Functional genomic analysis of frataxin deficiency reveals tissue-specific alterations and identifies the PPARγ pathway as a therapeutic target in Friedreich’s ataxia

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INTRODUCTION

Friedreich’s Ataxia (FRDA, OMIM #229300) is an inherited autosomal recessive disorder characterized by progressive neurologic disability, cardiomyopathy and, in some patients, diabetes mellitus (1). FRDA is caused by partial deficiency of the mitochondrial protein frataxin (2). Though the function of frataxin is still partly controversial, there is general agreement that it is involved in cellular iron homeostasis and that its deficiency results in multiple enzyme deficits, mitochondrial dysfunction, and oxidative damage. Complete absence of frataxin is incompatible with life in higher organisms, as demonstrated by the embryonic lethality observed in systemic and conditional gene knock-out models (3–6).

Residual frataxin amounts vary between 5 and 35% of normal levels in FRDA patients, and are little more than 50% in heterozygous carriers, who show no sign of disease (2). A clear pattern of selective vulnerability to this partial loss of frataxin can be observed in FRDA. In the nervous system, neurodegeneration affects the large primary sensory neurons in the dorsal root ganglia and their axons in peripheral nerves and in the dorsal columns of the spinal cord, the spinocerebellar pathways, the dentate nucleus in the cerebellum, the...
distal part of the corticospinal tract and, to a variable extent, the auditory and visual pathways. Cardiac involvement is present in most patients at the preclinical level, and 45–63% of FRDA patients have hypertrophic cardiomyopathy, which can cause premature death (7,8). Overt diabetes is present in 14–19% of FRDA patients, and glucose intolerance is seen in 24–40% (9–11). Skeletal muscle is only sub-clinically involved in FRDA, as a reduced ATP production can be detected with MR spectroscopy (12), but the causal metabolic pathways have not been identified.

Reducing frataxin expression in mice to levels similar to those observed in FRDA (KIKO mice) induced a biochemical CNS phenotype (13) but not clinical abnormalities (14). To study the selective vulnerability to frataxin deficiency in a model where no confounding non-specific or secondary degenerative changes are expected to occur, we have utilized frataxin-deficient mice to investigate gene expression profiles in the heart, a major target of FRDA pathology, and in skeletal muscle, only sub-clinically affected in the human disease. This unbiased genome-wide analysis, which we further extended to liver and cellular models, confirmed the hypothesis that frataxin deficiency causes tissue-specific changes in several metabolic pathways. Involvement of the peroxisome-proliferator activator receptor gamma (PPARγ)/PPARγ coactivator 1 alpha (Pgc1a) pathway in several cell types may serve as key regulator of FRDA pathogenesis, including enhanced risk of diabetes and cardiomyopathy.

RESULTS

Functional genomic analysis of frataxin deficient mice

We analyzed tissue from heart and skeletal muscle from four KIKO mice compared with control littermates. We used two array platforms (Agilent G4121A and Illumina Mouse RefSeq Expression arrays, see Materials and Methods), in order to increase our detection power. Unsupervised array clustering clearly separates the two tissues (Fig. 1A), confirming distinct expression profiles. Because cardiac involvement is most clinically evident, we expected more changes in the cardiac tissue than skeletal muscle. However, in KIKO mice versus WT littermates, we found more differentially expressed (DE) genes in skeletal muscle (n = 321) than in heart (n = 174) (Fig. 1B and C), suggesting a level of complexity in the tissue-specific response to frataxin deficiency that we had not anticipated. We observed a preponderance of downregulated genes in both tissues. This confirms previous reports in this (13) and other (15) FRDA models, and suggests that frataxin deficiency induces a prevalent downregulation of gene expression. Furthermore, we observed distinct expression patterns in each muscle type, consistent with distinct downstream effects of frataxin deficiency in these distinct tissues.

Three-hundred and twenty-one probes (corresponding to 308 unique gene names) were DE between KIKO and WT skeletal muscle. The most DE genes are reported in Supplementary Material, Table S1, and the complete gene list is reported in Supplementary Material, Table S2. The top Gene Ontology (GO) categories (Supplementary Material, Table S1; Fig. 1D) include lipid metabolism (overall upregulated), transcriptional regulation and signal transduction. Ingenuity Pathway analysis (Supplementary Material, Fig. S1) showed that a number of targets of the transcriptional regulator Srebp1 (including Acsa2, Sed2 and Aacs) were also upregulated in skeletal muscle. We also observed increased levels of Sox3, which has been linked to insulin resistance (16) and diabetes (17). Interestingly, we found a significant upregulation of the cytosolic malic enzyme 1 (Mod1), confirming a previous report of increased activity in FRDA muscle biopsies (18). Some contractile proteins were also dysregulated (mostly downregulated) in skeletal muscle, including Tnn1, My13, Tnn1c. We observed an over-representation of downregulated contractile proteins expressed in slow-twitch fibers, which would lead to a prevalence of fast, more anaerobic, less-oxidative fiber type, in contrast to the signature observed in cardiac muscle (see in what follows).

One-hundred and seventy-four probes (corresponding to 169 unique gene names) showed differential expression in heart (Supplementary Material, Tables S3 and S4). The top GO categories (Supplementary Material, Table S3; Fig. 1E) include ‘regulation of muscle contraction’ (overall downregulated), ‘oxidoreductase activity’ and ‘regulation of cell cycle’. Ingenuity Pathway analysis (Supplementary Material, Fig. S2) showed an overrepresentation of mitogen-activated kinase (Mapk) targets, confirming the involvement of this pathway as an early biochemical change in response to frataxin deficiency (13). Most strikingly, in heart a number of core proteins of the muscle-fiber contractile apparatus showed significant downregulation, including Myh4, Tnm2 and Tnn2 (Supplementary Material, Table S3). Downregulated genes were mostly associated with aerobic, fast-twitch myofibers (e.g. Atp2al, Tnn2, Tnm2, Tnnt3)—an opposite trend compared with skeletal muscle. Atp2al (also known as sarcoplasmic/endoplasmic reticulum calcium ATPase 1, Serc1) is involved in calcium sequestration and muscular excitation/contraction. Downregulation of SERCAs has been associated with cardiomyopathy in a number of studies (19), and its upregulation is beneficial for cardiac function in animal models (20). Only 6% of DE genes in heart were also dysregulated in skeletal muscle, indicating a differential effect of frataxin deficiency on these two metabolically distinct muscle tissues.

We selected 14 genes to represent a cross-section of DE genes expressed at different levels and in different tissues, for validation by qPCR. We confirmed ~71% of the targets (Fig. 1F)—a high confirmation rate, considered the magnitude of the observed changes and the sensitivity of qPCR.

Taken together, these data suggest an upregulation of lipodigenic enzymes in skeletal muscle, and a shift in fiber-type composition in both heart and skeletal muscle, in opposing directions. In heart, these expression changes, together with changes in calcium-related genes (21), are compatible with early signs of cardiomyopathy, whereas in skeletal muscle (a major insulin target) these data suggest a derangement of fuel metabolism related to early changes occurring in insulin resistance and type 2 diabetes (T2D). Pgc1a and the sterol-responsive element binding protein 1 (Srebp1) are master regulators of lipid biosynthesis and breakdown (22,23). Pgc1a is also known to control the muscle fiber-type ratio (24), and both factors are involved in insulin resistance and diabetes (25–27). Since the dysregulation of these two master transcriptional regulators provides a parsimonious explanation
for the tissue-specific transcriptional changes that we observed, we hypothesized that they are directly affected by frataxin deficiency. Further supporting this hypothesis, we found that other genes whose expression is affected by \(Pgc1a\) [e.g. \(Mmp9\) (28), \(Fabp3\) (29), \(Socs3\) (30)] are also dysregulated.

We generated cellular models of frataxin deficiency using short hairpin RNA interference (shRNAi) and followed the effects of transient frataxin downregulation on a set of target genes identified in the microarray study. In such models, expression changes occurring over a short time period (12–72 h) can be more confidently attributed to a direct, primary effect of frataxin deficiency, rather than a secondary degenerative process due to long-term cellular changes.

Transfection of C2C12 myoblasts with an anti-frataxin shRNA (shRNAi\(^{fxn}\)) induced an average 50% downregulation of the \(Fxn\) transcript and protein that started at 12 h, peaked at 24–32 and lasted up to 72 h (Fig. 2A and B). In these cells, we observed dysregulation of a set of genes already known to be downstream of frataxin deficiency, such as \(Isu\) (31) and \(Mthf\) (15), thus validating them as a cellular model of frataxin deficiency. Consistent with our interpretation of the microarray findings in KIKO skeletal muscle, we found a coordinate downregulation of \(Pgc1a\) and upregulation of \(Srebp1\)
Accordingly, two out of three tested lipogenic genes that were upregulated in KIKO skeletal muscle (Elovl3, Acas2, Acly) also showed a significant upregulation in shRNAiFxn-transfected C2C12 cells, peaking at 48 h after transfection (24 h after frataxin downregulation, Fig. 2A). In addition, PPAR-binding protein (Pparbp), which was downregulated in brains of KIKO mice and fibroblasts from FRDA patients (13), was also downregulated.

To check the specificity for skeletal muscle-derived cells of the above changes, as suggested by microarray data, we generated shRNAFxn-transfected HL-1 cardiomyocytes. Though these cells showed a 40–50% frataxin mRNA downregulation, no significant changes in lipogenic genes occurred, whereas Pparbp and Pgc1a showed a non-significant trend towards upregulation (Fig. 2B), in concordance with the KIKO heart expression profiling findings.

We next tested the hypothesis that the changes observed in skeletal muscle represent systemic metabolic changes consistent with fuel shift and potential insulin resistance. Because liver is a major player in fuel metabolism and insulin response, we analyzed global patterns of gene expression in the liver of 3 KIKO mice compared to 3 WT mice using microarrays. This
analysis identified 182 DE probes in KIKO versus WT mice (Supplementary Material, Fig. S3 and Table S5). Strikingly, by GO analysis, lipid metabolism was among the top categories showing a significant enrichment in liver (Supplementary Material, Fig. S3B). Many dysregulated genes were shared with skeletal muscle (e.g. Acac, Acas2). In addition, other lipid-related genes showed liver-specific changes (Supplementary Material, Table S5), consistent with a systemic metabolic rearrangement towards increased lipogenesis in frataxin deficiency. These observations further suggest that Pgc1a and Srebpl are likely to play a central role in this transcriptional response. To summarize this concept, a potential model that is parsimonious with these data is depicted in Supplementary Material, Figure S4.

FRDA patients are insulin resistant

The finding that gene expression profiles in KIKO mice skeletal muscle and liver were suggestive of decreased insulin sensitivity prompted us to test insulin sensitivity in 14 non-diabetic FRDA patients by intravenous glucose-tolerance testing and minimal model analysis of glucose kinetics. Confirming previous reports (9,32), we found that non-diabetic FRDA patients were insulin resistant compared with controls (insulin sensitivity index \( S_1 = 20 \pm 2 \) in patients versus \( 34 \pm 3 \times 10^{-2} \text{min}^{-1}/(\mu U/ml) \) in healthy controls matched for age and body mass index, \( n = 14 \) in each group, \( P < 0.005, \) Fig. 2E and G). Furthermore, insulin sensitivity was significantly inversely correlated with the number of GAA repeats in the smaller FRDA allele (\( r = -0.55, P < 0.05, \) Fig. 2F) in FRDA patients, indicating that it is directly linked to the degree of frataxin deficiency. To exclude that the severity of neurological impairment, possibly because of the resulting reduced motor activity, rather than the degree of frataxin depletion was the cause of insulin resistance, we compared the score on a neurological impairment rating scale ranging from 0 (normal) to 42 (most severe impairment) between the 50% more insulin sensitive FRDA patients (\( n = 7 \)) and the 50% more insulin resistant patients (\( n = 7 \)), and found no significant difference (Fig. 2G), suggesting that frataxin deficiency directly causes insulin resistance, independent of physical impairment.

The PPARγ pathway is dysregulated in frataxin deficient mice and FRDA patients

We assessed whether Pgc1a is dysregulated in additional cell types, including CNS cells, strengthening the causal connection between frataxin and Pgc1a downregulation. We cultured neural precursor cells (NPCs) from the subventricular zone (SVZ) from homozygous \( Fxn^{GAA}_{230} \) (KIKI) mice (33), KIKO mice and WT littermates and quantified \( Fxn \) and \( Pgc1a \) transcript levels using RT-qPCR. \( Pgc1a \) transcript levels were decreased by ~25% in KIKI- and by ~70% in KIKO-derived NPCs (Fig. 3A).

To extend these observations to human patients, we studied lymphoblastoid cell lines and primary skin fibroblasts from FRDA patients. In both cell types we observed a 70–80% downregulation of \( PGC1A \) relative to normal controls (Fig. 3A). Furthermore, when we examined results from FRDA fibroblasts, a strong correlation emerged between \( PGC1A \) and \( FXN \) expression, normalized to controls (\( r^2 = 0.9, P < 0.001, \) Fig. 3B).

PPARγ manipulation affects frataxin levels

\( Pgc1a \) plays a key role in mitochondrial biogenesis and function by promoting the expression of several genes involved in mitochondrial DNA replication and in oxidative phosphorylation (34). The importance of frataxin for normal mitochondrial function makes it a possible target of PGC1A, prompting us to test whether PGC1A manipulation can affect frataxin levels. We downregulated PGC1A in human fibroblasts using a specific siRNA and observed a significant decrease in frataxin mRNA and protein levels after a 72-h transfection with \( siRNA^*_{Pgc1a} \) in both FRDA patients and controls (Fig. 3C and D). Thus, a positive feedback loop between PGC1A and frataxin levels appears to exist. We hypothesize that, when frataxin is primarily abnormally low, as in FRDA, the resulting impairment of this feedback loop is likely to further aggravate frataxin deficiency. Conversely, a pharmacological intervention to activate PGC1A should help re-establishing the positive feedback and therefore increase frataxin levels. The recent report that the potent PPARγ agonist Azelaloyl-PAF (35) is able to increase frataxin levels in FRDA cells (36) supports this model.

DISCUSSION

Neurodegenerative diseases affect specific subsets of neurons and in some cases non-nervous system structures as well, but the reasons underlying such selective cellular vulnerability are largely unknown. Reduced frataxin levels in FRDA are likely to have some downstream consequences that are the same in all cell types, along with consequences and adaptive responses that are cell and tissue-specific. We took an unbiased, genome-wide approach to obtain clues on FRDA pathogenesis and selective vulnerability. We analyzed how global gene expression profiles are affected in the heart (affected in FRDA), skeletal muscle and liver (not clinically affected) from a mouse model expressing frataxin at levels (about 30–35% of normal) corresponding to mildly affected FRDA patients, and yet not showing obvious clinical or pathological abnormalities. We observed the same basic pattern of dysregulation in skeletal muscle and liver, consistent with their roles in energy metabolism. However heart muscle, which is significantly affected by myopathy in the human disease, showed changes suggesting a fiber-type switch and dysregulation of contractile proteins, possibly consistent with cardiomyopathy.

Dysregulation of the PPARγ/PGC1A pathway was observed in tissues from animal and cellular models of frataxin deficiency, as well as in cells from FRDA patients, suggesting that this is a general downstream effector of frataxin deficiency. Pgc1a is the most studied of the peroxisome proliferator activated receptor co-activators (PGCs), a family of transcriptional co-activators that regulate mitochondrial biogenesis, energy substrate and utilization, and oxidative metabolism (23). In KIKO skeletal muscle and liver, our results
indicate that Pgc1a activity is downregulated, as shown by the increased expression in both tissues of a set of genes involved in lipogenesis that are normally repressed by Pgc1a, including Acas2, Scd2, Acly, Aacs, Elovl3. These changes are also supported by the simultaneous upregulation of the transcription factor Srebp1. In skeletal muscle, changes in expression of contractile proteins that would result in increased glycolytic fast-twitch fibers and decreased slow, oxidative fibers type I and IIa, provide further evidence of downregulated Pgc1a activity (37). Pgc1a downregulation, and the related Srebp1 upregulation, are known to occur in insulin resistance and diabetes (38,39), along with reduced mitochondrial oxidative phosphorylation and lower oxidative-to-glycolytic muscle fiber ratio (40,41). The increased risk of diabetes in FRDA patients is therefore likely to be a consequence of the downregulation of Pgc1a in key tissues for insulin response and fuel metabolism control. FRDA patients are insulin resistant before being diabetic (9), and higher incidence of glucose intolerance and insulin resistance has been reported in family members of FRDA patients (42,43). However, other reports suggest a primary beta-cell involvement in FRDA (44), and a pathogenic mechanism primarily involving beta-cell failure has been proposed based on studies in other mitochondrial disorders (45,46) and on the pancreatic conditional frataxin knock-out (6). Here, we confirm that insulin resistance is present in all tested non-diabetic FRDA patients, strongly supporting the hypothesis suggested by our gene expression data that insulin target tissues are relevant in the pathogenesis of diabetes in FRDA. Furthermore, we show here for the first time that the degree of insulin resistance of FRDA patients correlates with GAA repeat size (hence to lower residual frataxin levels) and is independent of neurological impairment, in agreement with the hypothesis that it is a direct consequence of frataxin deficiency.

Though the direct mechanistic link between frataxin and PGC1A remains to be completely defined, it is very likely related to mitochondrial dysfunction caused by frataxin deficiency. Mitochondrial dysfunction also occurs in T2D (47), as shown by 30% reduction of ATP production in skeletal muscle, associated with increased intramyocellular lipid (IML) content and decreased aerobic/anaerobic muscle fiber ratio (40,48). IML content, a consequence of increased lipid synthesis, is strongly correlated with glucose intolerance and insulin resistance in diabetic patients and can trigger insulin resistance (26). No data are available on IMLs in FRDA muscle, but the finding of increased cytosolic malic enzyme activity (18), in perfect concordance with gene expression data from KIKO mice, at least suggests increased lipogenesis.

Figure 3. Genetic and pharmacologic modulation of the PPARγ pathway affects frataxin levels in vitro. (A and B) Pgc1a levels are correlated with frataxin levels in neural precursor cells from mouse models and in cells from patients with FRDA. (A) qPCR quantification of Fxn (white bars) and Pgc1a (blue bars) in neural precursor cells from the subventricular zone (SVZ-NPC) in wild-type (WT, n = 3), KIKI (n = 3) and KIKO (n = 3) mice, and in fibroblasts and lymphoblasts from FRDA patients (n = 4) and normal controls (n = 3). KIKI mice express ~75% of normal frataxin levels, and KIKO ~30%. Pgc1a mRNA expression levels are reduced in all these cell lines, in a degree which is proportional to Fxn downregulation. Bars represent the average of six replicates, error bars represent the standard error. P < 0.05 for all the comparisons; (B) Relative Pgc1a levels are strongly correlated with relative Fxn mRNA levels (r² = 0.9, P < 0.001); qPCR quantification in fibroblasts from controls (open circles, n = 3) and FRDA patients (filled circles, n = 4). (C and D) PGC1A downregulation via siRNA reduces frataxin protein levels in control fibroblasts, and further in FRDA fibroblasts. Western blotting analysis (C) and quantification (D) of PGC-1A (top) and FNX (bottom) protein 72 h after transfection with siRNAPgc1a. Control and FRDA are fibroblasts from healthy controls and patients, respectively. All experiments were performed on n = 4 FRDA cell lines and n = 3 control cell lines. Bars represent the average of six replicates, error bars represent the standard error. P < 0.05 for all comparisons.
and a metabolic shift similar to T2D. We propose therefore that in FRDA, like in T2D, a state of insulin resistance and associated metabolic changes long precedes the onset of clinically overt diabetes, which is eventually due to pancreatic beta-cell failure. Beta-cell failure may in turn be accelerated by intrinsic mitochondrial dysfunction due to the gene defect (Fig. 4).

In contrast with skeletal muscle, we observed a downregulation of fast fibers in cardiac muscle, which is eventually due to pancreatic beta-cell failure. Beta-cell failure may in turn be accelerated by intrinsic mitochondrial dysfunction due to the gene defect (Fig. 4).

In conclusion, we have identified distinct pattern of gene expression changes in metabolically distinct tissues in FRDA, tricarboxylic acid cycle; IMLs, intra-myocellular lipids.

Figure 4. Diabetes in FRDA and type-2 diabetes may have a final common pathway. In normal conditions (left panel), lipid breakdown and biosynthesis are tightly controlled by the two master regulators Pgc-1a and Srebp-1. In type-2 diabetes (center), increased dietary lipids provided to the skeletal muscle can overload TCA and divert long-chain-CoA species into lipid precursors than can be converted in lipids, leading to an increased lipid intramyocellular content, which in turn leads to reduced glucose transport, reduced glycogen synthesis and ultimately to insulin resistance. In FRDA (right), deficits of TCA enzymes occur, as well as decreased activity of complexes I–II–III of the respiratory chain. An ineffective TCA and mitochondrial OXPHOS would lead to TCA overflow, favoring a metabolic shift toward the redirection of citrate from the TCA cycle and electron transport chain to processing into fatty acids. In mitochondrial diseases and in FRDA in particular, the oxidative stress can contribute to the diabetes pathogenesis at both (i) skeletal muscle and liver (insulin targets) and (ii) the beta-cell level, leading to overt diabetes mellitus. TCA, tricarboxylic acid cycle; IMLs, intra-myocellular lipids.

Diabetes can have opposite changes in different tissues is supported by the fact that its overexpression induces uncoupled respiration in adipose tissue and coupling in cardiac myocytes (51). Pgc-1a binding to tissue-specific transcription factors, enabling the activation of diverse metabolic programs in different tissues (23,55), offers an explanation for these differences and the distinct metabolic effects observed in skeletal and cardiac muscle in this model of frataxin deficiency (Supplementary Material, Fig. S4).

This model was supported by recent reports that (i) PGC1A overexpression in skeletal muscle cells (56) and (ii) treatment of FRDA cells with the PPARγ agonist Azelaoyl-PAF (36) are able to increase frataxin levels. PPARγ agonists such as rosiglitazone and pioglitazone are in clinical use as oral antidiabetics. The latter molecule may be of particular interest because of its capacity to cross the blood–brain barrier. Clearly, the opposite dysregulation of Pgc-1a in the heart imposes caution for any attempt to clinically test such a drug in FRDA. Maybe not surprisingly, cardiac toxicity is a known side effect of this class of drugs.

In conclusion, we have identified distinct pattern of gene expression changes in metabolically distinct tissues in
a model of frataxin deficiency. These data suggest Pgc1a as a key regulator of gene expression that is directly affected by frataxin deficiency and in turn affects frataxin expression. Further studies (such as frataxin overexpression) are needed to characterize the relationship between Fxn and Pgc1a. Downregulation of Pgc1a is the rule in several frataxin-deficient cell types, including skeletal muscle, liver, lymphoblasts, fibroblasts and possibly the pathologically relevant CNS cells, engaging a feedback loop that leads to even lower frataxin levels, reduced antioxidant defenses and mitochondrial function. Such a mechanism appears to be responsible in particular for the vulnerability to diabetes of FRDA patients. In these cell types, upregulation of Pgc1a using PPARγ agonists may be an appealing therapeutic approach.

MATERIALS AND METHODS

Animals and tissue samples
Frataxin knockout/knockin (Frda230GAA/−, KIKO) and knockin/knockin (Frda230GAA/230GAA, KIKI) mice were obtained as described (14). Four 6-month-old male KIKO and three 6-month-old male KIKI mice were compared with age and gender-matched WT littermates. Total RNA from skeletal muscle (gastrocnemius), heart and liver was extracted by acid phenol extraction (Trizol, GIBCO/BRL) as recommended by the manufacturer.

Microarrays
Sample preparation and array hybridization and scanning were performed according to established protocols (detailed in Supplementary Material). Quality control, differential expression, and cross-platform microarray analysis were performed using Bioconductor packages in the R statistical environment (see Supplementary Material for a detailed description). The microarray data have been deposited in the NCBI Gene Expression Omnibus database (GEO, www.ncbi.nlm.nih.gov/geo) with the GEO series accession number GSE15849.

Cell culture
C2C12 cells were obtained from ATCC (Manassas, VA) and HL-1 cells were a kind gift from the Claycomb Lab (School of Medicine, LSU, New Orleans, LA). Fibroblasts primary cell cultures were obtained from skin biopsies of FRDA patients and healthy controls after receiving written informed consent. All the patients were homozygous for the GAA repeat expansion (500–1700 repeats), and were being treated with Idebenone 5 mg/kg. Epstein Barr virus-transformed lymphoblast cell lines GM15850 were from a FRDA patient (650–1030 GAA repeats; NIGMS, Human Genetic Cell Repository at the Coriell Institute, Camden, NJ, USA) and GM15851 from one unaffected control. Mouse neuronal precursor cells (NPC-SVZ) were obtained from wild-type and from KIKI/KIKO mice. Brain layer of tissue surrounding the ventricles was cultured in the media containing 20 ng/ml recombinant human EGF and 10 ng/ml recombinant human bFGF (Peprotech, NJ, USA). Derived neurospheres were plated in a fresh growth medium and were assessed for their character-istics of self-renewal and multipotentiality. Cells were transfected using standard procedures, detailed in Supplementary Material.

qPCR experiments
Real-time quantitative PCR experiments were performed as described previously (13) and in Supplementary Material, Methods.

Western blot and luciferase assays
Western blot and luciferase assays were performed using standard procedures, detailed in Supplementary Material, Methods.

Assessment of physical impairment in FRDA patients
Speech, upright stability, upper limb coordination, and lower limb coordination scores from the FARS (57) scale were used.

Assessment of insulin sensitivity in FRDA patients and healthy controls
FRDA patients and healthy volunteers provided written informed consent. After an overnight fast, subjects underwent an intravenous glucose-tolerance test to measure insulin sensitivity. Glucose (0.3 g/kg) was infused intravenously, followed by an insulin infusion (0.025 U/kg) 20 min later, and blood was sampled at times −15, −5 and −1 min before intravenous glucose administration, and at 2, 3, 4, 5, 6, 8, 10, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160 and 180 min (58). Plasma glucose was measured using the glucose oxidase method (DiaSys, Holzheim, Germany). Plasma insulin concentrations were measured by ELISA (INSI-CTK immunoradiometric analysis; Diasorin, Saluggia, Italy). Insulin sensitivity was quantified as the insulin sensitivity index (SI) based on the glucose and insulin data using the minimal model of glucose kinetics (59).

SUPPLEMENTARY MATERIAL
Supplementary Material is available at HMG online.

ACKNOWLEDGEMENTS

The authors thank Andrew Chen, Marie-Anne Neef and Maren Engelhardt for technical assistance; Eric Wexler, Raj Ratan and Peter Tontonoz for helpful discussions; Audrey Begu, Chantal Depondt and Françoise Féry for help with the Friedreich’s ataxia patient studies; Satyan Chintawar for help with neural precursor cells; Coriell Cell Repositories for providing FRDA and control fibroblast and lymphoblast cell lines; and the Claycomb lab for providing the HL-1 cell line.

Conflict of Interest statement. None declared.
FUNDING

This work was supported by a research grant from Friedreich’s Ataxia Research Alliance/MDA Seek-A-Miracle (to G.C. and D.H.G.); the Dr Miriam and Sheldon G. Adelson Medical Research Foundation (AMRF, to D.H.G. and G.C.); the Waverly Smith Memorial fund gift (to D.H.G.); the National Ataxia Foundation (to M.C.); grants from Fondazione Gofar (Italy), Fondazione CRT (Italy), French Friedreich Ataxia Association (AFAAF), FNRS, and Belgian Ministry for Scientific Policy (Interuniversity Attraction Poles Program 6) to M.P.; and by the National Institutes of Health [grant number NS34192 to M.P.]. D.M. received a fellowship from the Waverly Smith Memorial fund gift (to M.C.); the Fonds National de la Recherche Scientifique (FNRS) - Fonds de la Recherche Scientifique Medicale (FRSM) (to M.C.); grants from Fondazione Gofar (Italy), Fondazione CRT (Italy), French Friedreich Ataxia Association (AFAAF), FNRS, and Belgian Ministry for Scientific Policy (Interuniversity Attraction Poles Program 6) to M.P.; and by the National Institutes of Health [grant number NS34192 to M.P.]. D.M. received a fellowship from the Waverly Smith Memorial fund gift (to M.C.); the Fonds National de la Recherche Scientifique (FNRS) - Fonds de la Recherche Scientifique Medicale (FRSM) (to M.C.); grants from Fondazione Gofar (Italy), Fondazione CRT (Italy), French Friedreich Ataxia Association (AFAAF), FNRS, and Belgian Ministry for Scientific Policy (Interuniversity Attraction Poles Program 6) to M.P.; and by the National Institutes of Health [grant number NS34192 to M.P.]. 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