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SHORT COMMUNICATION

Plasma neurofilament light chain as a biomarker for fatal familial insomnia

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Abstract

Background and purpose: Fatal familial insomnia is a rare hereditary prion disease associated with the D178N-129M *PRNP* mutation. Early diagnosis is difficult, because the clinical syndrome may overlap with affective disorders. In addition, most known cerebrospinal fluid biomarkers for prion diseases and magnetic resonance imaging do not show a good diagnostic accuracy for fatal familial insomnia. In this context, data on plasma biomarkers are scarce.

Methods: We analyzed levels of neurofilament light chain, glial fibrillary acidic protein, chitinase-3-like protein 1, calcium-binding protein B, and total tau protein in six serial plasma samples from a patient with fatal familial insomnia. Subsequently, plasma neurofilament light chain was analyzed in $n = 25$ patients and $n = 19$ controls. The diagnostic accuracy and associations with disease stage and duration were explored.

Results: Among all biomarker candidates in the case study, only neurofilament light chain levels showed a constant evolution and increased over time. They discriminated fatal familial insomnia from controls with an area under the curve of 0.992 (95% confidence interval [CI] = 0.974–1) in the case–control study. Higher concentrations were associated with methionine homozygosity at codon 129 *PRNP* ($p = 0.006$), shorter total disease duration (rho = −0.467, $p = 0.019$, 95% CI = −0.790 to −0.015), and shorter time from sampling to death (rho = −0.467, $p = 0.019$, 95% CI = −0.773 to −0.019).

Conclusions: Plasma neurofilament light chain may be a valuable minimally invasive diagnostic biomarker for fatal familial insomnia after clinical onset. Most important, stage-related increase and association with disease duration indicate potential as a prognostic marker and as a surrogate marker in clinical trials.

KEYWORDS
biomarker, fatal familial insomnia, neurofilament light chain, plasma, prion disease

INTRODUCTION

Fatal familial insomnia (FFI) is a rare hereditary prion disease associated with the D178N-129M *PRNP* mutation, primarily thalamic pathologic changes, and fatal outcome. The survival time is influenced by the polymorphism of methionine/valine (M/V) at codon 129 *PRNP* [1]. Early FFI is characterized by sleep disorder, nonspecific psychiatric, and vegetative symptoms [2]. Thus, early diagnosis may

be difficult, even in known mutation carriers. Unfortunately, cerebrospinal fluid (CSF) and imaging biomarkers for sporadic Creutzfeldt–Jakob disease (sCJD) [3] do not perform well in the diagnosis of FFI [4]. The only sensitive diagnostic marker is thalamic hypometabolism in positron emission tomography [5], a laborious method. Minimally invasive, blood-based biomarkers are needed for early diagnosis and trial monitoring. Candidates include markers for neuroaxonal injury such as total tau and neurofilament light chain (NfL), as well as glial markers. Some of these proteins were shown to be of diagnostic value when measured in plasma of patients with sCJD [6,7] and some genetic prion diseases [8]. Data regarding FFI are scarce.

We analyzed plasma samples from a patient with FFI at various time points before and after disease onset to demonstrate the evolution of candidate biomarker proteins. Subsequently, we analyzed plasma samples from a historical cohort of FFI patients to evaluate the diagnostic accuracy and potential prognostic utility of plasma NfL, a neuron-specific protein that is able to indicate neuroaxonal injury in neurodegenerative diseases [9].

METHODS AND MATERIALS

FFI and control patients

A patient with D178N *PRNP* mutation was followed from 3 years before to 8 months after FFI onset in the outpatient clinic of the Göttingen University Medical Center. Six consecutive plasma samples were analyzed for NfL, glial fibrillary acidic protein (GFAP), chitinase-3-like protein 1 (YKL-40), calcium-binding protein B (S100B), and total tau.

Subsequently, patients with prion disease and D178N *PRNP* mutation were selected from the biobank of the German National Reference Center for Transmissible Spongiform Encephalopathies when plasma samples and clinical data were available ($n = 28$), excluding the patient from the case study. Healthy carriers of D178N ($n = 5$), P102L ($n = 2$), and 120 base pair insert ($n = 2$) *PRNP* mutation (HMC), as well as patients with neuropsychiatric disorders (depression, $n = 7$; psychosis, $n = 1$, polyneuropathy, $n = 2$), in whom FFI or other neurodegenerative disorders had been considered during the diagnostic process (neuropsychiatric controls [NPC]), were assigned as controls.

Biomarker analyses

Plasma NfL, total tau, and GFAP levels were measured with SIMOA assays (Quanterix). MicroVueYKL-40 EIA ELISA kit from Quidel was used for quantification of YKL-40 and LIAISON SANGTEC 100 for S100B. Manufacturers' instructions were followed. Test performers were blind to clinical information and clinical investigators to test performance.

Statistical methods

Comparisons were performed with multivariate linear regression models, including age and sex, followed by Tukey contrasts for multiple comparisons. Data were normalized by log transformation. Receiver operating characteristic (ROC) curves and area under the curve (AUC) with 95% confidence intervals (CIs) were calculated. Associations of NfL levels were explored with Spearman correlation. SPSS and *R* multComp were used for analyses and Excel for graphic items. Statistical significance was considered at $p < 0.05$.

Ethical standards

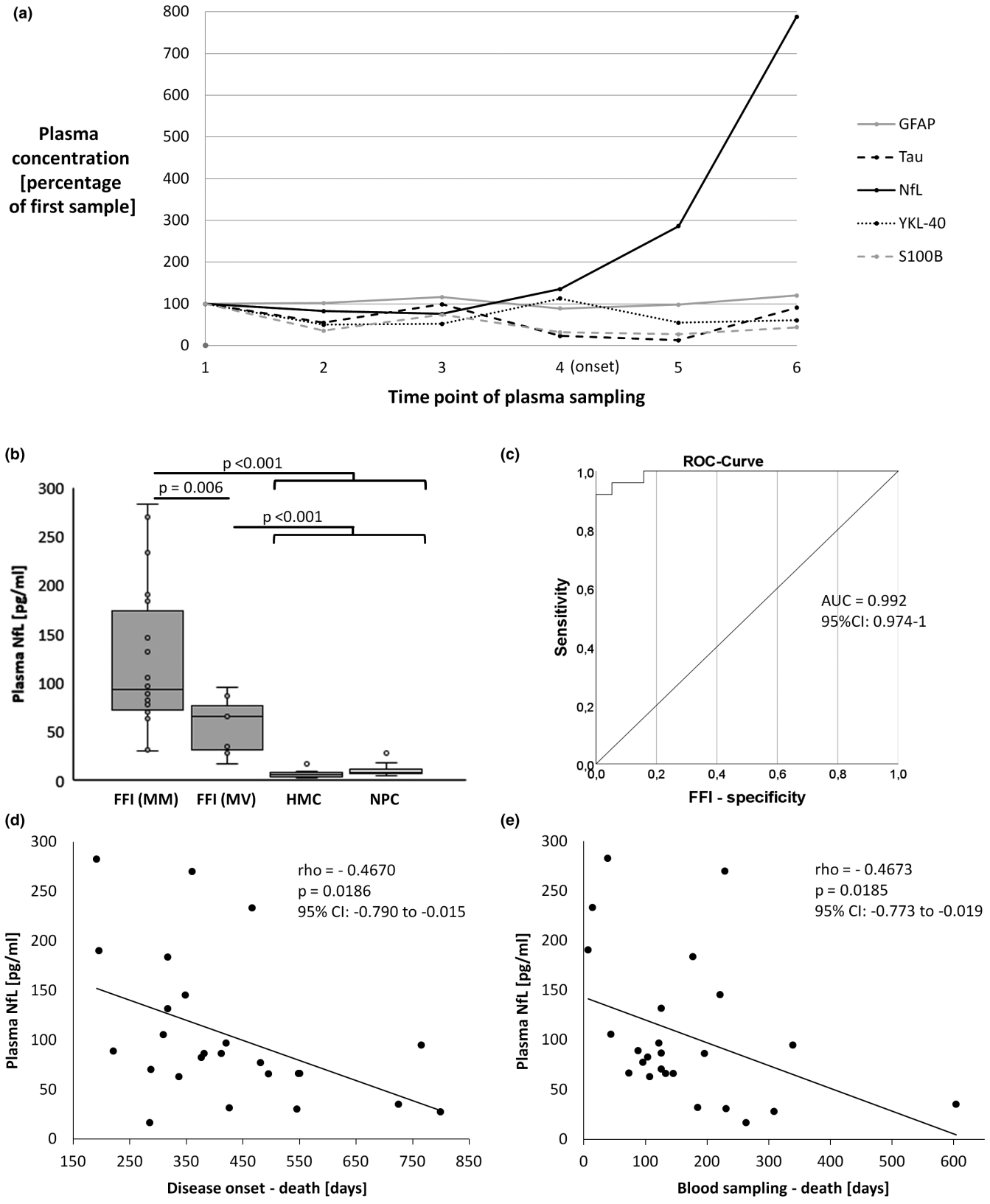
The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines, approved by the Göttingen University Medical Center ethics committee (No. 11/11/93). All patients or their legal representatives gave informed consent.

RESULTS

Case report and evaluation of biomarker candidates

Longitudinal evaluation of plasma NfL, GFAP, YKL-40, S100B, and total tau in a D178N (codon 129: MM) mutation carrier revealed that only NfL concentrations showed a stage-related increase. We obtained plasma close to the exact point of clinical onset. The patient

FIGURE 1 Evolution of plasma biomarker candidates in a case of fatal familial insomnia (FFI). (a) Development of plasma biomarker levels (percentage) in relation to Time Point 1 (100%). Time Point 1: 3 years before onset; Time Point 2: 2 years before onset; Time Point 3: 1 year before onset; Time Point 4: clinical onset (very mild sleep disturbance); Time Point 5: 4 months after onset (insomnia, hyperhidrosis, depressive mood); Time Point 6: 8 months after onset (mild dementia, ataxia, upper limb rigor). GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; S100B, calcium-binding protein B; tau, total Tau protein; YKL-40, chitinase-3-like protein 1. (b) Box plots displaying NfL concentrations in FFI MM, FFI MV, and control patients. To improve readability, logarithmic scale was chosen for the y-axis. HMC, healthy *PRNP* mutation carriers; MM, methionine homozygosity at codon 129 *PRNP*; MV, methionine/valine heterozygosity at codon 129 *PRNP*; NPC, neuropsychiatric controls. Bars and *p*-values above boxes indicate *p*-values from linear regression and Tukey contrasts. (c) Receiver operation characteristic (ROC) curve and area under the curve (AUC) with 95% confidence intervals (CIs) in the discrimination of FFI patients and all controls (HMC plus NPC). (d) Scatter plot displaying association between plasma NfL (pg/ml) and time from disease onset to death (days). Spearman coefficients (rho), 95% CIs, and corresponding *p*-values are indicated. (e) Scatter plot displaying association between plasma Nfl (pg/ml) and time from blood uptake to death (days). Spearman coefficients (rho), 95% CI, and corresponding *p*-values are indicated



visited the outpatient clinic for a routine appointment and reported very mild sleep disturbance during the prior week. No further symptoms were present. The NfL level was almost twice as high (9.8 pg/ml) as 1 year before (5.49 pg/ml). At follow-up (4 months later; the patient showed insomnia, vegetative, and affective symptoms but no focal neurological signs or dementia), NfL levels had increased further (20.75 pg/ml) and peaked in the last sample (57.15 pg/ml) 8 months after onset (Figure 1a, Table S1).

Diagnostic accuracy of NfL and association with disease duration of FFI

From $n = 28$ patients with D178N *PRNP* mutation, $n = 25$ showed an FFI phenotype and three presented with a CJD syndrome. Among FFI cases, $n = 18$ were homozygous for methionine at codon 129 *PRNP* (MM, mean = 124.35 pg/ml, SD = ±73.21) and $n = 7$ were heterozygous (MV, mean = 52.19 pg/ml, SD = ±27.85). Patients with CJD syndrome showed lower NfL concentrations (63.49 pg/ml, SD = ±30.63) than FFI patients (102.15 pg/ml, SD = ±71.95) but were excluded from further analyses due to the low case number. Mean NfL levels were similar in HMC with D178N mutation (6.53 pg/ml, SD = ±2.07), other *PRNP* mutations (7.28 pg/ml, SD = ±6.81), and NPC (10.88 pg/ml, SD = ±7.26; Table 1).

A pooled control group (HMC and NPC) was used for comparison models and ROC analyses. FFI-MV patients showed longer total disease duration ($p = 0.023$) and lower NfL concentrations ($p = 0.006$) than FFI-MM patients (Figure 1b). Both FFI groups showed higher NfL than controls ($p < 0.001$). ROC analyses displayed an AUC of 0.992 (95% CI = 0.974–1) in the discrimination of all FFI patients from controls (Figure 1c). Regarding subgroups, NfL discriminated FFI-MM with an AUC of 1 (95% CI = 1–1) and FFI-MV with an AUC of 0.970 (95% CI = 0.912–1) from controls.

Samples from patients in the first disease tertial showed low NfL mean concentrations (25.90 pg/ml, SD = ±13.12) compared to the second (105.65 pg/ml, SD = ±73.89) and third (118.14 pg/ml, SD = ±74.66) tertials. Due to the low case number in the first group ($n = 2$), statistical comparisons were not performed (Table 1). Spearman calculations showed significant negative correlations between NfL and total disease duration (rho = −0.4670, $p = 0.0186$, 95% CI = −0.790 to −0.015; Figure 1d), as well as time from blood sampling to death (rho = −0.4673, $p = 0.0185$, 95% CI = −0.773 to −0.019) in FFI cases (Figure 1e), but no significant correlation between NfL and time from onset to blood sampling (rho = −0.214, $p = 0.305$, 95% CI = −0.677 to 0.264) was observed.

DISCUSSION

Most prion disease CSF biomarkers and magnetic resonance imaging show low diagnostic accuracy in FFI [4]. In contrast, CSF NfL was reported to have good diagnostic accuracy for prion diseases with the D178N *PRNP* mutation [10] and is also elevated in sporadic fatal insomnia [11]. Previous studies on plasma biomarkers for prion diseases included only few individuals with FFI. Nonetheless, probable elevation of plasma NfL was reported in FFI [6,8] but not in healthy D178N *PRNP* mutation carriers [12]. In our FFI patient, NfL increased over time, whereas other biomarker candidates showed no clear tendency. Although, for example, total tau showed a decrease during the observation time, we did not consider this alteration as relevant, because the course offers no clear tendency and all concentrations were at a generally low level.

Our retrospective study showed a significant elevation of plasma NfL in symptomatic FFI patients. Although NfL was significantly higher in FFI-MM than in FFI-MV, an excellent discriminatory value versus controls could be observed for both groups, indicating a high potential value of plasma NfL as a diagnostic biomarker. Additionally, we validated a longer disease duration in FFI-MV patients compared to FFI-MM patients [1]. Higher NfL may be associated with faster disease progression in the FFI-MM group. Control groups with other encephalopathic syndromes were not available. However, compared to previous investigations, mean concentration in FFI (102.15 pg/ml)

TABLE 1 Demographic and NfL data in the study cohort

Group	*n*	Sex, female/male	Age, median (min–max)	Disease duration, median days (min–max)	Plasma NfL, mean pg/ml ± SD
Controls	19	13/6	59 (32–73)	–	8.97 ± 6.27
D178N *PRNP* mutation	5	4/1	49 (32–70)	–	6.53 ± 2.07
Other *PRNP* mutations	4	3/1	48 (36–60)	–	7.28 ± 6.81
Other disorders	10	6/4	63 (48–73)	–	10.88 ± 7.26
Cases [D178N-FFI]	25	9/16	53 (24–70)	381 (178–765)	102.15 ± 71.95
FFI-MM	18	7/11	51 (24–65)	343 (178–550)[a]	124.35 ± 73.21
FFI-MV	7	2/5	67 (43–70)	495 (285–765)[a]	52.19 ± 27.85
First disease tertial	2	1/1	– (52–70)	– (285–679)	25.90 ± 13.12
Second disease tertial	11	5/6	46 (24–69)	391 (226–765)	105.65 ± 73.89
Third disease tertial	12	3/9	57 (27–70)	370 (178–550)	118.14 ± 74.66
Cases [D178N-gCJD][b]	3	1/2	57 (52–60)	654 (453–661)	63.49 ± 30.63

Note: Total disease duration was divided into three time spans (tertials), and patients were assigned according to time of blood sampling.

Abbreviations: FFI, fatal familial insomnia; gCJD, genetic Creutzfeldt–Jakob disease; M, methionine at codon 129 *PRNP*; min–max, minimum–maximum; NfL, neurofilament light chain; V, valine at codon 129 *PRNP*.

[a]Comparison of median disease duration with Mann–Whitney *U* test showed longer duration in FFI-MV (U:25, *z*-score = −2.270, $p = 0.023$).

[b]In gCJD, two patients had codon 129 MM genotype (NfL = 97.97 and 36.88 pg/ml) and one patient had codon 129 MV genotype (NfL = 56.63 pg/ml).

was lower than what had been shown for sCJD (349.7 pg/ml) and nonneurodegenerative encephalopathies (182.2 pg/ml). In contrast, it was two- to threefold higher than in Alzheimer disease (34.9 pg/ml) and other dementia syndromes [7].

Most strikingly, the current observation shows significant association of plasma NfL with FFI stage (time from sampling to death) and duration, in line with observations in other neurodegenerative disorders [13]. To our knowledge, we investigated the largest symptomatic cohort with D178N *PRNP* mutation regarding plasma biomarkers. On the other hand, the cohort was still too small to perform certain subgroup analyses. Only two samples in the retrospective study were from the first disease tertial. Those showed higher NfL concentrations than HMC but still lower levels than FFI patients in later stages. Unfortunately, the case study could not show apparent elevation of NfL prior to disease onset. Thus, fluorodeoxyglucose positron emission tomography may have superior sensitivity in preclinical and very early clinical stages [5].

As a marker of neuroaxonal damage, plasma NfL is elevated in various neurologic diseases [9] and its specificity depends on the diagnostic context. Regarding FFI, affective disorders that may cause behavioral changes or sleeping disturbance are important differential diagnoses in early disease stages. Our control group was chosen accordingly. Individuals from families with known genetic prion diseases have a high risk for such disorders, because they suffer from higher stress and anxiety levels than controls [14]. In this context, plasma NfL may be useful for diagnosis of disease manifestation or as an individual screening test before positron emission tomography is applied. Most importantly, the stage-related increase and the strong association with survival time corroborate the potential role of plasma NfL as an urgently needed surrogate marker [15] in clinical trials for genetic prion diseases. The prognostic potential of plasma NfL in FFI may be improved by consideration of the codon 129 genotype. Here, we performed an exploratory evaluation in a small cohort. Larger investigations using international cohorts are needed to characterize the biomarker further.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Peter Hermann: Conceptualization (equal), formal analysis (lead), investigation (equal), writing–original draft (lead). **Sezgi Canaslan:** Investigation (equal), writing–review & editing (equal). **Anna Villar-Piqué:** Investigation (equal), writing–review & editing (equal). **Timothy Bunck:** Formal analysis (supporting), investigation (equal), writing–review & editing (equal). **Stefan Goebel:** Investigation (equal), writing–review & editing (equal). **Franc Llorens:** Investigation (equal), writing–review & editing (equal). **Matthias Schmitz:** Investigation (equal), writing–review & editing (equal). **Inga Zerr:** Conceptualization (equal), formal analysis (supporting), supervision (lead), writing–review & editing (equal).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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