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Novel Diagnostic Paradigms for Friedreich Ataxia

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Abstract

Friedreich ataxia is the most common inherited ataxia, with a wide phenotypic spectrum. It is generally caused by GAA expansions on both alleles of *FXN*, but a small percentage of patients are compound heterozygotes for a pathogenic expansion and a point mutation. Two recent diagnostic innovations are further characterizing individuals with the phenotype but without the classic genotypes. First, lateral-flow immunoassay is able to quantify the frataxin protein, thereby further characterizing these atypical individuals as likely affected or not affected, and providing some correlation to phenotype. It also holds promise as a biomarker for clinical trials in which the investigative agent increases frataxin. Second, gene dosage analysis and the identification of affected individuals with gene deletions introduce a novel genetic mechanism of disease. Both tests are now clinically available and suggest a new diagnostic paradigm for the disorder. Genetic counseling issues and future diagnostic testing approaches are considered as well.

Keywords

diagnosis; exon deletion; frataxin; Friedreich ataxia; next-generation sequencing

Friedreich ataxia is the most commonly inherited ataxia syndrome, accounting for half of the inherited progressive ataxias and three-quarters of those with onset before age 25.¹ It is inherited in an autosomal recessive manner, and appears most prevalent among those of European, Middle Eastern, North African, and Indian descent.² Since it was first characterized in 1863,³ its diagnosis historically depended on clinical findings using

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Author Contributions

JF and KB conducted the background literature searches, wrote the first draft of the manuscript, and provided critical review. ED conducted the scientific studies, and provided critical review of the manuscript. DL contributed to drafting and revising the manuscript and supervised the scientific studies.

Declaration of Conflicting Interests

The authors declare no conflicts of interest relating to authorship or publication of this article.

Ethical Approval

The Institutional Review boards of the Children's Hospital of Philadelphia and the University of California at Los Angeles approved the sample collection for frataxin protein analysis as well as the collection of demographic and neurological data. Written informed consent was obtained before any procedures were performed.

established criteria, namely onset before age 20, truncal and limb ataxia, and loss of deep tendon reflexes, followed by loss of proprioceptive and vibratory senses and dysarthria. The development of cardiomyopathy and scoliosis is not pathognomonic, but highly characteristic of the condition.^{4,5} Upon the discovery of *FXN* in 1996 and the introduction of confirmatory genetic testing,⁶ the wide phenotypic variability of the condition (discussed by Delatycki and colleagues in this issue) became more apparent, with up to 25% of individuals with homozygous GAA expansion mutations (approximately 95% to 98% of affected individuals) exhibiting clinical findings that do not perfectly match the classic clinical picture.^{7–10} For example, individuals of Acadian descent with Friedreich ataxia appear to have a milder disease course while exhibiting the classic expansion genotype.¹¹

Molecular studies of the pathogenic GAA expansion have explained some of this phenotypic range, as they have shown the repeat expansion to be inversely related to age of onset and severity of disease, such that individuals with lower GAA repeat expansions often exhibit a later age of onset, sometimes well into adulthood, and often have a milder, variant phenotype.¹² Compound heterozygotes (with a pathogenic GAA expansion on one allele and a point mutation on the second) make up a minority of those with Friedreich ataxia (2% to – 5%) and often exhibit a nonclassical phenotype as well.^{13,14} Less severe phenotypes are associated with some of the missense mutations affecting the amino terminal portion of the protein, such as G130V, R165C, R165P, and D122Y. These individuals often exhibit spastic paraparesis with minimal ataxia, brisk reflexes, absence of scoliosis and cardiomyopathy, normal speech, and slower progression of disease.^{14,15}

While no *FXN* point mutation homozygotes have been identified to date, this genetic combination seems theoretically possible. Either 2 loss-of-function alleles may be embryonically lethal or the phenotype of those individuals may lie so far outside the associated features of Friedreich ataxia that the condition is not even considered within the differential diagnoses.

Two recent advances have changed the diagnostic landscape for Friedreich ataxia, and potentially further expanded an already broad spectrum of disease. The first is the introduction of an immunoassay-based test that measures the concentration of the frataxin protein in both whole blood and buccal cells, and readily identifies differences in frataxin levels between controls, carriers, and affected individuals.^{16–18} This test has multiple applications to clinical care and research.^{19,20} It is especially useful as an adjunct diagnostic test that can easily, noninvasively, and inexpensively help the clinician determine whether Friedreich ataxia should remain under diagnostic consideration, and further supports a diagnosis of Friedreich ataxia in those individuals without confirmatory genetic testing in the context of suggestive clinical findings and frataxin in the affected range. In addition, this assay holds promise as a biomarker for future clinical trials in which frataxin levels are increased following introduction of a potentially therapeutic agent, such as the proof-of-concept study of recombinant human erythropoietin recently undertaken in Austria,¹⁹ and may be useful in monitoring patients with Friedreich ataxia when exposed to other potentially hazardous agents, as discussed by Deutsch and colleagues in this issue.

The second development is the identification of whole exon deletions within *FXN* via multiplex ligation-dependent probe analysis. This technique and the subsequent identification of affected individuals who are compound heterozygous with intragenic deletions and GAA expansions^{17,21} suggest a wider range of disease-causing mutation types than previously believed. This additional DNA-based diagnostic tool may provide definitive genetic diagnosis for a number of individuals previously characterized as “Friedreich-like” without confirmatory genetic testing by existing DNA-based testing.

We discuss these novel diagnostic techniques for Friedreich ataxia in this paper, and a diagnostic testing algorithm for Friedreich ataxia incorporating them is proposed to guide the clinician when considering a diagnosis of Friedreich ataxia. In addition, the phenotype of the individuals who are compound heterozygotes for intragenic exon deletions is further characterized. The counseling implications of these new diagnostic tools are explored. Lastly, potential future approaches to diagnosis of this condition are considered.

Protein-based Immunoassay

Several research groups have used Western blots, electrochemiluminescence, or lateral-flow immunoassay to reliably quantify frataxin levels among various cell types (see Table 1).^{16–18,22,23} Frataxin levels have been reproducibly quantified in controls, carriers, and affected individuals, including those with late-onset Friedreich ataxia or a point mutation. While the various techniques clearly and reproducibly distinguish the frataxin levels between affected and unaffected individuals, the lateral-flow immunoassay appears to be the least laborious to perform.¹⁷

Some information on phenotype can be inferred from the frataxin assay that may be useful in some instances for counseling patients and families. Various studies support an inverse relationship between frataxin and GAA repeat number, and a direct correlation with age of onset.^{17,18} Frataxin levels do not appear to correlate with current severity of disease, indicating they reveal biologic severity of disease but are not impacted by clinical status.¹⁷ The immunoassay is less informative for individuals with late-onset disease and those with point mutations. Individuals with late-onset Friedreich ataxia have some overlap of frataxin levels with carriers and controls^{17,18} and their phenotype cannot be predicted on the basis of their frataxin.¹⁸ Ultimately, the mean value from serial frataxin testing in late-onset individuals or those with borderline results may reflect the most accurate measure, somewhat akin to repeat creatine kinase sampling once used to determine carrier status in Duchenne muscular dystrophy before the advent of genetic testing.²⁴

Frataxin studies of individuals with point mutations have also shown that protein levels do not correlate with the phenotypic severity associated with those mutations.¹⁷ While most point mutations tested had frataxin levels firmly in the affected range, individuals with the R165P and R165C mutations had values well into the carrier range.^{17,18} It has been suggested that some point mutations produce a dysfunctional frataxin that is immunoreactively normal, allowing for the assay to detect it as residual frataxin.¹⁸ More extensive molecular studies of Friedreich ataxia point mutations may further explain this finding.

Lateral flow immunoassay for frataxin is now commercially available²⁵ and has the potential to serve as an important diagnostic tool for the clinician, inexpensively and rapidly, supporting or refuting the diagnosis of Friedreich ataxia in many cases. While it can be equivocal with some point mutation patients, it nonetheless compels the clinician to pursue additional genetic testing for those individuals with the characteristic phenotype but only one identified pathogenic GAA expansion, those whose genetic testing has been altogether unrevealing, or those with an atypical phenotype for which Friedreich ataxia is under diagnostic consideration. Such atypical patients may be candidates for expanded genetic testing to include gene dosage studies. Indeed, frataxin levels in the affected range in the context of the disease phenotype may be sufficient for those individuals to soundly transition from probably to definitive diagnosis.

Gene Dosage Studies

The first case of a patient with Friedreich ataxia due to an exon deletion in *FXN* and a pathogenic GAA expansion was reported in 2004 by Zülke and colleagues. The combination approach of the research-based frataxin immunoassay and gene dosage studies via multiplex-dependent ligation analysis has been used to further characterize atypical patients with the disease phenotype but without two known *FXN* mutations.¹⁷ This approach by Deutsch and colleagues has identified another individual heterozygous for a pathogenic GAA expansion and an intragenic deletion of exons 2 and 3. Her frataxin level was in the affected range (19.5%), and like the other individual with a deletion of exon 5a, her disease features and progress have been similar to the classic phenotype.¹⁷ The phenotypes of both individuals with identified exon deletions in *FXN* are summarized in Table 2.

This approach on subsequent subjects has revealed an unpublished case of another deletion of exon 5 in an 11-year-old boy with 2.1% frataxin compared with controls, a GAA repeat size of 867, and classic disease with a severe presentation. His younger brother has recently developed disturbances in gait and loss of deep tendon reflexes, and is undergoing similar diagnostic testing to confirm his disease status (Lynch DL, personal communication). Three other atypical subjects with a suggestive presentation and only one identified mutation have had frataxin in the affected range but unrevealing gene dosage studies.¹⁷ The clinical presentation, single identified mutation, and low frataxin in the context of normal gene dosage studies in these patients indicate an undetected second mutation resulting in disease. Additional genetic testing to detect mutations in introns, the promoter, or elements regulating gene expression may be warranted to identify the presumed second mutation.

Diagnostic Testing Algorithm for Friedreich Ataxia

Figure 1 outlines a suggested diagnostic testing approach to diagnosis using these novel techniques. For individuals with a clinical presentation suggestive of Friedreich ataxia, targeted GAA expansion analysis should be pursued, which will detect the vast majority of affected individuals. If no expansions are identified on testing but the presentation, ethnic descent, and family history are consistent with Friedreich ataxia, quantitative frataxin protein analysis should be pursued to determine whether additional genetic testing is warranted. Indeed, if those individuals do not have frataxin levels in the affected range, other inherited ataxia syndromes should be considered. Should only one expansion be identified on testing, many commercial laboratories can reflexively sequence the gene to identify any nonsense mutations, more common splice site errors, as well as missense mutations, which appear to be mainly in the highly conserved, carboxy-terminal domain of the protein.²⁶ If sequencing is normal, quantitative frataxin protein analysis should be performed to determine if the patient is a coincidental carrier of Friedreich ataxia or instead has a second unidentified mutation, bearing in mind that individuals with late-onset disease or some point mutations may have equivocal frataxin results. Individuals with one identified mutation and low frataxin are ideal candidates for gene dosage studies, which are now clinically available in Europe and the United States.²⁷ If gene dosage studies are negative, but the patient and family desire confirmatory genetic testing for consideration in clinical drug trials or family planning purposes, they may opt to pursue next-generation sequencing, which remains largely a research tool at present, but likely will become a viable and affordable commercial option within the foreseeable future.

Genetic Counseling Implications

The addition of the frataxin immunoassay and genetic testing for exon deletions can improve diagnosis and genetic counseling for cases where standard genetic testing has not been confirmatory. However, the small number of patients with identified gene deletions at

present makes predicting the long-term prognosis and associated phenotype difficult for genetic counseling purposes. Nonetheless, testing for other family members interested in learning their carrier status can be easily pursued, and prenatal testing is possible. Since frataxin levels are associated with age of onset and disease severity and can be used to confirm diagnosis. Patients who lack genetic diagnosis but have low frataxin and the disease phenotype should be presumed to have Friedreich ataxia. However, genetic counseling for these patients is limited at present, as the absolute value of frataxin is not the only determinant of disease,¹⁸ and prenatal genetic counseling for these families is more complex, as fetal frataxin analysis is not yet possible and genetic testing using existing genetic diagnostic techniques will be similarly unrevealing.

Future Approaches

Individuals with Friedreich ataxia exhibit a wide spectrum of disease features, and substantial differences in age at onset have been noted within a single generation of the same family.²⁸ Indeed, there are likely various influences outside *FXN* itself that may alter the disease course. The Acadian variant supports this hypothesis; the disease appears to progress at a slower rate and is overall less severe than in non-Acadian individuals with the same genotype.¹¹ Until now, identifying these causal genetic variants within the genome has been cumbersome and elusive. However, whole exome sequencing, involving genome capture and massive parallel sequencing, is quickly transcending from a research-based tool to one available in clinical practice.²⁹ This next-generation DNA sequencing platform allows the use of custom captures to examine whole regions of the genome for disease-modifying variants, and has already been applied to rare disease.³⁰ These genetic variants will likely provide additional insight into the function and pathophysiology of *FXN*, and in the process become novel targets in therapeutic development for the recovery of the frataxin protein to carrier or control levels.¹⁷ Next-generation sequencing will also allow for closer examination of the *FXN* gene itself for mutations that remain undetected by current molecular testing technology. While whole exome sequencing will identify mutations in the coding regions of other genes, whole genome sequencing will be necessary to provide information on variants within the non-coding regions of the genome. The end goal of these studies in Friedreich ataxia is the identification of all disease-related variants within *FXN* itself as well as the vast genome, therefore obviating the need for frataxin protein analysis as a diagnostic tool in the future. However, proper interpretation of the immense amount of resulting data will be challenging, and solutions to this daunting task are being developed, such as the creation of databases for the accumulated data and scientific algorithms to predict their significance. Frataxin may be a useful biomarker in the determination of the functional consequences of any identified variants long after its utility as a diagnostic test has passed. Once the significant challenge of data interpretation has been overcome, identifying and characterizing these new causal variants will vastly expand the current understanding of disease pathophysiology, introduce new therapeutic targets, allow more precise genetic counseling, and provide truly personalized medicine based on the genomic profile.

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References

1. Harding AE. Classification of the hereditary ataxias and paraplegias. *Lancet*. 1983; 1:1151. [PubMed: 6133167]
2. Labuda M, Labuda D, Miranda C, et al. Unique origin and specific ethnic distribution of the Friedreich ataxia GAA expansion. *Neurology*. 2000; 54(12):2322–2324. [PubMed: 10881262]
3. Friedreich N. Über degenerative Atrophie der pinalen Hinterstränge. *Virchows Arch Pathol Anat*. 1863; 26:391.
4. Geoffrey G, Barbeau A, Breton G, et al. Clinical description and roentgenologic evaluation of patients with Friedreich's ataxia. *Can J Neurol Sci*. 1976; 3:279–286. [PubMed: 1087179]
5. Harding AE. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain*. 1981; 104:589–620. [PubMed: 7272714]
6. Campuzano V, Montermini L, Molto MD, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science*. 1996; 271:1423–1427. [PubMed: 8596916]
7. Dürr A, Cossee M, Agid Y, et al. Clinical and genetic abnormalities in patients with Friedreich's ataxia. *N Engl J Med*. 1996; 335:1169–1175. [PubMed: 8815938]
8. McCabe DJ, Ryan F, Moore DP, et al. Typical Friedreich's ataxia without GAA expansions and GAA expansion without typical Friedreich's ataxia. *J Neurol*. 2000; 247(5):346–355. [PubMed: 10896266]
9. Schöls L, Amoiridis G, Przuntek H, et al. Friedreich's ataxia. Revision of the phenotype according to molecular genetics. *Brain*. 1997; 120(Pt 12):2131–2140. [PubMed: 9448568]
10. Filla A, De Michele G, Coppola G, et al. Accuracy of clinical diagnostic criteria for Friedreich's ataxia. *Mov Disord*. 2000; 15(6):1255–1258. [PubMed: 11104216]
11. Montermini L, Richter A, Morgan K, et al. Phenotypic variability in Friedreich ataxia: role of the associated GAA triplet repeat expansion. *Ann Neurol*. 1997; 41:675–682. [PubMed: 9153531]
12. Bhidayasiri R, Perlman S, Pulst SM, Geschwind DH. Late-onset Friedreich ataxia: phenotypic analysis, magnetic resonance imaging findings, and review of the literature. *Arch Neurol*. 2005; 62:1865–1869. [PubMed: 16344344]
13. De Castro M, Garcia-Planells J, Monros E, et al. Genotype and phenotype analysis of Friedreich's ataxia compound heterozygous patients. *Hum Genet*. 2000; 106:86–92. [PubMed: 10982187]
14. Cossee M, Dürr A, Schmitt M, et al. Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes. *Ann Neurol*. 1999; 45:200–206. [PubMed: 9989622]
15. Forrest SM, Knight M, Delatycki MB, et al. The correlation of clinical phenotype in Friedreich ataxia with the site of point mutations in the FRDA gene. *Neurogenetics*. 1998; 1:253–257. [PubMed: 10732799]
16. Willis JH, Isaya G, Gakh O, et al. Lateral-flow immunoassay for the frataxin protein in Friedreich's ataxia patients and carriers. *Molr Genet Metab*. 2008; 94:491–497.
17. Deutsch EC, Santani AB, Perlman SL, et al. A rapid, noninvasive immunoassay for frataxin: Utility in assessment of Friedreich ataxia. *Mol Genet Metab*. 2010; 101:238–425. [PubMed: 20675166]
18. Saccà F, Puorro G, Antenora A, et al. A combined nucleic acid and protein analysis in Friedreich ataxia: implications for diagnosis, pathogenesis and clinical trial design. *PLoS One*. 2011; 6(3):1–9.
19. Boesch S, Sturm B, Hering S, et al. Friedreich's ataxia: clinical pilot trial with recombinant human erythropoietin. *Ann Neurol*. 2007; 62:521–524. [PubMed: 17702040]

20. Nachbauer W, Wanschitz J, Steinkellner H, et al. Correlation of frataxin content in blood and skeletal muscle endorses frataxin as a biomarker in Friedreich ataxia. *Mov Disord.* 2011; 26(10): 1935–1938. [PubMed: 21692115]
21. Zühlke CH, Dalski A, Habeck M, et al. Extension of the mutation spectrum in Friedreich's ataxia: detection of an exon deletion and novel missense mutations. *Eur J Hum Genet.* 2004; 12:979–982. [PubMed: 15340363]
22. Campuzano V, Montermini L, Lutz Y, et al. Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. *Hum Mol Genet.* 1997; 6(11):1771–1780. [PubMed: 9302253]
23. Steinkellner H, Scheiber-Mojdehkar, Goldenberg H, Sturm B. A high throughput electrochemiluminescence assay for the quantification of frataxin protein levels. *Analytica Chimica Acta.* 2010; 659:129–132. [PubMed: 20103114]
24. Percy ME, Andrews DF, Thompson MW. Serum creatine kinase in the detection of duchenne muscular dystrophy carriers: effects of season and multiple testing. *Muscle Nerve.* 1982; 5:58–64. [PubMed: 7057807]
25. Mayo Foundation for Medical Education and Research, Mayo Clinic Mayo Medical Laboratories. Test catalog, Test ID FFRWB: Friedreich Ataxia, Frataxin, Quantitative, Whole Blood. 2012 Jan 03. [Online]. Available: <http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretative/60477>
26. Pandolfo M, Pastore A. The pathogenesis of Friedreich ataxia and the structure and function of frataxin. *J Neurol.* 2009; 256(Suppl 1):9–17. [PubMed: 19283345]
27. National Center for Biological Information (NCBI). GeneTests. Friedreich Ataxia Clinical Testing. 2012 Jan 03. [Online]. Available: http://www.ncbi.nlm.nih.gov/sites/GeneTests/lab/clinical_disease_id/2227?db=genetests
28. Klopstock T, Chahrokh-Zahed S, Holinski-Feder E, et al. Markedly different course of Friedreich's ataxia in sib pairs with similar GAA repeat expansions in the frataxin gene. *Acta Neuropathol.* 1999; 97:139–142.
29. Majewski J, Schwartzentruber J, Lalonde E, et al. What can exome sequencing do for you? *J Med Genet.* 2011; 48:580–589. [PubMed: 21730106]
30. Bamshad MJ, Ng SB, Bigham AW, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev.* 2011; 12:745–755.
31. Teer JK, Mullikin JC. Exome sequencing: the sweet spot before whole genomes. *Hum Mol Genet.* 2010; 19(R2):R145–R151. [PubMed: 20705737]

Figure 1. Proposed Diagnostic Algorithm for Friedreich Ataxia Testing

Abbreviations: FRDA¹, homozygous GAA expansions; FRDA², compound heterozygous with a GAA expansion and a point mutation; FRDA³, compound heterozygous with a GAA expansion and an intragenic deletion; FRDA⁴, compound heterozygous with a GAA expansion/ point mutation and an unidentified mutation in the context of low frataxin; FRDA⁵, homozygous point mutations (not yet reported); FRDA⁶, compound heterozygous with a point mutation and an intragenic deletion (not yet reported). FRDA, Friedreich ataxia; GAA, GAA expansion; GD, gene dosage; PM, point mutation.

Table 1

Average Frataxin Protein Levels in Controls, Known Carriers, and Patients

Study	Method	Cell source	% frataxin	relative to controls
			Carriers	Affected
Campuzano <i>et al</i> , 1997	Western blot	Lymphoblasts	ND	4–29 (n = 7)
Willis <i>et al</i> , 2008	Lateral flow immunoassay	Lymphoblasts	64 (n = 4)	29 (n = 7)
Steinkellner <i>et al</i> , 2010	Electrochemiluminescence	Lymphocytes	ND	27 (n = 11)
Deutsch <i>et al</i> , 2010	Lateral flow immunoassay	Buccal cells	50.5 (n = 81)	21.1 (n = 195)
		Whole blood	81.7 (n = 18)	32.2 (n = 52)
Saccà <i>et al</i> , 2011	Lateral flow immunoassay	PBMC	68.7 (n = 33)	35.8 (n = 36)

Abbreviations: ND, not determined; PBMC, peripheral blood mononuclear cells.

Table 2Clinical Features in Patients With *FXN* Exon Deletions

Study	Zühlke <i>et al</i> 2004	Deutsch <i>et al</i> 2010
Genotype (GAA repeats/ exon deletion)	820/ del 5a	600/del 2–3
Age of onset	9	10
Gait ataxia	+	+
Limb ataxia	+	+
Age wheelchair-bound	15	14
Proprioceptive deficits	+	+
Vibratory sense deficits	+	+
Lower limb weakness		–
Dysarthria	+	–
Loss of deep tendon reflexes	+	+
Hypertrophic cardiomyopathy	+	+
Kyphoscoliosis	+	+
Optic atrophy		–
Diabetes		–
Pes cavus	+	+
Hearing loss		–
Urinary dysfunction		–
Thenar atrophy		+