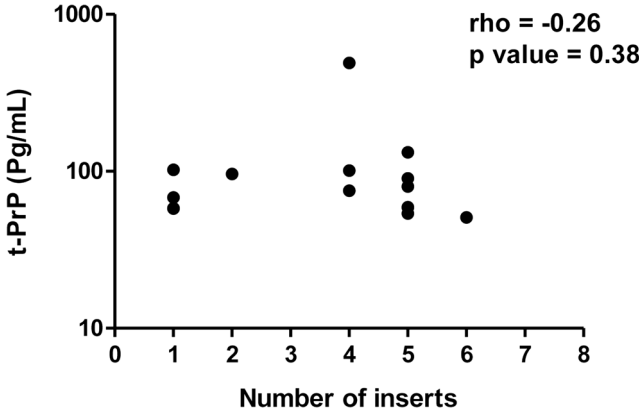


Fig. 2 Influence of the number of octapeptide repeat insertions (OPRI) in the prion protein gene (*PRNP*) on CSF t-PrP concentrations. Association analysis between t-PrP concentrations and number of OPRI in the *PRNP* gene. Spearman rank correlation coefficients were used

between different cohorts, only cohorts including enough cases to perform normality tests were used.

Data Availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

CSF t-PrP in Prion Disease of Different Aetiologies

CSF t-PrP concentrations were assessed in ND, sCJD, iCJD and gPD cases (Table 1). Compared to ND, mean t-PrP levels were lower in all types of prion diseases, with the exception of gPD associated with V180I, K194E and Y218N mutations (one case of each was available), and the R208H mutation (four cases available). Other mutations and variants also displayed similar concentrations to those reported in ND (P102L, P105L and N173K).

In order to assess if there were differences in t-PrP levels among diagnostic groups, t-PrP concentrations in ND, sCJD, iCJD and the four most prevalent forms of genetic prion diseases (E200K, V210I, D178N-M and P120L mutations) were further analysed in a multiple comparison test. Compared to ND, t-PrP was lower in sCJD ($p < 0.001$), iCJD ($p < 0.01$) and in gPD associated with E200K ($p < 0.001$), V210I ($p < 0.01$) and D178N-M mutations ($p < 0.001$), but not in P102L cases (Fig. 1a). Lowest t-PrP concentrations were found in iCJD (95 ± 71 ng/mL) and E200K (98 ± 77 ng/mL) (Table 1), but no statistical differences among different forms of prion diseases were detected.

AUCs for the discrimination of sCJD, iCJD and gPD E200K, V210I and D178N-M from ND ranged from 0.69 to 0.83, indicating moderate potential for a diagnostic test (Fig. 1b). In contrast, t-PrP levels showed no diagnostic value in distinguishing P102L from ND (AUC = 0.57, $p = 0.40$) (Fig. 1b).

CSF from gPD cases was obtained from different countries (see "Methods"). No differences were found when comparing t-PrP concentrations from different cohorts for the E200K, D178N-M and V210 mutations (Suppl. Fig. 1).

Established CSF prion disease biomarkers such as 14-3-3, tau [26, 27] and RT-QuIC [28] show a high diagnostic accuracy in the discrimination of non-prion disease cases from sCJD and gCJD E200K and V210I. However, they present limited value in the diagnosis of genetic prion disease associated with D178N-M. We recently reported elevated CSF NFL in D178N-M cases and showed moderate diagnostic potential for t-PrP in this analysis; therefore, we explored the ability of NFL/t-PrP ratio for improving the discrimination of D178N-M from ND. The AUC value in D178N-M cases using the NFL/t-PrP ratio (AUC = 0.97, 95% CI 0.95–0.99) was superior to that obtained by t-PrP only (AUC = 0.78, 95% CI 0.70–0.86, $p < 0.001$), but not relatively increased when compared to NFL alone (AUC = 0.96, 95% CI 0.93–0.98, $p = 0.2$).

The presence of octapeptide repeat insertions (OPRI) in the N-terminal region of the *PRNP* gene is linked with genetic prion diseases. High clinical and neuropathological heterogeneity in ORPI carriers is associated to the number of insert mutations (one to nine) [29–31] with the likelihood that low OPRI numbers are not pathogenic [32]. Mean t-PrP levels in cases with OPRI mutations were lower than in ND cases ($p = 0.003$) (Table 1). However, no association between CSF t-PrP levels and number of inserts was detected in our study population ($p = 0.38$) (Fig. 2).

Associations of Demographic and Genetic Parameters with t-PrP Concentrations

In prion diseases, CSF t-PrP levels were associated neither with age at onset ($p = 0.10$) (Fig. 3a) nor with sex ($p = 0.21$) (Fig. 3b). Lack of association with age and sex was also detected in the ND group (data not shown). CSF t-PrP concentrations were not statistically different between prion disease cases harbouring methionine/methionine [MM] (129 ± 107 ng/mL, $n = 219$), methionine/valine [MV] (114 ± 88 ng/mL, $n = 99$) and valine/valine [VV] (112 ± 85 ng/mL, $n = 59$) at codon 129 of the *PRNP* gene ($p = 0.58$) (Fig. 2c). Similarly, no differences were detected between different sCJD molecular subtypes in the subset of cases with neuropathological prion disease confirmation and available prion type (MM1/MV1 110 ± 80 pg/mL, $n = 55$; MV2 84 ± 61 pg/mL, $n = 6$; VV2 88 ± 77, $n = 16$) ($p = 0.16$) (Fig. 3d). To investigate whether t-PrP concentrations are associated with different pathological phenotypes among a mutation type, D178N-M and E200K cases were stratified according to their codon 129 *PRNP* genotype, which influences their clinico-

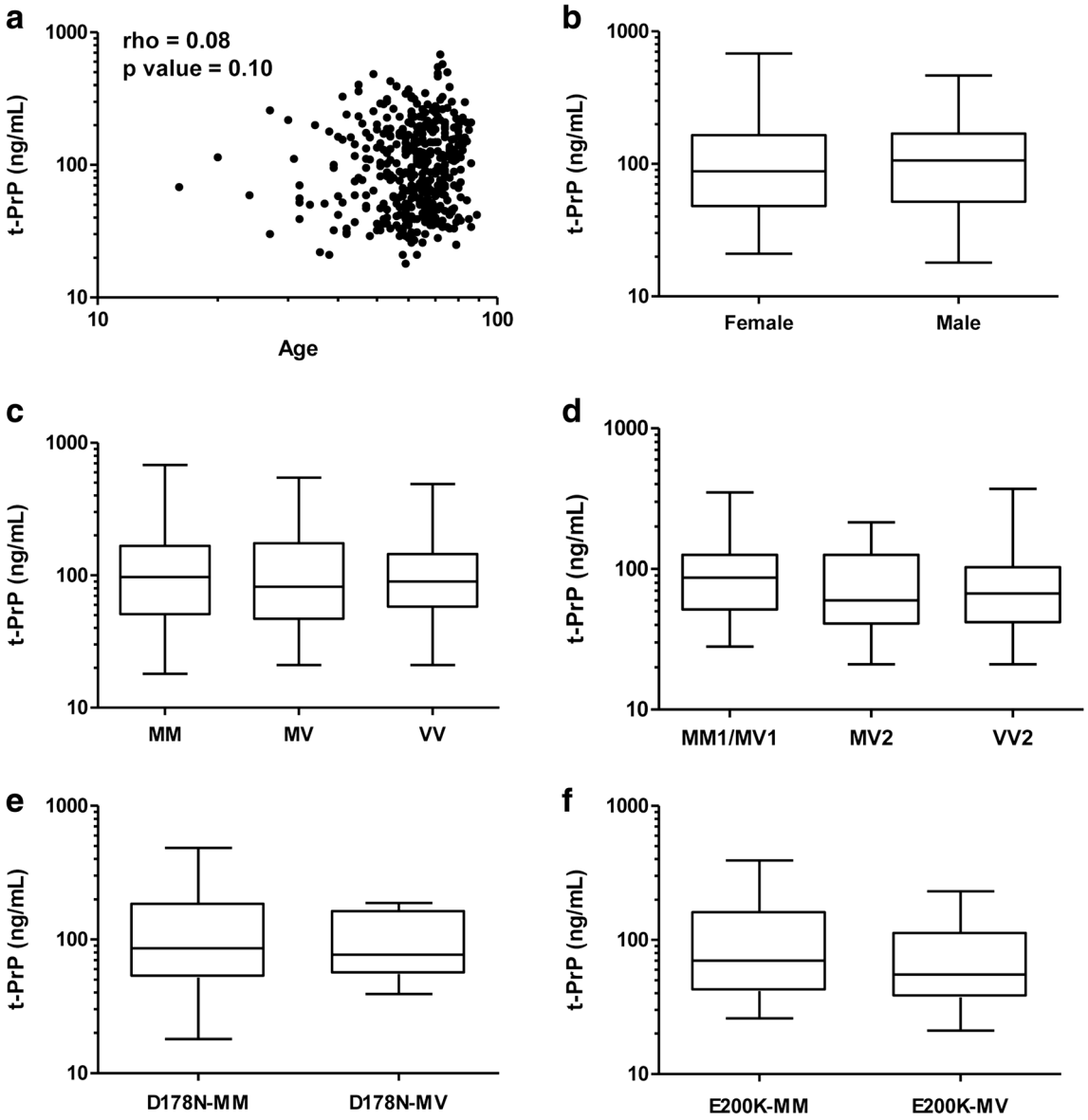


Fig. 3 Influence of demographic and PRNP codon 129 genetic factors on CSF t-PrP concentrations in prion diseases. **a** Association analysis between t-PrP concentrations and age at disease onset (in years) in all prion disease cases (sCJD, iCJD and gPD). Spearman rank correlation coefficients were used. **b** t-PrP in prion diseases stratified by sex. **c** t-PrP concentrations in prion diseases stratified by prion protein gene (*PRNP*) codon 129 polymorphism (M methionine, V valine) in probable and definite prion disease cases. **d** t-PrP concentrations in definite sCJD cases stratified by sCJD molecular subtypes. **e** t-PrP concentrations in D178-MM and MV cases. **f** t-PrP concentrations in E200K-MM and MV cases. Kruskal-Wallis test followed by Dunn's post hoc tests (correction for multiple testing) was applied for multiple comparisons and Mann-Whitney *U* test for two group comparisons

pathological features [33, 34]. t-PrP levels were neither different between D178-MM and -MV cases (Fig. 3e) nor between E200K-MM and -MV cases (Fig. 3f).

CSF t-PrP along Disease Stages

t-PrP levels were quantified in sequentially repeated lumbar punctures (LPs) obtained from 20 sCJD cases (2 LPs available in 19 cases and 3 LPs available in 1 case). To normalise time intervals between LPs, samples were grouped into three categories according to whether they underwent LP in the first (time of LP to disease onset/total duration of the disease < 0.33), second (0.33–0.66) or third (> 0.66) stage of the disease, as previously reported [35, 36]. In 15 LPs, t-PrP concentrations were lower in the follow-up LP compared to the initial estimate, while in 5 LPs, t-PrP concentrations were higher at advanced disease stages (Fig. 4). Using a multi-level mixed linear model, a decrease in t-PrP of 24.5 per unit in disease stages was calculated (95% CI 12.8–48.8, $p = 0.005$).

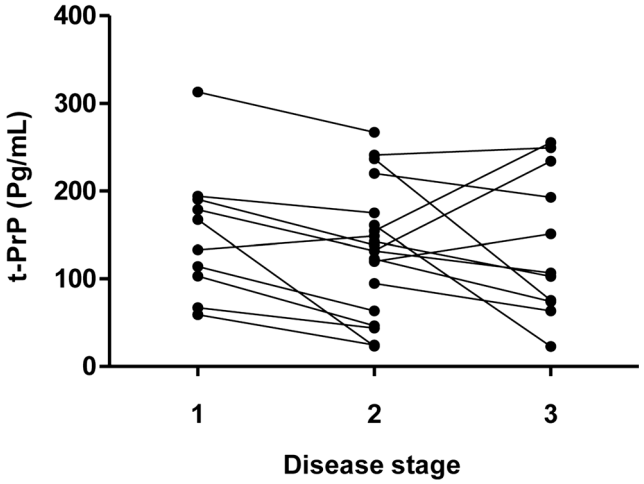


Fig. 4 Association between CSF t-PrP levels and disease duration in sCJD patients. t-PrP concentrations in serial lumbar punctures (LPs) in sCJD cases at different stages of the disease. Samples were grouped in three categories according to whether they underwent LP in the first (< 0.33), second (0.33–0.66) or third (> 0.66) stage of an individual's disease

CSF in Pre-Clinical PRNP Mutation Carriers

t-PrP concentrations were analysed in a subset of asymptomatic *PRNP* mutation carriers from the UCSF cohort and were descriptively compared with symptomatic cases from the same cohort (symptomatic, UCSF), with the whole population of cases included in the present study (symptomatic, ALL) and with the ND cases (Suppl. Fig. 2). For some cases, serial LPs from the mutation carriers were available.

In E200K, pre-symptomatic carriers (17 LPs from 14 cases) displayed t-PrP concentrations similar to ND controls (Suppl. Fig. 2) and higher than those detected in symptomatic cases in the UCSF cohort (1 case) and in the whole cohort of E200K patients (Fig. 5a). In D178N-M cases, pre-symptomatic carriers showed similar levels like symptomatic D178N-M patients from the UCSF and the whole D178N-M cohort, although only six LPs from three individuals were available (Fig. 5b), limiting any meaningful conclusions. In P102L carriers, t-PrP concentrations were similar between pre-symptomatic (two cases available) and symptomatic patients, in agreement with the absence of alterations between ND and symptomatic cases described above (Fig. 5c).

Discussion

In this study, we report the comparative signatures of CSF t-PrP concentrations across the spectrum of prion diseases. While decreased t-PrP concentrations are well-reported in sCJD cases [13–16, 18], data on genetic and iatrogenic cases have hitherto been quite limited and in most of the cases, restricted to single case reports. Our study validates previous observations of decreased CSF t-PrP in sCJD cases compared to controls and other non-neurodegenerative neurological conditions evaluated by ELISA [13–16,] and by immunoblotting techniques [18]. Additionally, although the total number of samples was relatively low, our data clearly point to the presence of reduced t-PrP concentrations in iCJD.

Low t-PrP concentrations were observed in genetic prion diseases associated with mutations E200K, D178N-M and V210I, but not in P102L cases supporting that among gCJD and GSS-S-associated mutations, t-PrP levels were not homogeneous. Genetic CJD patients with V180I, K194E and R208H mutations showed normal t-PrP concentrations, while as did GSS-S patients with P102L, P105L and Y218N mutations. The rest of the mutations displayed decreased or intermediate t-PrP levels. In this regard, well-known disease causing mutations such as A117V, P102L, E200K and D178N-M [37] showed diverse t-PrP concentrations, while some mutations with no strong evidence of increased risk for developing the disease (e.g. A133V, V176G, I215V, P238S) displayed reduced t-PrP levels. Although these results should be interpreted with caution due to the low number of cases (statistical analysis was carried out for the most prevalent forms only), our data suggest the presence of highly heterogeneous profiles, which are not associated with disease phenotype. Indeed, D178N-M cases, displaying a specific clinicopathological phenotype compared to sCJD, iCJD and gCJD E200K and V210I, presented low t-PrP concentrations. Additionally, no differences on t-PrP levels were detected between D178N-MM and -MV cases, although both groups show distinct clinical features and neuropathological profiles [33, 38]. Moreover, similar t-PrP levels were detected between E200K-MM and -MV cases, despite the presence of different types of PrP depositions in the brain tissue, with a synaptic pattern for MM subjects and granules and plaque-like structures for MV subjects [34].

Overall, the analysis of CSF signatures demonstrated high variation in t-PrP concentrations across the diagnostic groups. This, together with a moderate diagnostic accuracy in discriminating prion disease cases from ND controls (AUC of 0.76 for sCJD), argues against the use of t-PrP quantification alone as a diagnostic biomarker for prion diseases in clinical practice. However, the tentative decrease of t-PrP concentrations along disease duration observed in serial lumbar punctures from the same patients indicates that, for a given case, longitudinal alterations on t-PrP levels may have a potential role in the evaluation of disease progression, as well as in the evaluation of the efficacy of a potential therapeutic intervention. In this regard, other CSF prion biomarkers such as tau, alpha-synuclein and NFL have been able to predict disease duration [36, 39, 40], but show stable concentrations along disease progression [35, 36, 40]. These markers reflect pathological alterations associated with the neurodegeneration process such as neuro-axonal degeneration and white matter involvement. In contrast, decreased t-PrP levels may reflect primary alterations in the molecular process

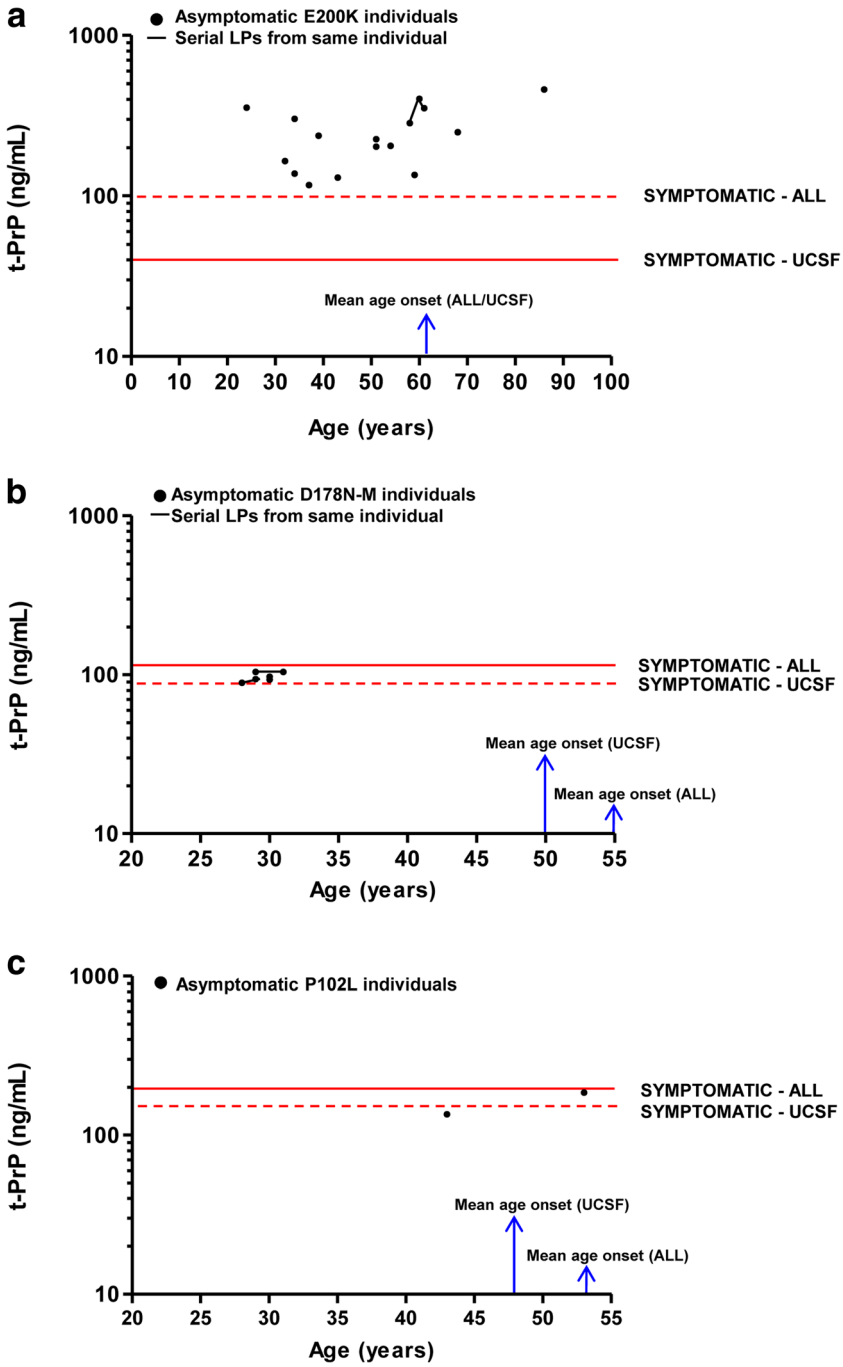


Fig. 5 CSF t-PrP concentrations in asymptomatic genetic prion diseases. CSF t-PrP concentrations and age of LP in pre-symptomatic *PRNP* mutation carries for the E200K (**a**), D178N-M (**b**) and P102L (**c**) mutations from the UCSF cohort. Black spots indicate a LP. Black spots connected with a black line indicating serial LPs from the same patient. Red lines indicate mean t-PrP concentrations for symptomatic cases from all the cases analysed in the present study (symptomatic, ALL). Dashed red lines indicate mean t-PrP concentrations for symptomatic cases from the UCSF cohort (symptomatic, UCSF). Mean age at onset from UCSF cohort and from all cases are indicated with a blue arrow

associated with the formation of prions. Broadly similar to amyloid-beta42 levels in AD, the decrease of CSF t-PrP concentrations in prion cases is speculated to be a consequence of the misfolding of the cellular prion protein (PrPc) into PrPsc with consequent "trapping" of the protein in aggregates, limiting the amount of soluble PrP filtering to the CSF. Based on this hypothesis, it could be assumed that CSF t-PrP levels would be highly dependent on prion disease aetiology and sCJD subtypes, which differ with respect to the neuropathological profiles and type of PrPsc aggregates [16, 21, 34]. For instance, in D178N-M cases, CSF t-PrP concentrations are decreased despite the reduced levels of PrPsc in the brain parenchyma, which is usually only detectable in the entorhinal cortex and in some cases with CJD-type alterations in the deep regions of the temporal cortex. Therefore, the absence of differences in CSF t-PrP concentrations between prion diseases displaying different types of PrP brain aggregates indicates that additional factors appear to explain the singular signatures of CSF t-PrP across the spectrum of prion diseases. Interestingly, decreased PrP expression was found in the two most prevalent sCJD subtypes (MM1 and VV2) [16],

as well as in the thalamus and entorhinal cortex of D178N-M cases [41] causing a reduction of PrP levels in the brain, and potentially in the CSF. Moreover, the decrease in CSF t-PrP could be associated with a decrease in proteinase-sensitive intermediate PrP isoforms not detected by the ELISA t-PrP assay.

In pre-symptomatic carriers of the D178N-M mutation, t-PrP concentrations were similar to those detected in symptomatic cases and lower than those in ND. This indicates that, at least in D178N-M, t-PrP could be a potential pre-clinical biomarker of the pathology. In contrast, pre-symptomatic E200K carriers harboured t-PrP higher than in clinical cases and similar to values measured in NDs. Although the low number of cases available impedes a statistical evaluation and, therefore, results remain descriptive, several aspects should be underlined. First, the presence of reduced t-PrP concentrations is not a general observation for all mutations. Whether these results are associated with mutation-specific disease duration, low number of cases, data dispersion and/or confounders such as heterogeneity of age at onset needs to be further explored in larger cohorts. Therefore, we cannot exclude t-PrP as a potential pre-clinical biomarker for other mutation types or prion disease types. Second, despite the absence of studies addressing the quantification of t-PrP levels in pre-symptomatic cases, we recently detected lower t-PrP concentrations in the CSF of pre-clinical and clinical naturally occurring scrapie [42], which would support the idea that reduced t-PrP concentrations may happen at pre-clinical and early prion disease stages.

Conclusions

Herein, we report the largest and most complete study of the levels of CSF t-PrP across the spectrum of prion diseases, including multiple cases of gPD associated with a broad range of *PRNP* mutations. We have confirmed previous data demonstrating reduced CSF t-PrP in sCJD.

Although the diagnostic potential of CSF t-PrP alone is perhaps limited, its combination with other biomarkers may heighten its utility. Importantly, the observed CSF t-PrP decline along disease progression in sCJD and in pre-symptomatic carriers of certain mutations such as D178N-M cases deserves further investigation towards potential translational application in clinical practice.

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Authors' Contributions AV-P, MS, IZ and FL designed the study. AV-P, MS and FL performed experiments. AV-P, MS, AK, IZ and FL analysed data and interpreted the results. IL, OC, CS, SS, AL, AP, IS, IF, EM, D.Z, MP, IB, MC, SJC, MDG, RS-V and IZ contributed to samples and/or technical expertise. FL and AV-P wrote the manuscript draft. All authors critically revised the manuscript and approved its content before submission.

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Compliance with Ethical Standards

Ethics Approval and Consent to Participate The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines, and was approved by all local Ethics committees. All study participants or their legal guardians provided written informed consent.

Consent for Publication Not applicable.

Competing Interests Dr. Lachmann reports he is a representative of AJ Roboscreen GmbH, Leipzig, Germany.

Abbreviations GSS-S, Gerstmann–Sträussler–Scheinker syndrome; ROC, Receiver operating characteristic; OPRI, Octapeptide repeat insertion; PRNP, Prion protein gene; gCJD, Genetic Creutzfeldt-Jakob disease; iCJD, Iatrogenic Creutzfeldt-Jakob disease; vCJD, Variant Creutzfeldt-Jakob disease; FFI, Fatal Familial Insomnia; NFL, Neurofilament light; PrPscz, PrPsc: Prion protein scrapie; LP, Lumbar puncture; AUC, Area under the curve; sCJD, Sporadic Creutzfeldt-Jakob disease; CSF, Cerebrospinal fluid; ELISA, Enzyme-linked immunosorbent assays; ND, Neurological diseases; t-PrP, Total prion protein

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