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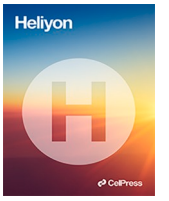

# Clinical stage and plasma neurofilament concentration in adults with Friedreich ataxia

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ABSTRACT

*Objectives:* Friedreich Ataxia (FRDA) is the most common recessive ataxia disorder. Yet, little is known of the prevalence in Sweden. In the future, there may be effective disease-modifying therapies, and use of clinical rating scales as well as possible biomarkers in serum or cerebrospinal fluid may be of importance. We evaluated the axonal protein neurofilament light in plasma (*p*-NfL) as a possible biomarker for disease severity in FRDA.

*Materials & methods:* We searched for all possible genetically confirmed FRDA cases in the Västra Götaland Region (VGR) of Sweden, and investigated each patient clinically and obtained blood sample for analysis of *p*-NfL.

*Results:* We found eight patients corresponding to 1/170.000 adults in the VGR, and 5 of these participated in the study. Three out of the five FRDA patients displayed a small or moderate increase in the *p*-NfL value, compared to the age-adjusted cut-offs for *p*-NfL established in the Clinical Neurochemistry Laboratory at our hospital. The two others were the oldest and most severely affected, displayed normal values according the cut-off values. The cohort is too small to make any statistically significant correlation between the five *p*-NfL values with regard to disease severity.

*Conclusions:* FRDA is less prevalent in our region of Sweden than could be assumed. In concordance with previous studies from other authors, we find that *p*-NfL may be increased in patients with FRDA, but less so in older more clinically affected patients. Thus, we conclude that on an individual basis, *p*-NFL is of uncertain clinical value as a suitable biomarker.

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## 1. Introduction (Background)

Friedreich ataxia (FRDA) is an autosomal recessive disorder in most cases caused by bi-allelic pathological GAA trinucleotide repeat expansions present on both alleles in the frataxin (*FXN*) gene [1]. Patients with FRDA usually have symptom onset at age 10–15 years with progressive walking difficulties, impaired coordination, dysarthria, and spasticity. This reflects loss of neurons predominantly of long fibre tracts in the spinal cord, e.g., dorsal root ganglia, dorsal spinocerebellar pathways, posterior columns, pyramidal tracts, as well as peripheral sensory and motor neurons [2]. Other manifestations are diabetes mellitus in some 30 % of patients and cardiomyopathy, which is present in two thirds of the patients. Cardiomyopathy is the main cause of death in FRDA [3] contributing to a reduced life expectancy.

FRDA is the most common form of autosomal recessive ataxia, with a reported prevalence of between 1:20 000–1: 750 000 in Western and Nordic populations [4,5]. Recently, omaveloxalone was approved in the US, based on a phase II study by Lynch and coworkers [6] There is thus a need for evaluation of potential biomarkers suitable for repeated monitoring of disease activity.

It is today possible to measure several biomarkers in cerebrospinal fluid (CSF), which are increased in many different types of brain injuries and disorders of various etiologies [7]. One of these is neurofilament-light chain (NfL) protein, an axonal protein, the CSF levels of which are increased in neuroaxonal degeneration [8]. There are strong indications that a blood-based sampling for these CNS-related proteins can replace CSF analysis [9,10]. Plasma-NfL (*p*-NfL) has been examined in different forms of spinocerebellar ataxias (SCA) [11,12], as well as in recently published work on FRDA [13–15]. Zeitlberger et al. [13], Hayer et al. [14], and Clay et al. [15] found increased *p*-NfL in FRDA patients compared to matched controls. Longitudinal data showed that the *p*-NfL values were stable over time [14,15], and all three publications showed that the *p*-NfL levels were inversely correlated with age in FRDA patients [13–15].

**Table 1**
Clinical characteristics of five patients with Friedreich Ataxia, FRDA.

| Participant | A | B | C | D | E |
|---|---|---|---|---|---|
| Age at investigation | 29 | 33 | 55 | 57 | 40 |
| Age at onset | 14 | 13 | 15 | 15 | 14 |
| Disease duration | 15 | 20 | 40 | 42 | 26 |
| MRI findings | Normal cerebellum | Normal cerebellum | Moderate cerebellar atrophy | Moderate cerebellar atrophy | Normal cerebellum |
| Neuropathic pain | Slight discomfort | No | No | No | No |
| Willis-Ekbom disease | No | No | No | No | No |
| Scoliosis | No | No | Yes | Yes | Yes |
| Spasticity | No | No | Yes | Yes | Yes |
| ECG (electro cardiography) | Without remarks | Without remarks | Without remarks | T-inversion only | Without remarks |
| Echocardiography | Without remarks | Without remarks | Without remarks | Slightly reduced EF. | Without remarks |
| Hearing loss | No | No | Yes | No | Yes |
| Depression (history of) | No | Yes | Yes | No | No |
| Sleeping problems | No | No | Yes but no OSAS | Yes OSAS | No |
| FARS | FARS range 0–6 (increasing level of disability) | | | | |
| ;Functional staging | 4.0 | 2.5 | 5.0 | 5.0 | 5.0 |
| ;ADL | 14 | 13 | 28 | 26 | 27 |
| ;neurologic exam | | | | | |
| ¨bulbar | 1 | 1 | 5 | 2 | 6 |
| ¨upper limb | 14 | 12 | 28 | 25 | 22 |
| ¨lower limb | 6 | 7 | 16 | 16 | 16 |
| ¨peripheral | 22 | 9 | 16 | 18 | 18 |
| ¨upright stability (with footwear) | 25 | 1 | 27 | 27 | 27 |
| ¨total neurologic exam | 68 | 30 | 92 | 88 | 89 |
| ;PATA rate (repetitions of the syllables "PA-TA" during a 10 s interval) | 17 | 22 | 12,5 | 10,5 | 7 |
| ;9 Peg hole right (seconds) | 47,5 | 29,5 | unable to perform | unable to perform | 440 |
| ;9 Peg hole left (seconds) | 65,5 | 36,5 | unable to perform | unable to perform | 490 |
| SARA | SARA range 0–40 (increasing level of ataxia score) | | | | |
| ;gait | 7 | 1 | 8 | 8 | 8 |
| ;stance | 5 | 0 | 6 | 6 | 6 |
| ;sitting | 1 | 0 | 4 | 4 | 4 |
| ;speech | 1 | 1 | 4 | 4 | 3 |
| ;finger chase | 2 | 1 | 3 | 1,5 | 2 |
| ;nose finger | 0 | 1 | 3 | 2,5 | 1 |
| ;alt.move | 3 | 1 | 3 | 3 | 3 |
| ;heel shin | 2 | 2 | 4 | 4 | 4 |
| ;Total SARA | 21 | 7 | 35 | 33 | 31 |

FARS = Friedreich ataxia rating scale, SARA = Scale for the Assessment and Rating of Ataxia, OSAS = Obstructive Sleep Apnoea Syndrome, EF = Ejection fraction.

In 2019, we set up the present cross-sectional study with the intention to include a cohort of all adult patients living within the Västra Götaland region (VGR) in Sweden, a region in the south west of Sweden with 1.7 million inhabitants. We aimed to estimate the number of FRDA patients in VGR, to describe the clinical stages and symptoms of these patients, and further to investigate if there was any correlation between levels of NfL in spinal fluid or serum and disease duration or the clinical stage of patients.

## 2. Methods

The study followed the tenets of the Declaration of Helsinki, and it was conducted with the approval of the Swedish Ethical Review Authority (2019-01659). Patients were identified by a diagnosis-based search in the Västra Götaland region general electronic medical records system, using the term "Friedreich ataxia", and we also had a direct contact with doctors at all adult neurological clinics in the region and the department of adult cardiology at Sahlgrenska University Hospital where the FRDA patients are seen for follow up regarding their cardiac function. We also conducted a search for FRDA patients via the Department of Clinical Genetics, Sahlgrenska, which is the only genetics laboratory in VGR, thereby identifying all FRDA-positive samples. All patients who were included were genetically confirmed and all had bi-allelic GAA expansions. The minimum prevalence of FRDA patients was calculated based on the number of verified patients with FRDA aged 18 years or older and the present number of adult inhabitants in VGR. Potential participants were contacted via telephone and requests for participation were sent out via letter. Written consent was obtained.

Participants were all seen at an out-patient visit by one and the same doctor at the neurology clinic (MJ). Information about their medical history was obtained via the medical records and the participants' medical history. Patients underwent clinical examinations using ataxia grading scales. We choose two such validated scales: Friedreich ataxia rating scale (FARS) [16], and Scale for the Assessment and Rating of Ataxia (SARA) [17]. FARS is disease-specific for FRDA [16]. SARA is a widely validated scale for use in various cerebellar ataxia diseases and could possibly be the scale best suited to monitor disease progression although SARA mainly captures the severity of cerebellar dysfunction [17–19].

None of the patients accepted a lumbar puncture, thus blood samples were obtained and were sent to the certified neurochemistry lab at Sahlgrenska University Hospital and were stored at −80 °C pending batch analysis by board-certified laboratory technicians who were blinded to clinical data. The samples were analyzed for plasma levels of NfL using the NF-light kit and ultrasensitive Single molecule array (Simoa) technology, according to instructions by the manufacturer (Quanterix, Billerica, MA).

The laboratory results were compared to the age-adjusted cut-offs for *p*-NfL established in the Clinical Neurochemistry Lab, Sahlgrenska University Hospital, Mölndal, Sweden, which are based on upper 95 % confidence interval (CI) for values in healthy individuals without history, symptoms or signs of psychiatric or neurological disorders [20]. These cut-offs are <9 pg/mL for the age group 18–40 years (n = 266), <12 pg/mL for the age group 41–50 years (n = 303), and <16 pg/mL for the age group 51–60 years (n = 689). The participant's ages at investigation were 29–57 years.

## 3. Results

We identified eight adult patients with genetically confirmed Friedreich ataxia living in the Västra Götaland Region by Dec 31, 2017. Five were patients at Sahlgrenska University Hospital, two from a regional hospital and one in a large outpatient clinic. This corresponds to 1:170 000 in the adult population. Out of these eight patients, we managed to come in direct contact with seven, and five of these accepted to participate in the clinical part of the study. None of the participants wanted to undergo a lumbar puncture, but all five agreed to provide a blood sample. Clinical data appears in Table 1. Patients A and B had relatively mild clinical symptoms, while patients C, D and E were more severely affected as evidenced by the ratings according to FARS (score = 5) and SARA ratings of 35, 33 and 31, respectively. Patient A had disease duration of 15 years, used walking aids and had some assistance in ADL activities. Patient B had 20 years disease duration, walked and performed all activities slowly but independently. Patients C, D and E had disease durations of 40, 42 and 26 years and were all using a wheelchair, had severe disability and needed assistance for all activities in daily life. Only one patient (D) had mild cardiac affection, none had diabetes. Signs of neuropathy were not specifically noted, but is included in the line "peripheral" in FARS section in Table 1. Medications used included betablocker (A, D, E), lamotrigine (C), baclophen (C), oxascand (E)and insulin (C). None of the patients used any FRDA specific medication.

The patients had *p*-NfL levels between 11 and 16 pg/mL (Table 2). This was a value considered to be within the normal range in patients C and D due to a higher normal range. Notably, these two patients (C, D) were the most severely affected with regard to ataxia rating. Patients A, B and E displayed a moderate increase in the *p*-NfL value. Our cohort was too small to make any statistically analysis regarding correlation between disease severity as measured by the different ataxia rating scales, and the *p*-NfL levels.

## 4. Conclusions (Discussion)

Friedreich ataxia is a rare disorder with internationally varied prevalence figures. In our cohort, we included eight patients

**Table 2**
P-NfL values (Quanterix Assay), corresponding to A-E participants in Table 1.

| | A | B | C | D | E |
|---|---|---|---|---|---|
| Plasma-NfL (Normal values age dependent) | 12 ng/L (<9) | 14 ng/L (<9) | 11 ng/L (<16) | 13 ng/L (<16) | 16 ng/L (<9) |

corresponding to 1:170 000 in the adult population of VGR. The prevalence of FRDA is in most populations higher, but our figure is at about the same level as shown in data from Norway [4]. It may well be so that, despite our ambitions to find all adult cases, some patients living in VGR were not retrieved: they might not be genetically diagnosed alternatively diagnosed in other parts of Sweden and moved into the VGR.

Our data indicate that *p*-NfL may be increased in patients with FRDA. This is in accordance with recent studies that have shown increased levels of *p*-NfL in patients with FRDA compared to matched controls [13–15]. The *p*-NfL levels are also relatively stable over 1–2 years [13–15]. As our cohort is small, we cannot examine a possible correlation between the *p*-NfL values and other clinical parameters.

The two oldest patients in our cohort had values within the normal range. This finding is in line with earlier results suggesting an inverse correlation of *p*-NfL with age in FRDA patients [13–15], also reviewed and discussed in [19]. In FRDA *p*-NfL did not correlate with disease severity [13–15]. This is in contrast with findings in other disorders, e.g., SCA [11,12] Why FRDA exhibits a NfL profile over time which is in contrast to that of SCA is not obvious. The underlying mechanism behind this unclear. Since NfL is found in large myelinated axons of both the central and the peripheral nervous system, one possible explanation might be that early in the disease process peripheral neurones are affected but less so in later stages, thus contributing less to NfL levels later in the disease process. One different aspect might be that early in the disease process at younger ages, elevated NfL might reflect an increased synthesis of the protein [21]. Thus, there is a need for future studies in order to better understand the processes behind NfL elevation in FRDA to increase its interpretability in clinical trials and practice.

## Ehtics declaration

This study was reviewed and approved by Swedish Ethical Review Authority, with the approval number: 2019-01659.
All patients provided informed consent for the publication of their anonymised case details.

## Data availability statement

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

## Ehtics declaration

This study was reviewed and approved by Swedish Ethical Review Authority, with the approval number: 2019-01659.
All patients provided informed consent to participate in the study.
All patients provided informed consent for the publication of their anonymised case details.

## Declaration of competing interest

HZ has served at scientific advisory boards and/or as a consultant for AbbVie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB. (BBS), which is a part of the GU Ventures Incubator Program.

CL has served as a consultant, at advisory boards for PTC therapeutics and Biogen.

MJ declare no financial or other conflicts of interest in relation to this work.

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## References


[1] V. Campuzano, L. Montermini, M.D. Moltò, et al., Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion, Science 271 (1996) 1423–1447.

[2] C.A. Galea, A. Huq, P.J. Lockhart, et al., Compound heterozygous FXN mutations and clinical outcome in friedreich ataxia, Ann. Neurol. 79 (3) (2016) 485–495, https://doi.org/10.1002/ana.24595.

[3] A.Y. Tsou, E.K. Paulsen, S.J. Lagedrost, et al., Mortality in friedreich ataxia, J. Neurol. Sci. 307 (1–2) (2011 Aug 15) 46–49, https://doi.org/10.1016/j.jns.2011.05.023. Epub 2011 Jun 8. PMID: 21652007.

[4] K. Bürk, Friedreich Ataxia: current status and future prospects, Cerebellum Ataxias 4 (4) (2017), https://doi.org/10.1186/s40673-017-0062-x. Published 2017 Apr 7.

[5] I.M. Wedding, M. Kroken, S.P. Henriksen, et al., Friedreich ataxia in Norway - an epidemiological, molecular and clinical study, Orphanet J. Rare Dis. 10 (2015) 108, https://doi.org/10.1186/s13023-015-0328-4. Published 2015 Sep. 4.

[6] D.R. Lynch, M.P. Chin, M.B. Delatycki, S.H. Subramony, M. Corti, J.C. Hoyle, S. Boesch, W. Nachbauer, C. Mariotti, K.D. Mathews, et al., Safety and efficacy of omaveloxolone in Friedreich ataxia (MOXIe study), Ann. Neurol. 89 (2021) 212–225.

[7] R. Constantinescu, H. Zetterberg, B. Holmberg, L. Rosengren, Levels of brain related proteins in cerebrospinal fluid: an aid in the differential diagnosis of parkinsonian disorders, Parkinsonism Relat. Disorders 15 (3) (2009) 205–212, https://doi.org/10.1016/j.parkreldis.2008.05.001.

[8] M. Khalil, C.E. Teunissen, M. Otto, F. Piehl, M.P. Sormani, T. Gattringer, C. Barro, L. Kappos, M. Comabella, F. Fazekas, A. Petzold, K. Blennow, H. Zetterberg, J. Kuhle, Neurofilaments as promising biomarkers in neurological disorders, Nat. Rev. Neurol. 14 (10) (2018) 577–589.

[9] O. Hansson, S. Janelidze, S. Hall, N. Magdalinou, A.J. Lees, U. Andreasson, N. Norgren, J. Linder, L. Forsgren, R. Constantinescu, H. Zetterberg, K. Blennow, Swedish BioFINDER study. Blood-based NfL: a biomarker for differential diagnosis of parkinsonian disorder, Neurology 88 (10) (2017 Mar 7) 930–937, https://doi.org/10.1212/WNL.0000000000003680. Epub 2017 Feb 8. PMID: 28179466; PMCID: PMC5333515.

[10] K. Blennow, H. Zetterberg, Fluid biomarker-based molecular phenotyping of Alzheimer's disease patients in research and clinical settings, Prog Mol Biol Transl Sci 168 (2019) 3–23, https://doi.org/10.1016/bs.pmbts.2019.07.006.

[11] C. Wilke, E. Haas, J. Faber, et al., Neurofilaments in spinocerebellar ataxia type 3: blood biomarkers at the preataxic and ataxic stage in humans and mice, EMBO Mol. Med. 12 (7) (2020), e11803, https://doi.org/10.15252/emmm.201911803.

[12] G. Coarelli, F. Darios, E. Petit, et al., Plasma neurofilament light chain predicts cerebellar atrophy and clinical progression in spinocerebellar ataxia, Neurobiol. Dis. (2018), https://doi.org/10.1016/j.nbd.2021.105311.

[13] A.M. Zeitlberger, G. Thomas-Black, H. Garcia-Moreno, et al., Plasma markers of neurodegeneration are raised in friedreich's ataxia, Front. Cell. Neurosci. 12 (2018) 366, https://doi.org/10.3389/fncel.2018.00366. Published 2018 Oct 30.

[14] S.N. Hayer, I. Liepelt, C. Barro, et al., NfL and pNfH are increased in Friedreich's ataxia, J. Neurol. 267 (5) (2020) 1420–1430, https://doi.org/10.1007/s00415-020-09722-6.

[15] A. Clay, K.M. Obrochta, R.K. Soon Jr., C.B. Russell, D.R. Lynch, Neurofilament light chain as a potential biomarker of disease status in Friedreich ataxia, J. Neurol. 267 (9) (2020) 2594–2598, https://doi.org/10.1007/s00415-020-09868-3.

[16] S.H. Subramony, W. May, D. Lynch, et al., Measuring Friedreich ataxia: interrater reliability of a neurologic rating scale, Neurology 64 (2005) 1261–1262.

[17] T. Schmitz-Hübsch, S. Tezenas du Montcel, L. Baliko, et al., Scale for the assessment and rating of ataxia. Development of a new clinical scale, Neurology 66 (2006) 1717–1720.

[18] K. Bürk, S.R. Schulz, J.B. Schulz, Monitoring progression in Friedreich ataxia (FRDA): the use of clinical scales, J. Neurochem. 126 (Suppl 1) (2013) 118–124, https://doi.org/10.1111/jnc.12318.

[19] K. Reetz, I. Dogan, A.S. Costa, et al., Biological and clinical characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) cohort: a cross-sectional analysis of baseline data, Lancet Neurol. 14 (2) (2015) 174–182, https://doi.org/10.1016/S1474-4422(14)70321-7'.

[20] J. Simrén, U. Andreasson, J. Gobom, et al., Establishment of reference values for plasma neurofilament light based on healthy individuals aged 5-90 years, Brain Commun 4 (4) (2022 Jul 4) fcac174, https://doi.org/10.1093/braincomms/fcac174.

[21] B. Frempong, R.B. Wilson, K. Schadt, D.R. Lynch, The role of serum levels of neurofilament light (NfL) chain as a biomarker in friedreich ataxia, Front. Neurosci. 15 (2021), 653241, https://doi.org/10.3389/fnins.2021.653241. Published 2021 Mar 2.