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ORIGINAL COMMUNICATION

Frataxin analysis using triple quadrupole mass spectrometry: application to a large heterogeneous clinical cohort

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Abstract

Background Friedreich ataxia is a progressive multisystem disorder caused by deficiency of the protein frataxin; a small mitochondrial protein involved in iron sulfur cluster synthesis. Two types of frataxin exist: FXN-M, found in most cells, and FXN-E, found almost exclusively in red blood cells. Treatments in clinical trials include frataxin restoration by gene therapy, protein replacement, and epigenetic therapies, all of which necessitate sensitive assays for assessing frataxin levels.

Methods In the present study, we have used a triple quadrupole mass spectrometry-based assay to examine the features of both types of frataxin levels in blood in a large heterogenous cohort of 106 patients with FRDA.

Results Frataxin levels (FXN-E and FXN M) were predicted by GAA repeat length in regression models (R^2 values = 0.51 and 0.27, respectively), and conversely frataxin levels predicted clinical status as determined by modified Friedreich Ataxia Rating scale scores and by disability status (R^2 values = 0.13–0.16). There was no significant change in frataxin levels in individual subjects over time, and apart from start codon mutations, FXN-E and FXN-M levels were roughly equal. Accounting for hemoglobin levels in a smaller sub-cohort improved prediction of both FXN-E and FXN-M levels from R^2 values of (0.3–0.38 to 0.20–0.51).

Conclusion The present data show that assay of FXN-M and FXN-E levels in blood provides an appropriate biofluid for assessing their repletion in particular clinical contexts.

Keywords GAA repeat length · Friedreich Ataxia Rating scale · Disability status · Frataxin blood levels · Mitochondrial protein

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Introduction

Friedreich ataxia (FRDA), the most common inherited ataxia, is usually (> 96%) caused by a GAA triplet repeat (GAA-TR) expansion in intron 1 of the *FXN* gene [1, 2]. Most people have less than 9 GAA triplets on both *FXN* genes, but an expansion of 40 to 1700 triplets on both alleles results in FRDA [2]. About 4% of people with FRDA carry an expansion on one allele and a point mutation or a deletion on the other. Heterozygous carriers of one pathogenic allele are asymptomatic throughout life. Clinical features of FRDA include ataxia, cardiomyopathy, scoliosis, and diabetes [3–5].

The expanded GAA repeats in *FXN* partially silences the gene leading to decreased transcription of the mRNA for frataxin, a small mitochondrial protein [6, 7]. This leads to decreased levels of frataxin protein (< 10% of normal); point mutations causing disease similarly lead to loss of functional

frataxin [8, 9]. Usually such point mutations lead to unstable frataxin protein or loss of translation initiation, although a few missense mutations may produce dysfunctional frataxin that is present at near-normal levels [8–11]. Frataxin exists in at least 2 forms in humans: a mature form (FXN-M) synthesized in most cells and largely targeted to mitochondria; and isoform E (FXN-E), made from an mRNA with a different exon 1 through alternative splicing [12, 13]. FXN-E is largely found in erythrocytes and has no mitochondrial targeting sequence. Most point mutations affect both forms, although point mutations in exon 1 can selectively spare FXN-E [11–13].

Restoration of sufficient frataxin represents a crucial approach for future treatment of FRDA, leading to a need for sensitive assessment of frataxin levels. In FRDA, frataxin levels are decreased in all tissues; consequently, assessment of frataxin in unaffected tissue can serve in some therapeutic approaches as a marker of levels in affected tissues. A variety of immunologically based assays for frataxin have been developed across different platforms [14–19]. Their sensitivity varies greatly, although in most cases the results correlate with expected markers of disease severity. Reproducibility has also been suboptimal and depends on the nature of the antibody used in the assay [20]. We have previously reported assessment of frataxin using LC coupled to either a high resolution mass spectrometer [13, 21, 22] or to a low resolution triple quadrupole mass spectrometer [23]. In the present study, we have used this latter approach [23] to assess the levels of both frataxin isoforms in blood from a large heterogeneous cohort of patients with FRDA and their relationship to clinical features of FRDA.

Methods

Patients

All procedures were approved by the Children's Hospital of Philadelphia (CHOP) IRB. Subjects were selected from 2 ongoing studies of the natural history of FRDA:FA-CHILD, which focuses on children with FRDA; and FACOMS, a more general study examining all persons with FRDA [24, 25]. Subjects were evaluated at CHOP, the University of California Los Angeles, or the University of Florida. The total number of subjects was 106, 13 of whom provided samples for serial analysis. Blood samples were collected into EDTA purple top vacutainer tubes and frozen at − 80 °C immediately until frataxin assays were performed. Clinical data (including demographics, most recent mFARS scores, and most recent functional disability stage (FDS) were available from the FA-CHILD or FACOMS database [26]. FXN-M and FXN-E assays were performed as previously described [23].

Statistical analyses

Data analysis was performed using STATA SE/17 software. Linear regressions and Student's t-tests were used where appropriate.

Results

The population for this study was diverse, including subjects from 11 to 68 years of age and GAA1 repeat lengths from 101 to 1000 (Supplementary Table 1). 59% of the subjects were female. As reported previously, GAA1 length correlated with age of onset [2–5], and neurological measures such as mFARS and FDS were predicted by age and GAA1 length in models accounting for age, GAA1 and sex [24, 25]. In general, there were low but still significant correlations ($p < 0.005$) between mFARS and age, GAA1, and age of onset, consistent with the diversity in duration and genetic severity in the cohort (Supplementary Table 2). Still, the correlation of age of onset and GAA1 with age demonstrates that there is some selection in the participants in that not all ages of a given GAA1 length are equally represented in the cohort. Sex did not influence neurologic measures, and GAA1 length was equal between sexes (Supplementary Table 2).

We then examined the relationship between frataxin levels and clinical parameters. Frataxin E (FXN-E) and Frataxin M (FXN-M) levels correlated moderately with age and more highly with age of onset (AOO) and GAA1 (Supplementary Table 2). FXN-E and FXN-M also correlated with each other at a higher level ($R^2 = 0.75$) than with AOO or GAA1. In addition, there was a small correlation of FXN-E (but not FXN-M) with sex (females having lower values). Since GAA1 and age correlate inversely with each other, we then performed multiple linear regression to identify their independent effects (along with effects of sex). In regression models examining the effect of sex, GAA1 and age on FXN-M and FXN-E levels, the combination of variables predicted FXN-E ($R^2 = 0.51$; $p < 0.0001$) and FXN-M ($R^2 = 0.27$; $p < 0.0001$) (Table 1). Separating the effects of each variable, both FXN-M and FXN-E were predicted by GAA1 length with high levels of significance, and with lesser significance by age. For both FXN-M and FXN-E, older age led to a higher frataxin level, with predictions of frataxin increasing by 1% per year for FXN-M and 2% per year for FXN-E (based on regression coefficients). In addition, female sex marginally ($p = 0.09$) was associated with a lower FXN-E (but not FXN-M) level.

In addition, we examined if FXN-M and FXN-E predicted clinical severity (Tables 2, 3). In regression models

Table 1 Frataxin levels: Regression with GAA/Age /Sex

	FXN-M	FXN-E
Model	0.27 (<0.0001)	0.51 (<0.0001)
GAA1	−0.0023 ± 0.0006 (<0.001)	−0.0081 ± 0.0013 (<0.001)
Age	0.023 ± 0.010 (0.029)	0.074 ± 0.022 (0.001)
Sex	−0.085 ± 0.22 (0.70)	−0.81 ± 0.48 (0.090)

Multiple linear regression analysis was performed for evaluation of the dependent variables FXN-M and FXN-E as predicted by age, GAA1, and sex. R^2 values for the models are given along with regression coefficients for each variable (p values given in parentheses). Overall, FXN-E models had higher R^2 values that FXN-M, and GAA1 and age were both predictors of frataxin levels

Table 2 Linear regression of mFARS scores by age sex, and FXN levels

	FXN-M	FXN-E
Model	0.13 (0.0004)	0.16 (0.0001)
Sex	−1.39 ± 2.99 (0.64)	−3.02 ± 3.00 (0.32)
Age	0.56 ± 0.13 (<0.001)	0.58 + 0.13 (<0.001)
FXN	−3.93 + 1.27 (0.003)	−1.48 + 0.40 (<0.001)

Multiple linear regression analysis was performed for evaluation of the dependent variable mFARS score as predicted by age, sex, and frataxin levels (either FXN-M or FXN-E). R^2 values for the models are given along with regression coefficients for each variable (p values given in parentheses). R^2 values for both models were significant but relatively low, and frataxin values predicted mFARS scores (as did age)

Table 3 Linear regression of Functional Disability scores by age sex, and FXN levels

	FXN-M	FXN-E
Model	0.16 (0.0001)	0.17 (<0.0001)
Sex	0.14 ± 0.23 (0.54)	0.044 ± 0.23 (0.85)
Age	0.049 ± 0.010 (<0.001)	0.050 ± 0.010 (<0.001)
FXN	−0.26 ± 0.098 (0.008)	−0.093 ± 0.031 (0.004)

Multiple linear regression analysis was performed for evaluation of the dependent variable functional disability score as predicted by age, sex, and frataxin levels (either FXN-M or FXN-E). R^2 values for the models are given along with regression coefficients for each variable (p values given in parentheses). R^2 values for both models were significant but relatively low, and frataxin values predicted mFARS scores (as did age)

examining age, sex, and frataxin levels, age predicted mFARS and FDS values with high significance while sex had no effect. FXN-M and FXN-E both predicted mFARS and FDS scores, with FXN-E having more significance.

Although small, the linear regression results predicted that frataxin levels might change over time. We thus assessed repeated samples for the same subject separated by one year over the course of up to 4 years in 13 subjects. We observed no systematic change over this period (Fig. 1), and the mean level of change at each year was not different from zero for either FXM or FXN E. In addition, the correlation between change in FXN-M and FXN E was $r = 0.71$ ($p = 0.015$) showing the year-to-year variability was tightly linked.

Previously, we had noted that individuals with mutation of the start codon carried normal levels of FXN-E, owing to its synthesis from a different splice variant [17]. We examined individuals with other point mutations (Supplementary Table 3). Those with mutations within exon 1 had preserved levels of FXN-E but not FXN-M. Interestingly, such individuals had mFARS scores matching their pathologically low FXN-M levels, not the preserved FXN-E levels.

We then investigated whether normalization to an internal blood-specific protein to account for volume changes might be useful for improving associations. We used a smaller sub-cohort in which hemoglobin values were available. Hemoglobin clinically provides a marker of acute hydration state. Demographic values in this sub-cohort were similar to the overall study, and the cohort was 57% women (Supplementary Table 4). Although the smaller sample size ($n = 27$) made R^2 values in linear regressions lower than in the complete cohort, relationships between frataxin levels and age, GAA1 and sex were generally similar, with FXN-M levels being less well predicted than FXN-E levels (Tables 4, 5). We then assessed whether the inclusion of hemoglobin would increase the R^2 values and significance of the linear regression model. Including hemoglobin values improved the R^2 value for the prediction of FXN-M in blood from 0.033 (NS) to 0.20 ($p = 0.06$), and hemoglobin itself predicted FXN-M levels ($p = 0.022$). Similarly, including hemoglobin improved the R^2 for modeling FXN-E values from 0.38 ($p = 0.003$) to 0.51 ($p = 0.0005$), and the significance of GAA1. Furthermore, hemoglobin itself predicted FXN-E levels, and its inclusion in the model removed the independent effect of sex, suggesting that the effect of sex is mediated by differences in hemoglobin levels between men and women.

Discussion

In this study, FXN-E and FXN-M levels determined by a triple quadrupole LC–MS assay predicted neurological outcomes, demonstrating their utility as a potential biomarker of disease status. In addition, such levels were predicted by GAA1 length, again consistent with the use of frataxin as a marker of disease status. The present results are more consistent than our previous results from lateral flow assays in several ways [17]. First, while results from lateral flow assays predict neurological and genetic variables in FRDA, such results required more subjects (several hundred) and

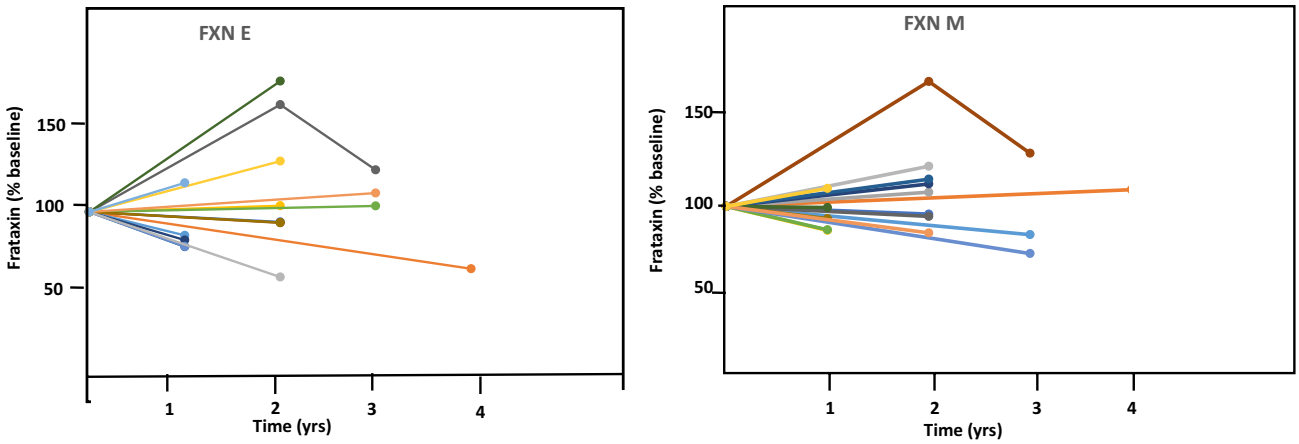

Fig. 1 Frataxin values over time. FXN-M and FXN-E were measures multiple times over several years in a group of subjects with FRDA. No consistent change was observed in either FXN-M or FXN-E

Table 4 Effect of Hemoglobin on FXN-M values

Variable	Coefficient	Significance
Age	0.0024 + 0.039	0.95
GAA1	−0.0028 + 0.0020	0.18
Sex	−0.59 + 0.51	0.25
Hb included		
Age	−0.021 + 0 0.036	0.56
GAA1	−0.0031 + 0.0018	0.10
Sex	0.40 + 0.61	0.52
Hemoglobin	0.028 + 0 0.011	0.022

Multiple linear regression analysis was performed for evaluation of the dependent variable FXN-M as predicted by age, sex, and frataxin levels (with or without hemoglobin as an additional independent variable). Regression coefficients for each are given along with *p* values. In this small cohort, only hemoglobin predicted FXN-M values

Table 5 Effect of Hemoglobin on FXN-E values

V	Coefficient	Significance
Age	−0.0051 ± 0.14	0.97
GAA1	−0.025 ± 0.007	0.003
Sex	−4.15 ± 1.87	0.037
HB included		
Age	−0.096 ± 0.1325	0.474
GAA1	−0.026 ± 0.007	0.001
Sex	−0.32 ± 2.23	0.886
Hemoglobin	0.108 ± 0.042	0.017

Multiple linear regression analysis was performed for evaluation of the dependent variable FXN-M as predicted by age, sex, and frataxin levels (with or without hemoglobin as an additional independent variable). Regression coefficients for each are given along with *p* values. In this small cohort, hemoglobin predicted FXN-M values, and the inclusion of hemoglobin as an additional independent variable improved the significance of GAA1

repeated assays in each subject to obtain consistent results [17]. The lateral flow assay also requires normalization to more factors (tissue protein, internal standards) for consistent results [17]. The present results utilized a single assay in a cohort of roughly the same size as typical medium-sized clinical trials. In addition, the variability of individual subjects across time was lower than the difference between patients and carriers (the proposed target of frataxin restoration trials), suggesting that the present assay can be used in serial analysis in clinical trial situations [17].

Still, there are several aspects to be considered in the use blood as a surrogate tissue in clinical trials or other studies in FRDA. First blood is a heterogeneous tissue, with multiple cell types contributing to frataxin levels [27]. Those cell types (RBC, platelets, and the multiple types of WBC) all vary in concentration in normal blood [28]. Consequently, normalizing values per mL of blood should allow a significant amount of variability (from about 25% for RBC to 33% for any single WBC type to 50% for platelets). Such normal variability in cell types could be a contributor to the variability in FXN measurements in blood. FXN-E is thought to be derived essentially only from RBC in whole blood [12]. Consequently, the variability in frataxin can be accounted for by normalizing with hemoglobin values, a marker in most people of the number of RBC (Tables 4, 5). This also explains the trend for women to have slightly lower FXN-E levels but not FXN-M levels. Unfortunately, multiple cell types contribute to FXN-M levels, making normalization very complex at best even if precise levels of different cell types were available. Thus, results in clinical studies may require interpretation based on blood counts and normalization of FXN-E values based on hemoglobin values. If frataxin levels are used as a marker in clinical studies of other tissues, similar considerations may be relevant,

In heart, most of the relevant frataxin is presumed to be in cardiomyocytes, However, the progressive fibrosis of FRDA cardiomyopathy might confound direct measurements of frataxin in cardiac tissue unless the exact level of fibrosis and fibroblast infiltration are considered [29]. In addition, although gliosis is not usually a prominent feature of FRDA, similar considerations might occasionally be relevant to frataxin measurements in brain.

Differences in cell type levels may explain some of the paradoxical statistical results. Although deficient FXN-E levels likely contribute minimally to neurological dysfunction in FRDA based on the severe phenotype of patients whose FXN-E level is spared, FXN-E levels correlate better with GAA1 than FXN-M and predict mFARS and FDS levels more significantly than FXN-M. This may reflect the lower variability in routine RBC levels as compared with WBC and platelets.

The predicted increase in both isoforms of frataxin in blood with age is interesting. In fibroblasts an expansion of 9 GAA repeats per year is predicted, which would logically lead to a fall in frataxin [30]. However, in vivo selection of cells over time likely would lead to enhanced survival of cells with shorter GAA repeats, as appears to occur following chemotherapy [31]. In the present study, we could not directly demonstrate a change in frataxin levels over time in subjects with FRDA, even though linear regression predicted such changes. One reason is that the predicted change (between 1 and 2% of the total) is far lower than the technical reproducibility of the assay and the variability of blood cell types in blood (Table 1; Supplementary Table 1) [25, 27, 28]. Based on the expected reproducibility, 10 years would be needed to potentially identify such increases even without accounting for the variability of blood cell types. Another possibility is that the predicted increase may represent a statistical aberration in the regression models, possibly because of the small size of the predicted effect. Thus, whether a small increase in frataxin actually occurs is unclear.

Overall, the present results from blood show that frataxin levels determined by the present assay provides useful biomarkers for therapies in which changes in blood frataxin levels are representative of changes in frataxin levels in target tissues. This would include agents acting on most tissues (gamma interferon, HDAC inhibitors like RPG2833, Tat-frataxin) but not most forms of gene therapy, in which specific tissues are targeted and many constructs lack promoters that are active in blood [32–36]. In addition, the novel gene targeted chimera (gene-TAC) agent DT-216 appears to transit the circulation too rapidly to produce measurable effects on blood frataxin. The present study is limited by the moderate size of the present cohort and the lack of truly affected solid tissues. However, the present LC–MS approach can be adapted for solid tissues (muscle, heart) to provide a methodology for extension of LC–MS to the assessment of frataxin levels from other therapeutic approaches such as gene therapy [36].

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-023-12118-x.

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Data availability The data that support the findings of this study are available from author D.R.L., upon reasonable request.

Declarations

Conflicts of interest None.

Ethical standard statement All procedures were approved by the Children's Hospital of Philadelphia (CHOP) IRB and conducted in line with the ethical rules for data collection.

References

1. Campuzano V, Montermini L, Molto MD, Pianese L, Cossee M, Cavalcanti F, Monros E, Rodius F, Duclos F, Monticelli A, Zara F, Canizares J, Koutnikova H, Bidichandani SI, Gellera C, Brice A, Trouillas P, De Michele G, Filla A, De Frutos R, Palau F, Patel PI, Di Donato S, Mandel JL, Cocozza S, Koenig M, Pandolfo M (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 271:1423–1427
2. Durr A, Cossee M, Agid Y, Campuzano V, Mignard C, Penet C, Mandel JL, Brice A, Koenig M (1996) Clinical and genetic abnormalities in patients with Friedreich's ataxia. N Engl J Med 335:1169–1175
3. Pandolfo M (2012) Friedreich ataxia. Handb Clin Neurol 103:275–294
4. Babady NE, Carelle N, Wells RD, Rouault TA, Hirano M, Lynch DR, Delatycki MB, Wilson RB, Isaya G, Puccio H (2007) Advancements in the pathophysiology of Friedreich's Ataxia and new prospects for treatments. Mol Genet Metab 92:23–35
5. Pandolfo M (2009) Friedreich ataxia: the clinical picture. J Neurol 256(Suppl 1):3–8
6. Chutake YK, Lam C, Costello WN, Anderson M, Bidichandani SI (2014) Epigenetic promoter silencing in Friedreich ataxia is dependent on repeat length. Ann Neurol 76:522–528
7. Chutake YK, Costello WN, Lam CC, Parikh AC, Hughes TT, Michalopulos MG, Pook MA, Bidichandani SI (2015) FXN Promoter Silencing in the Humanized Mouse Model of Friedreich Ataxia. PLoS ONE 10:e0138437
8. Cossee M, Durr A, Schmitt M, Dahl N, Trouillas P, Allinson P, Kostrzewa M, Nivelon-Chevallier A, Gustavson KH, Kohlschutter A, Muller U, Mandel JL, Brice A, Koenig M, Cavalcanti F, Tammaro A, De Michele G, Filla A, Cocozza S, Labuda M, Montermini L, Poirier J, Pandolfo M (1999) Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes. Ann Neurol 45:200–206
9. Galea CA, Huq A, Lockhart PJ, Tai G, Corben LA, Yiu EM, Gurrin LC, Lynch DR, Gelbard S, Durr A, Pousset F, Parkinson M, Labrum R, Giunti P, Perlman SL, Delatycki MB, Evans-Galea MV (2016) Compound heterozygous FXN mutations and clinical outcome in friedreich ataxia. Ann Neurol 79:485–495
10. McCormack ML, Guttmann RP, Schumann M, Farmer JM, Stolle CA, Campuzano V, Koenig M, Lynch DR (2000) Frataxin point

mutations in two patients with Friedreich's ataxia and unusual clinical features. J Neurol Neurosurg Psychiatry 68:661–664
11. Lazaropoulos M, Dong Y, Clark E, Greeley NR, Seyer LA, Brigatti KW, Christie C, Perlman SL, Wilmot GR, Gomez CM, Mathews KD, Yoon G, Zesiewicz T, Hoyle C, Subramony SH, Brocht AF, Farmer JM, Wilson RB, Deutsch EC, Lynch DR (2015) Frataxin levels in peripheral tissue in Friedreich ataxia. Ann Clin Transl Neurol 2:831–842
12. Guo L, Wang Q, Weng L, Hauser LA, Strawser CJ, Mesaros C, Lynch DR, Blair IA (2018) Characterization of a new N-terminally acetylated extra-mitochondrial isoform of frataxin in human erythrocytes. Sci Rep 8:17043
13. Wang Q, Laboureur L, Weng L, Eskenazi NM, Hauser LA, Mesaros C, Lynch DR, Blair IA (2022) Simultaneous Quantification of Mitochondrial Mature Frataxin and Extra-Mitochondrial Frataxin Isoform E in Friedreich's Ataxia Blood. Front Neurosci 16:874768
14. Deutsch EC, Oglesbee D, Greeley NR, Lynch DR (2014) Usefulness of frataxin immunoassays for the diagnosis of Friedreich ataxia. J Neurol Neurosurg Psychiatry 85:994–1002
15. Oglesbee D, Kroll C, Gakh O, Deutsch EC, Lynch DR, Gavrilova R, Tortorelli S, Raymond K, Gavrilov D, Rinaldo P, Matern D, Isaya G (2013) High-throughput immunoassay for the biochemical diagnosis of Friedreich ataxia in dried blood spots and whole blood. Clin Chem 59:1461–1469
16. Nachbauer W, Wanschitz J, Steinkellner H, Eigentler A, Sturm B, Hufler K, Scheiber-Mojdehkar B, Poewe W, Reindl M, Boesch S (2011) Correlation of frataxin content in blood and skeletal muscle endorses frataxin as a biomarker in Friedreich ataxia. Mov Disord 26:1935–1938
17. Deutsch EC, Santani AB, Perlman SL, Farmer JM, Stolle CA, Marusich MF, Lynch DR (2010) A rapid, noninvasive immunoassay for frataxin: utility in assessment of Friedreich ataxia. Mol Genet Metab 101:238–245
18. Willis JH, Isaya G, Gakh O, Capaldi RA, Marusich MF (2008) Lateral-flow immunoassay for the frataxin protein in Friedreich's ataxia patients and carriers. Mol Genet Metab 94:491–497
19. Steinkellner H, Scheiber-Mojdehkar B, Goldenberg H, Sturm B (2010) A high throughput electrochemiluminescence assay for the quantification of frataxin protein levels. Anal Chim Acta 659:129–132
20. Weng L, Wang Q, Yu S, Yang X, Lynch DR, Mesaros C, Blair IA (2019) Evaluation of antibodies for western blot analysis of frataxin protein isoforms. J Immunol Methods 474:112629
21. Weng L, Laboureur L, Wang Q, Guo L, Xu P, Gottlieb L, Lynch DR, Mesaros C, Blair IA (2020) Extra-mitochondrial mouse frataxin and its implications for mouse models of Friedreich's ataxia. Sci Rep 10:15788
22. Guo L, Wang Q, Weng L, Hauser LA, Strawser CJ, Rocha AG, Dancis A, Mesaros C, Lynch DR, Blair IA (2018) Liquid chromatography-high resolution mass spectrometry analysis of platelet frataxin as a protein biomarker for the rare disease Friedreich's Ataxia. Anal Chem 90:2216–2223
23. Rojsajjakul T, Wu L, Grady CB, Hwang WT, Mesaros C, Lynch DR, Blair IA (2023) Liquid Chromatography-Mass Spectrometry Analysis of Frataxin Proteoforms in Whole Blood as Biomarkers of the Genetic Disease Friedreich's Ataxia. Anal Chem 95:4251–4260
24. Lynch DR, Farmer JM, Tsou AY, Perlman S, Subramony SH, Gomez CM, Ashizawa T, Wilmot GR, Wilson RB, Balcer LJ (2006) Measuring Friedreich ataxia: complementary features of examination and performance measures. Neurology 66:1711–1716
25. Friedman LS, Farmer JM, Perlman S, Wilmot G, Gomez CM, Bushara KO, Mathews KD, Subramony SH, Ashizawa T, Balcer LJ, Wilson RB, Lynch DR (2010) Measuring the rate of progression in Friedreich ataxia: implications for clinical trial design. Mov Disord 25:426–432
26. Rummey C, Corben LA, Delatycki MB, Subramony SH, Bushara K, Gomez CM, Hoyle JC, Yoon G, Ravina B, Mathews KD, Wilmot G, Zesiewicz T, Perlman S, Farmer JM, Lynch DR (2019) Psychometric properties of the Friedreich Ataxia Rating Scale. Neurol Genet 5:371
27. Lichtman MA, Kaushansky K, Prchal JT, Levi MM, Burns LJ, Linch DC (2022) Williams manual of hematology. MgGraw Hill, New York
28. Palka T, Koteja PM, Tota L, Rydzik L, Kopanska M, Kaczorowska I, Javdaneh N, Mikulakova W, Wolski H, Ambrozy T (2023) The Influence of Various Hydration Strategies (Isotonic, Water, and No Hydration) on Hematological Indices, Plasma Volume, and Lactate Concentration in Young Men during Prolonged Cycling in Elevated Ambient Temperatures. Biology (Basel) 12
29. Payne RM (2022) Cardiovascular Research in Friedreich ataxia: unmet needs and opportunities. JACC Basic Transl Sci 7:1267–1283
30. Long A, Napierala JS, Polak U, Hauser L, Koeppen AH, Lynch DR, Napierala M (2017) Somatic instability of the expanded GAA repeats in Friedreich's ataxia. PLoS ONE 12:e0189990
31. Deutsch EC, Seyer LA, Perlman SL, Yu J, Lynch DR (2012) Clinical monitoring in a patient with Friedreich ataxia and osteogenic sarcoma. J Child Neurol 27:1159–1163
32. Plasterer HL, Deutsch EC, Belmonte M, Egan E, Lynch DR, Rusche JR (2013) Development of frataxin gene expression measures for the evaluation of experimental treatments in Friedreich's ataxia. PLoS ONE 8:e63958
33. Seyer L, Greeley N, Foerster D, Strawser C, Gelbard S, Dong Y, Schadt K, Cotticelli MG, Brocht A, Farmer J, Wilson RB, Lynch DR (2015) Open-label pilot study of interferon gamma-1b in Friedreich ataxia. Acta Neurol Scand 132:7–15
34. Lynch DR, Hauser L, McCormick A, Wells M, Dong YN, McCormack S, Schadt K, Perlman S, Subramony SH, Mathews KD, Brocht A, Ball J, Perdok R, Grahn A, Vescio T, Sherman JW, Farmer JM (2019) Randomized, double-blind, placebo-controlled study of interferon-gamma 1b in Friedreich Ataxia. Ann Clin Transl Neurol 6:546–553
35. Strawser CJ, Schadt KA, Lynch DR (2014) Therapeutic approaches for the treatment of Friedreich's ataxia. Expert Rev Neurother 14:949–957
36. Rojsajjakul T, Hordeaux JJ, Chaudhury GR, Hinderer CJ, Mesaros C, Wilson JM, Blair IA (2023) Quantification of human mature frataxin protein expression in nonhuman primate hearts after gene therapy. Commun Biol. 6(1):1093