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Investigation of serum neurofilament light chain as a biomarker in Fabry disease

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Fabry disease (FD) constitutes a rare, X-linked lysosomal storage disorder affecting multiple organ systems, most notably heart, kidneys, and the central nervous system. Neurofilament light chains (NfL) have emerged as a prime candidate for a body fluid biomarker reflecting neuro-axonal injury. We aimed to evaluate its addition to the diagnostic and monitoring armamentarium in FD. Serum NfL concentrations (sNfL) were measured in 50 people with FD (PwFD) and 30 healthy control subjects (HC) using the Simoa© technology, followed by calculation of Z-scores adjusted for age and body mass index. In addition, clinical disease severity in PwFD was measured using the FOS-MSSI (Fabry outcome study – Mainz severity score index), which comprises clinical and paraclinical parameters. PwFD show elevated sNfL Z-scores compared to HC (PwFD: 1.12 [SD 1.5], HC: 0.01 [SD 1.2], $p < 0.001$). In PwFD, males showed higher sNfL Z-scores than females (1.75 [SD 1.5] vs. 0.73 [SD 1.4]). Importantly, sNfL Z-scores were increased in PwFD with ischemic white matter lesions of the CNS (1.5, SD 2.2 vs. 0.5, SD 2.9, $p = 0.03$). In our small cohort, sNfL Z-scores correlated fairly with FOS-MSSI (Kendall's-Tau [τ] = 0.25, $p = 0.01$), and, interestingly with serum creatinine (τ = 0.28, $p = 0.005$) and estimated glomerular filtration rate (eGFR, τ = -0.28, $p = 0.005$). Based on these exploratory results, sNfL might provide value as a biomarker of neuroaxonal damage in FD, possibly reflecting cerebrovascular injury. Additionally, the correlation of sNfL with renal function warrants further investigation.

Keywords Fabry disease, Neurofilament light, Glomerular filtration rate, Biomarkers, Cerebrovascular disorders

Fabry disease (FD; OMIM# 301500) is a complex, X-linked lysosomal storage disorder affecting multiple organ systems along a highly variable phenotypic spectrum. Variants in the *GLA* gene on Xq22.1 causing deficiency of the hydroxylase α-galactosidase A result in the accumulation of sphingolipids and their metabolites, mainly globotriaosylceramide (Gb-3) and globotriaosylsphingosine (Lyso-Gb_3). This, in turn, induces metabolic dysfunction and ultimately damage to vasculature, central and peripheral nervous system (PNS, CNS), heart and kidneys, among other organ systems[1]. Despite the X-linked inheritance, the disease burden in females reaches from asymptomatic to severe affection comparable to males, a fact that is attributed to skewed X-inactivation, the mechanism of which is subject of ongoing debate[2,3]. The incidence of FD was previously reported to be 1:117 000 [4], whereas more recent accounts from a European new born screening suggest a more common incidence of α-galactosidase deficiency of about 1:8000 [5].

The main neurological disease manifestations include cerebrovascular pathology with stroke, as well as peripheral neuropathy including small-fiber neuropathy[7]. Additionally, studies hinted on FD-patients being increasingly affected by microstructural white matter changes, neurodegenerative changes, as well as cognitive dysfunction and depression[8–10].

After establishing the diagnosis, two disease-specific forms of therapy are available: (i) three different formulations of intravenous enzyme replacement therapy (ERT) and (ii) an oral chaperone therapy. Both have shown efficacy in reducing disease burden and improving quality of life, as well as prevention of disease progression with stabilization of organ dysfunction and enhancement of lifespan[11–15].

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In PwFD, regular follow-up appointments including blood tests, imaging, ultrasound and clinical examinations are required to guarantee standard of care in the outpatient setting[22].

Neurofilament light-chain (NfL), an intermediary filament predominately expressed in myelinated axons and responsible for axonal diameter[16,17], has been established as a valuable diagnostic and prognostic tool in many neurological diseases in recent years[18]. The advent of fourth-generation detection assays now allows reliable detection of NfL in peripheral blood (serum or plasma), thus markedly facilitating sample acquisition. Large studies have looked at the distribution of NfL-concentrations in healthy individuals and suggested reference values partitioned for age and body mass index, an important milestone in establishing this biomarker for routine patient care[19,20]. Additionally, standardized Z-scores adjusted for age and body mass index have been published[21].

This study aimed to investigate the possible value of adding the biomarker sNfL to the diagnostic and monitoring armamentarium in the assessment of neurological disease manifestations in FD. We hypothesized that sNfL are increased in PwFD due to depositions of sphingolipids in all tissues, including peripheral and central nervous system.

Results

We analyzed the serum neurofilament light-chain concentration (sNfL) in 50 people with Fabry Disease (PwFD) and 30 healthy controls. Table 1 provides detailed information of both cohorts. The distribution of *GLA*-variants is shown in Fig. 1.

PwFD showed a higher mean sNfL Z-score (1.12, standard deviation [SD] 1.5) compared to healthy controls (0.01, SD 1.2, $p < 0.001$, Table 2; Fig. 2A).

In PwFD, males (1.75, SD 1.5) showed higher sNfL Z-scores than females (0.73, SD 1.4, $p = 0.022$), whereas there was no significant sNfL sex-difference in controls ($p = 0.497$, Fig. 2A; Table 2).

Severity of FD as measured by FOS-MSSI showed a fair positive correlation with sNfL Z-scores ($\tau = 0.25$, $p = 0.01$, Fig. 2D). The difference of sNfL Z-scores in PwFD with a neurological subscore of the FOS-MSSI-Neuro ≥ 1 and FOS-MSSI-Neuro $= 0$ was not significant. Comparison of presence and absence of ischemic white matter lesions (iWML) in MRI (according to a cerebrovascular score ≥ 3) revealed significantly elevated sNfL absolute values. PwFD with iWML ($n = 32$) were significantly older than PwFD without iWML ($n = 18$, $p = 0.005$, Table 3). Irrespective of this age-distribution with iWML predominance in older PwFD, however, sNFL Z-scores in PwFD with and without iWML remained significantly increased (1.5, SD 2.2 vs. 0.5, SD 2.9, $p = 0.03$, Fig. 2B; Table 4). The difference of sNfL Z-scores in PwFD without iWML and HC was non-significant (0.5, SD 2.9 vs. 0.01, SD 1.2, $p = 0.34$).

Lyso-Gb_3 concentrations were higher in males (13.9 ng/mL, IQR 18.1) than in females (3.4 ng/mL, IQR 3.5, $p < 0.001$). Sex-specific analysis yielded no significant correlation of Lyso-Gb3 with sNfL (females: $\tau = 0.10$, p-Value $= 0.424$; males: $\tau = 0.22$, p-Value $= 0.183$).

While we found significantly lower sNfL absolute values in treatment naïve PwFD (median 9.7 pg/mL, IQR 7.7) compared to those receiving any disease-specific therapy (ERT or chaperone-therapy, 16.3 pg/mL, IQR 20.6, $p = 0.015$), this difference failed to achieve significance when analyzing sNfL Z-scores (0.75, SD 1.6 vs. 1.48, SD 1.4; $p = 0.094$, Table 4). PwFD receiving disease-specific therapy were significantly older (49.3 years

	PwFD, $n = 50$	HC, $n = 30$	p-value
Female[a]	31 (62)	15 (50)	0.414[d]
Age, years[b]	44.3 (14.2)	41.3 (13.7)	0.336[e]
Age category Age 18 to < 51[a] Age 51 to < 61[a] Age 61 to < 70[a]	32 (64) 11 (22) 7 (14)	20 (66) 8 (27) 2 (7)	
BMI[b]	25.1 (4.4)	24.8 (4.4)	0.789[e]
Therapy Agalsidase alpha[a] Agalsidase beta[a] Migalastat[a] Lucerastat[a] none	10 (20) 3 (6) 11 (22) 1 (2) 25 (50)	NA	NA
Therapy-duration, months[c]	78 (155)	NA	NA
FOS-MSSI[c]	13 (14.8)	NA	NA
Mild (0–18)[a] Moderate (19–36) [a] Severe (> 36) [a]	29 (58) – Therapy 9/29 18 (36) – Therapy 13/18 3 (6) – Therapy 3/3		
Lyso-Gb_3 (ng/mL)[b]	8.5 (9.1)	NA	NA
Creatinine (mg/mL)[c]	0.80 (0.24)	NA	NA
eGFR (mL/min/1.73 m^2)[b]	91.9 (28.3)	NA	NA

Table 1. Characteristics of PwFD and healthy controls. PwFD: people with Fabry disease; HC: healthy controls; BMI: body-mass index; ERT: enzyme-replacement therapy; FOS-MSSI: Fabry Outcome Survey Mainz Severity Score Index; eGFR: estimated glomerular filtration rate (CKD-EPI 2021); [a]: absolute (percentage); [b]: mean (standard deviation); [c]: median (interqartile-range); [d]: χ^2-test; [e]: unequal variances t-test.

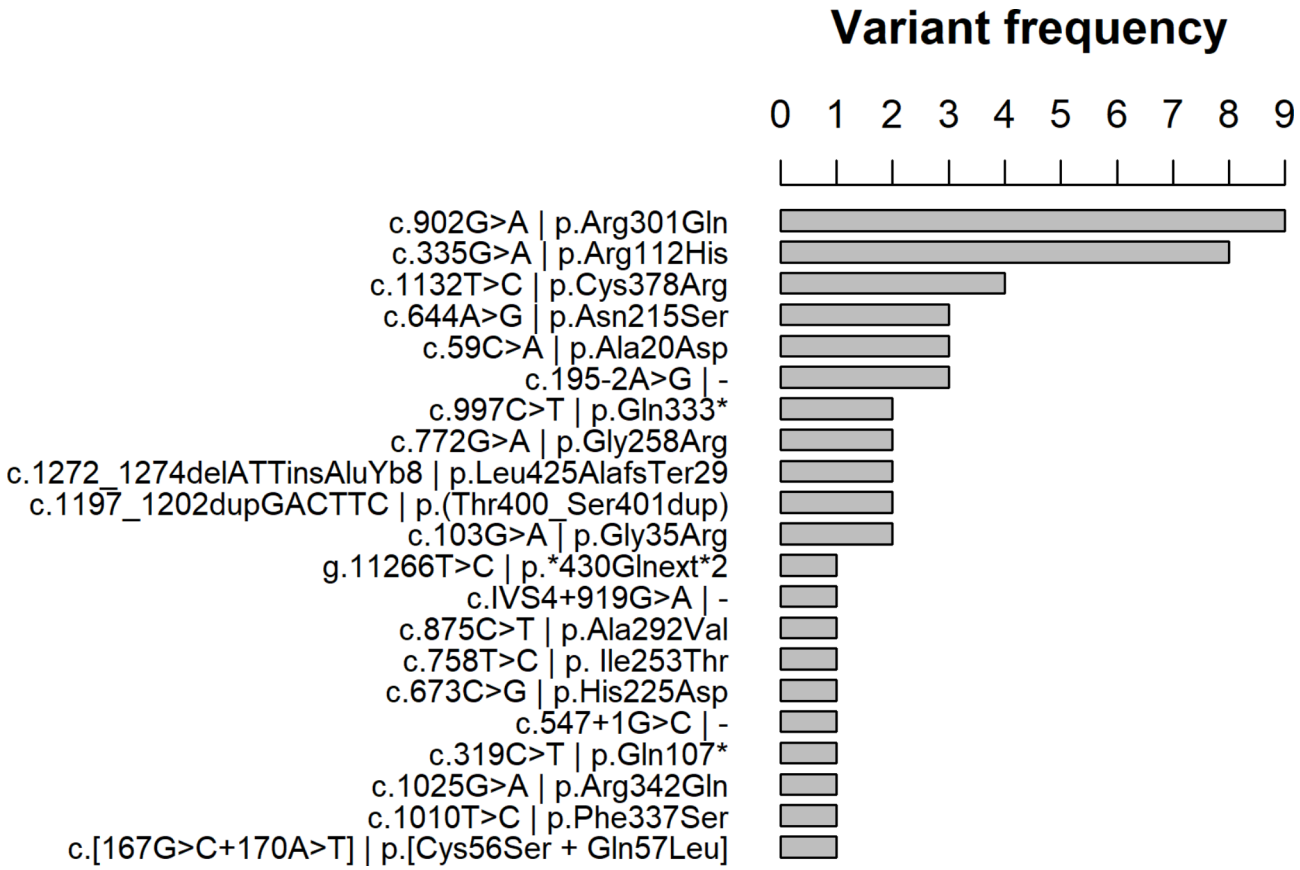

Fig. 1. Distribution of GLA-variants in the presented cohort, comprising a total of 21 unique variants from 27 families. Variants are listed with coding DNA (c.) and protein (p.) nomenclature, separated by "|".

	PwFD, $n=50$		**HC, $n=30$**		***p*-value**
	sNfL (pg/mL)[a]	Z-score[b]	sNfL (pg/mL)[a]	Z-score[b]	
all	12.3 (13.4)	1.12 (1.5)	8.0 (5.5)	0.01 (1.2)	**0.003**[c] | **<0.001**[d]
Age 18 to < 51	9.6 (10.2)	1.03 (1.7)	6.2 (5.1)	0.24 (1.3)	
Age 51 to < 61	13.1 (6.6)	1.05 (1.3)	9.4 (2.8)	-0.57 (0.7)	
Age 61 to < 70	35.5 (20.1)	1.6 (0.9)	15.6 (3.8)	0.41 (1.3)	
Male	21.9 (35.3)	1.75 (1.5)	7.4 (5.7)	0.19 (1.1)	
Female	11.0 (7.9)	0.73 (1.4)	9.3 (5.0)	-0.11 (1.2)	
p-value sex-diff	**0.018**[c]	**0.022**[d]	0.935 [c]	0.497[d]	

Table 2. sNfL and Z-scores in PwFD vs. HC. PwFD: people with Fabry disease; HC: healthy controls; [a]: median (interquartile-range), [b]: mean (standard-deviation), [c]: Wilcoxon rank sum test, [d]: welch two sample *t*-test.

[SD 12.2] vs. 39.4 years [SD 14.5], $p=0.012$, Table 3) and more severely affected with significantly higher FOS-MSSI (any specific therapy: median 21.0, IQR 16.0, no specific therapy: median 9.0, IQR 8.0, $p<0.001$, Table 3). Similarly, sNfL absolute values in PwFD showed a significant correlation with troponin T (TnT). However, TnT correlated even more strongly with age (data not shown). This is also reflected in the absence of significant group differences of sNfL Z-scores between PwFD with normal, borderline and elevated TnT (Table 4). There was no appreciable correlation of sNfL Z-scores or absolute sNfL values with duration of therapy (data not shown).

Mean sNfL Z-scores in PwFD with and without history of acroparesthesia did not differ significantly (0.9, SD 1.9 vs. 1.1, SD 1.1, $p=0.679$; available for $n=48$ PwFD, Table 4). While not available in all patients, mean sympathetic skin response latency to upper and lower extremities (SSRU: $n=36$, SSRL: $n=34$, respectively) as a surrogate marker for damage to autonomic nerves showed no correlation with the presence of acroparesthesia or sNfL-concentration in PwFD (NfL-Z-score: SSRU $\tau = -0.14$, $p=0.210$, SSRL $\tau = -0.019$, $p=0.870$; data on acroparesthesia not shown).

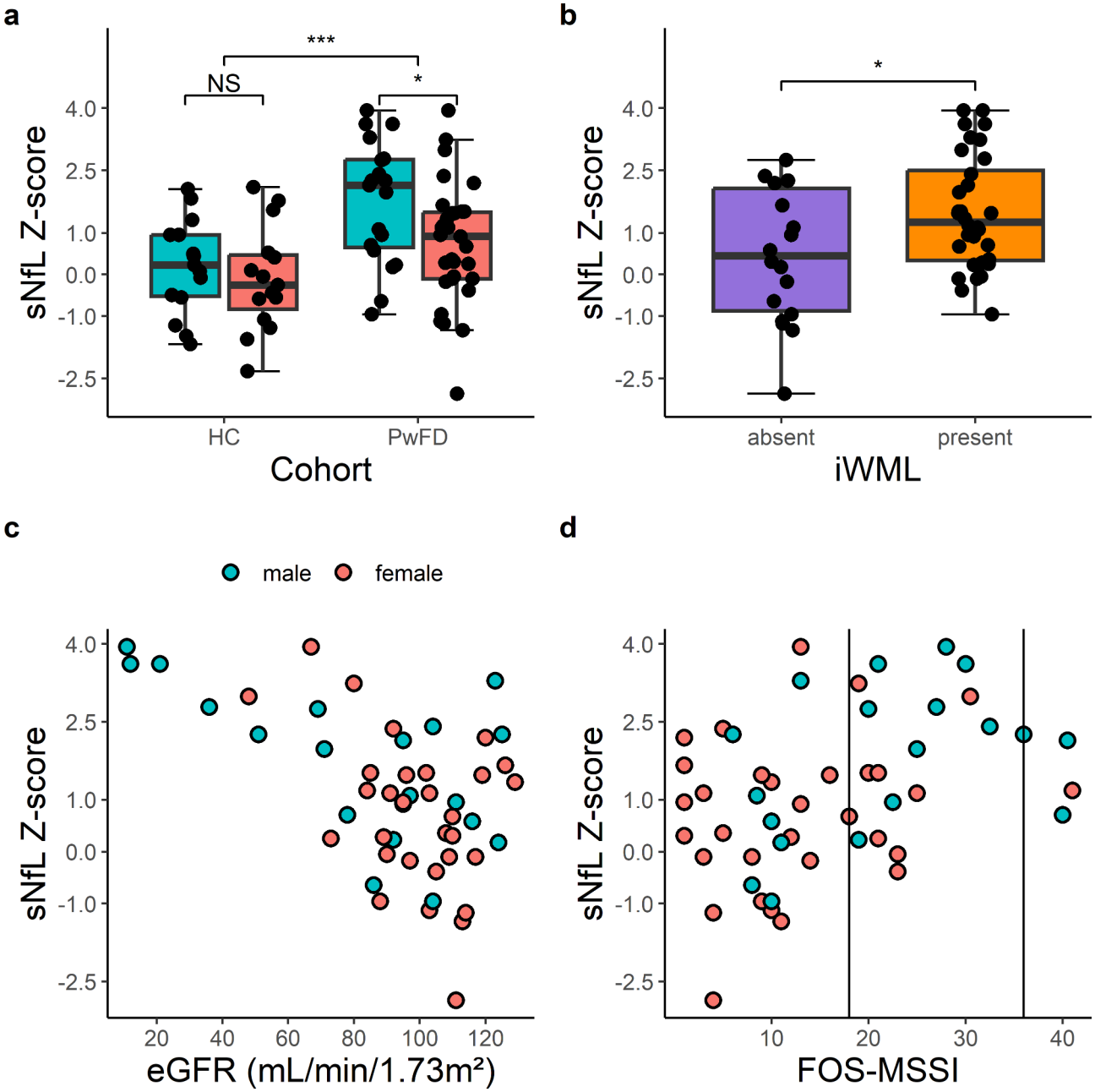

Fig. 2. sNfL in PwFD. (**A**): sNfL Z-score distribution between cohorts and sex, (**B**) sNfL Z-score in PwFD with respect to ischemic CNS lesions, (**C**) correlation of sNfL Z-scores with eGFR, (**D**) correlation of sNfL Z-scores with FOS-MSSI, vertical lines represent cut-off values for disease severity (0–18, 19–36 and > 36 refer to mild, moderate, and severe, respectively). HC: healthy control, PwFD: people with Fabry disease, iWML: ischemic white matter lesions, eGFR: estimated glomerular filtration rate (CKD-EPI 2021), sNfL: Z-score of serum neurofilament light chain concentration adjusted for age and BMI. Asterisks denote signifcance (*: $p < 0.05$, ***: $p < 0.001$, NS: $p \geq 0.05$).

SNfL Z-scores were significantly higher in PwFD with renal dysfunction (eGFR < 90mL/min/1.73 m^2, Table 4) and correlated significantly with serum-creatinine levels and eGFR ($\tau = 0.28$, $p < 0.01$, $\tau = -0.28$, $p < 0.01$, Fig. 2D). Interestingly, PwFD with iWML had significantly lower eGFR compared to those without iMWL (Table 3).

Absolute sNfL-concentrations show a moderate correlation with age ($\tau = 0.43$, $p < 0.001$). Upon analysis of Z-scores, there remained no residual correlation with age above Z-score-standardization (Pearson's $r = 0.23$, p-Value = 0.11).

	iWML present ($n=32$)	iWML absent ($n=18$)	p-value
Female[a]	19 (59.4)	12 (66.7)	0.837[d]
Age [b]	48.7 (12.4)	36.6 (14.2)	**0.005**[e]
BMI [b]	25.3 (4.1)	24.7 (5.0)	0.650[e]
Treated with disease specific therapy[a]	19 (59.4)	6 (33.3)	0.14[d]
eGFR [b]	86.0 (30.8)	102.4 (20.1)	**0.028**[e]
Lyso-Gb_3 [c]	8.2 (8.1)	9.5 (10.8)	0.653[f]
	Disease-specific therapy ($n=25$)	**No disease-specific therapy ($n=25$)**	
Female[a]	13 (52)	18 (72)	0.244[d]
Age [b]	49.3 (12.2)	39.4 (14.5)	**0.012**[e]
FOS-MSSI [c]	21.0 (16)	9.0 (8)	**< 0.001**[f]

Table 3. Demographic details for PwFD with and without iWML and disease-specific therapy. iWML: ischemic white matter lesions; eGFR: estimated glomerular filtration rate (CKD-EPI 2021); FOS-MSSI: Fabry Outcome Survey Mainz Severity Score Index. [a]: absolute (percentage); [b]: mean (standard deviation); [c]: median (interqartile-range); [d]: χ^2-test; [e]: unequal variances t-test; [f]: Wilcoxon rank sum test.

	sNfL (pg/mL)[a]	Z-score[b]	p-values
No therapy ($n=25$)	9.7 (7.7)	0.75 (1.6)	**0.015**[c]| 0.094[d]
Any therapy ($n=25$)	16.3 (20.6)	1.48 (1.4)	
FOS-MSSI 0 to 18 ($n=29$)	9.3 (5.5)	0.59 (1.5)	
FOS-MSSI 19 to 36 ($n=18$)	24.1 (32.3)	1.93 (1.4)	
FOS-MSSI > 36 ($n=3$)	17.1 (9.7)	1.34 (0.7)	
FOS-MSSI-Neuro > 0 ($n=40$)	14.7 (23.5)	1.17 (1.6)	0.108[c]| 0.520[d]
FOS-MSSI-Neuro = 0 ($n=10$)	9.5 (5.0)	0.89 (1.1)	
Ischemic lesions present ($n=32$)	16.2 (25.4)	1.5 (2.2)	**0.003**[c]| **0.030**[d]
Ischemic lesions absent ($n=18$)	9.0 (6.6)	0.5 (2.9)	
Renal dysfunction present (eGFR < 90 mL/min/1.73 m^2, $n=17$)	30.9 (48.6)	2.0 (1.6)	**< 0.001**[c]| **0.008**[d]
Renal dysfunction absent (eGFR ≥ 90 mL/min/1.73 m^2, $n=33$)	9.9 (7.7)	0.9 (1.3)	
Lyso-Gb_3 > 3.5 ng/mL ($n=31$)	16.0 (18.8)	1.36 (1.6)	0.110[c]| 0.139[d]
Lyso-Gb_3 ≤ 3.5 ng/mL ($n=19$)	9.7 (6.9)	0.72 (1.4)	
Normal troponin T (0–14 ng/L, $n=26$)	9.6 (9.5)	0.9 (1.6)	0.441[e]
Borderline troponin T (15–50 ng/L, $n=15$)	13.0 (13.3)	1.3 (1.3)	
Elevated troponin T (> 50 ng/L, $n=9$)	35.8 (35.4)	1.6 (1.8)	
Akroparesthesia* present ($n=20$)	10.9 (10.0)	0.90 (1.9)	0.583[c]| 0.679[d]
Akroparesthesia* absent ($n=28$)	12.9 (10.7)	1.10 (1.1)	

Table 4. sNfL and Z-score characterization in PwFD. eGFR: estimated glomerular filtration rate (CKD-EPI 2021). [a]: median (interquartile-range), [b]: mean (standard deviation), [c]: Wilcoxon rank sum test, [d]: unequal variances t-test, [e]: Analysis of Variance (ANOVA, based on Z-scores), *information on acroparesthesia unavailable for 2 patients.

Discussion

In this exploratory study, we aimed to investigate the potential of sNfL as a diagnostic and monitoring biomarker in FD.

In a large and representative cohort of PwFD, we found that sNfL-concentrations were (i) significantly higher in PwFD compared to healthy controls, (ii) significantly higher in PwFD with iWML, and (iii) correlated with clinical and paraclinical parameters of disease severity (FOS-MSSI, Fig. 2).

These findings are contrary to a small previous study, which found no significant difference in serum NfL levels in 12 female PwFD compared to 12 matched controls. However, sNfL concentrations were determined with ELISA and only one woman with FD was affected severely enough to receive ERT[23]. The different results reported in the current study may be explained by the inclusion of both men and women in our study. Additionally, our cohort was more severely affected, as indicated by the higher number of PwFD receiving disease specific therapy. Thirdly, we utilized the SIMOA technology in our analysis, compared to ELISA.

Unsurprisingly, in our study, sNfL was higher in male PwFD compared to female PwFD (Fig. 2), fitting the X-linked inheritance pattern. Similarly, the increasing absolute sNfL-concentration with higher age appears

easily conceivable for a lysosomal storage disease with accumulation of pathogenic metabolites over time. Upon consideration of sNfL Z-scores, however, no significant correlation above age adjustment was evident. This highlights the importance of incorporating standardized Z-scores into biomarker research, whenever possible.

The correlation of sNfL and FOS-MSSI appears equally plausible, although PwFD in the severe FOS-MSSI category were not those with the highest sNfL-concentrations (Table 4); as our cohort only included 3 PwFD in this group, analysis in a larger sample is required before definite statements regarding this can be permitted.

The neurological subscore of the FOS-MSSI does not seem to be the main driver behind this correlation, as sNfL Z-scores in PwFD with a neurological subscore of ≥ 1 and 0 show no significant difference (Table 4).

Importantly however, comparison of PwFD with and without ischemic white matter lesions on cerebral imaging (FOS-MSSI-cerebrovascular ≥ 3) yielded a significant difference in sNfL Z-scores ($p = 0.03$, Tables 3 and 4; Fig. 2). This increase of sNfL with the presence of iWML is in line with increased sNfL in people with ischemic stroke[24] and other diseases with involvement of the white matter and increased sNfL[18].

To further corroborate this finding, the difference of sNfL Z-scores in PwFD without iWML lesions and healthy controls was not significant ($p = 0.34$). While requiring confirmation and further characterization, this association of increased sNfL and white matter lesions might help in routine patient care to monitor CNS integrity, without requiring a costly annual MRI.

Although large fiber peripheral neuropathy has been linked to an elevation in sNfL[25,26], damage to small nerve fibers does not appear to cause an increase in sNfL[27]. Accordingly, we found no difference in sNfL-concentration in PwFD with respect to acroparesthesia, which is thought to be partly due to a small fiber neuropathy[28,29].

The significance of the difference of sNfL absolute values in PwFD receiving any disease-specific therapy compared to treatment naïve PwFD ($p = 0.015$) failed significance when analyzing Z-scores ($p = 0.094$). Indeed, the absence of disease-specific therapy most likely reflects mild disease-severity without relevant organ damage evident through a significantly lower FOS-MSSI, as well as younger age. On the other hand, improvement of increased sNfL with disease specific therapy seems equally plausible; and while we could not show any meaningful correlation of sNfL Z-scores with duration of therapy in analysis of a single timepoint, assessment of longitudinal change in sNfL will be required to clarify the association with disease specific therapy.

Interestingly, we found a fair correlation between sNfL Z-scores and serum creatinine and eGFR. A correlation of the concentration of NfL in blood (serum or plasma) with creatinine and eGFR has indeed been described in previous studies[30–32], maybe hinting at another, yet unknown mechanism by which an increase in blood-NfL is associated with a reduction in renal function, which – after all – has not been directly linked to neuro-axonal injury.

Although the elimination of sNfL in humans has not been understood in detail, urinary excretion seems unlikely, as NfL, is hardly detectable in urine[33] and does not appear elevated in patients with end-stage renal disease (eGFR < 10 mL/min/1.73 m²) requiring hemodialysis, who showed maximum sNfL-concentration below 20 pg/mL[34]. The considerable size of NfL with 68 kDa would also argue against renal filtration[35].

One possible route of degradation could lie in uptake by macrophages and/or the reticuloendothelial system and subsequent intracellular metabolism, similar to the proposed clearance of e.g. von Willebrand factor[36]. Hampered intracellular degradation with impaired lysosomal metabolism in a lysosomal storage disorder, however, could be envisaged to cause rising sNfL-levels over decades suffering from this disease.

On the other hand, eGFR has been shown to be a prominent biomarker for FD predicting Fabry-associated clinical events and disease progression[37,38]. A decrease in renal clearance might thus represent a general parameter of disease-severity.

While the size of our cohort considering the rarity of FD and the good clinical phenotyping can be considered as strengths of our study, we must acknowledge several limitations. Any differences and extrapolations based on subgroups have to be verified in larger samples as small sample sizes can easily interfere with statistical testing. A single-timepoint analysis of a biomarker in FD could fall short of detecting meaningful changes over time, hence a more detailed investigation including longitudinal data is required to further evaluate the role of sNfL as a biomarker in FD.

Methods

Patient recruitment and phenotyping

During routine annual visits in the outpatient FD clinic of the department of Neurology at the Medical University of Vienna, patients were presented with the option to participate in this study. After obtaining written consent, patients underwent examinations as per the FD outpatient protocol in collaboration with the departments of cardiology and nephrology, comprising neurological examination, blood sampling including determination of Lyso-Gb_3 (ng/mL) from dried blood spots via mass spectrometry, urinalysis, transthoracic echocardiogram, electrocardiogram, nerve conduction studies including sympathetic skin response, as well as calculation of the estimated glomerular filtration rate (eGFR, mL/min/1.73 m²) from age, sex and serum-creatinine, according to the CKD-EPI 2021 formula[39]. Magnetic resonance imaging of heart and brain was performed, according to state-of-the-art patientcare. Classification of disease severity was documented according to the Fabry Outcome Survey Mainz Severity Score Index (FOS-MSSI), where 0–18, 19–36 and > 36 referred to mild, moderate, and severe affection, respectively[40]. Parameters not objectively tested were scored by the treating physician based on history or patient reported outcomes.

Healthy controls were included upon informed consent for blood sampling, MR-imaging and received monetary compensation.

Inclusion criteria for PwFD consisted of a genetically confirmed diagnosis (pathogenic *GLA*-variant, not all variants found in our cohort have been unequivocally classified), age between 18 and 80 years and the ability to give informed consent.

Of 56 persons screened between 06/2020 and 06/2023, 6 had to be excluded due to non-pathogenic variants (2x c.427G > A | p.Ala143Thr; 2x c.937G > T | p.Asp313Tyr; 1x c.460 A > G | p.Ile154Val; 1x c.352 C > T | p.Arg118Cys).

The main inclusion criterion for healthy controls was the absence of a known neurological or severe systemic disorder or pathological findings in the neurological examination or magnetic resonance imaging of the brain.

Neurofilament light-chain measurement and calculation of Z-scores

Measurement of neurofilament light chains was conducted in duplicates as previously described[41]. After thawing for 60 min, the assay was performed according to the manufacturer's instructions and protocol using the Simoa® NF-light kits, calibrators and consumables in the Simoa® SR-X Analyzer (Quanterix, Lexington, MA, USA)[42]. Briefly, duplicate calibrators and single samples were equilibrated to room temperature and dispensed in 96-well plates. Subsequently, incubation with detectors and paramagnetic bead solutions was done according to the instructions. Washing and incubation was performed in the Simoa® microplate incubator and microplate washer. Sample readout and concentration analysis was calculated on the Quanterix SR-X Analyzer. According to the manual, determination of concentrations from 0 to 2000 pg/mL is considered reliable. Statistical analysis was performed including all sample data.

Standardized sNfL Z-scores adjusted for age and body mass index were calculated according to the literature[21].

Statistics

Statistical analysis was performed in R studio (Version 4.2.2, RStudio, Inc.). Categorical variables are reported with absolute counts and percentages, continuous variables are reported as mean and standard deviation or median and interquartile range, depending on the distribution of the values as assessed by Shapiro-Wilk-test. Univariate group comparisons were evaluated using the chi-square-test, the independent *t*-test (with Welch correction in case of unequal variances between groups), Mann-Whitney-U-test or ANOVA (for multiple groups) as appropriate. Bivariate correlations were analyzed by means of Pearson or Kendall, depending on normal distribution. P-values smaller than 0.05 were considered significant. Due to the exploratory nature of this study, no correction for repeated measurements was performed.

Conclusion

Analysis of PwFD reveals increased sNfL Z-scores compared to healthy controls. Among PwFD, higher sNfL Z-scores were found in the presence of ischemic white matter lesions in the CNS. Interestingly, while sNfL Z-scores are associated with disease-severity, also impaired renal function measured with serum-creatinine and eGFR appears to be fairly correlated with sNfL. Since the elimination of NfL is still not fully understood, maybe impaired lysosomal metabolism is involved in its observed increase in addition to cerebrovascular injury. Befitting the X-linked inheritance with random X-inactivation in females responsible for phenotypic variability, sNfL levels were higher in males than in females. As sNfL is easily obtainable and shows a robust correlation with disease severity in FD (FOS-MSSI, iWML) it might be a suitable biomarker for CNS injury and general disease severity in PwFD, although further examination is still required.

Data availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request by a qualified researcher and upon approval by the data-clearing committee of the Medical University of Vienna.

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Author contributions

MP acquired and analyzed data, recruited patients and conceived and drafted the manuscript; CG acquired data and revised the manuscript; GB helped with data analysis, interpretation and revised the manuscript; JR recruited patients and provided essential input concerning interpretation of the data; PA helped with acquiring data, designing measurements and revised the manuscript; TB provided essential feedback and manuscript revision; SG helped with acquiring data and revised the manuscript; GSP provided essential feedback and manuscript revision, PSR conceptualized the project, helped with data acquisition and interpretation and revision of the manuscript.

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Declarations

Competing interests

MP has received speaker or consulting honoraria from Amicus and participated in meetings sponsored by and received travel funding from Amicus, Merck, Novartis and Sanofi-Genzyme.CG has received speaker or consulting honoraria from Amicus therapeutics, Sanofi-Genzyme and Takeda pharmaceuticals and participated in meetings sponsored by and received travel funding from Amicus, Sanofi-Genzyme and Takeda PharmaceuticalsGB has participated in meetings sponsored by, received speaker honoraria or travel funding from Biogen, Celgene/BMS, Janssen, Lilly, Merck, Novartis, Roche, Sanofi-Genzyme and Teva, and received honoraria for consulting Biogen, Celgene/BMS, Janssen, Merck, Novartis, Roche, Sanofi-Genzyme and Teva. He has received unrestricted research grants from Celgene/BMS and Novartis.JR has received consulting fees from Takeda. PA has participated in meetings sponsored by, received speaker honoraria or travel funding from

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Ethical approval

The ethics committee at the Medical University of Vienna, Austria approved this study (EK1487/2020). Written informed consent was obtained from each patient, and study-procedures adhered to the guidelines set by the Declaration of Helsinki.

Additional information

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