

Heterozygous Expansion of the GAA Tract of the *X25/frataxin* Gene Is Associated With Insulin Resistance in Humans

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Friedreich's ataxia (FA) is an autosomal recessive disease that has been attributed to a GAA triplet repeat expansion in the first intron of the *X25/frataxin* gene. Impaired glucose tolerance is present in up to 39% of FA patients, and clinically apparent diabetes is seen in ~18% of the affected individuals. Subjects carrying the *X25/frataxin* GAA repeat in a heterozygous state do not develop FA and, therefore, represent an ideal model to study the underlying metabolic defects that contribute to the diabetes associated with this disorder. In the present study, we have compared 11 first-degree relatives of FA patients (i.e., parents or heterozygous siblings of FA patients) with matched normal control subjects to study the parameters of glucose metabolism. An oral glucose tolerance test revealed diabetes in one of the heterozygous subjects who was excluded from further analyses. Using an octreotide-based quantification of insulin sensitivity, 8 of the remaining 10 study subjects showed pronounced insulin resistance, reflecting a significant difference from the control group ($P = 0.001$). In conclusion, a heterozygous expansion of the *X25/frataxin* GAA repeat in healthy individuals is associated with insulin resistance and might be considered a genetic co-factor in the pathogenesis of mitochondrial subtypes of diabetes. *Diabetes* 49:1604–1607, 2000

Friedreich's ataxia (FA) is phenotypically characterized by progressive ataxia and other neurological alterations in association with hypertrophic cardiomyopathy. The vast majority of FA patients carry a homozygous expansion of an intronic GAA tract of the

X25/frataxin gene (1) leading to interference with the transcription of the frataxin gene (2) and subsequently decreasing expression of the frataxin protein (3). Frataxin is expressed in a wide variety of tissues, exhibits mitochondrial localization, and appears to be essential for embryonic development (4) in mice. Frataxin is most abundant in tissues with high metabolic activity, including skeletal muscle and brown fat (5). The characterization of the yeast frataxin homolog suggests a central role in oxidative phosphorylation (6). Furthermore, frataxin was proposed to regulate mitochondrial iron content (7,8) and to decrease oxidative damage to the cell (9,10). Frataxin is a mitochondrial protein that is significantly reduced in FA patients because of decreased transcription subsequent to expanded intronic GAA repeats on both copies of the *X25/frataxin* gene. Heterozygous carriers for this expansion are phenotypically normal but presumably exhibit a decreased expression of frataxin (2,11). In the present study, these heterozygous individuals were evaluated for alterations in glucose metabolism, specifically incidence of diabetes and insulin resistance.

First, first-degree relatives of FA patients were genotyped, as described in RESEARCH DESIGN AND METHODS, to assure the presence of GAA expansions, especially in siblings of the patients. Eleven subjects from 4 different unrelated families were analyzed (8 parents and 3 siblings of FA patients). As to be expected, all parents evaluated showed heterozygosity for the expansion of the GAA tract. Interestingly, one parent showed a premutation at 1 allele ($[GAA]_n = 38$) and a normal second allele ($[GAA]_n = 7$), whereas the FA-affected offspring showed 2 fully expanded alleles, which is consistent with our and others' previous observations (12,13) that the transmission of *X25/frataxin* expansions to the next generation can be highly unstable. Oral glucose tolerance tests (OGTTs) revealed 1 previously undiagnosed diabetic parent (65 years of age, BMI 27.2 kg/m²) in this heterozygous group. This individual was excluded from further analyses. The remaining 10 relatives showed normal OGTTs (data not shown). All other characteristics of this group are listed in Table 1. Control individuals were matched for sex, age, and BMI, had normal OGTTs, and tested negative for a family history of diabetes. HbA_{1c} (a long-term indicator of average blood glucose) and fasting glucose, as well as fasting insulin, were normal in both groups (Table 1).

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FA, Friedreich's ataxia; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction.

TABLE 1
Proband characteristics

	Sex (F/M)	Age (years)	BMI (kg/m ²)	HbA _{1c} (%)	Fasting glucose (mmol/l)	Fasting insulin (mU/l)	Frataxin expansion (GAA) _n
Expanded carriers	6/4	46.6 ± 4.61	25.1 ± 0.94	5.2 ± 0.18	4.8 ± 0.17	12.8 ± 1.90	683 ± 80.1
Normal individuals	6/4	46.4 ± 4.46	25.4 ± 0.71	5.3 ± 0.18	4.8 ± 0.20	14.2 ± 1.50	7.3 ± 0.37
<i>P</i>	NA	NS	NS	NS	NS	NS	<0.001

Data are *n* or means ± SE, unless otherwise stated. NA, not available.

Next, phenotyping of the study subjects and control patients was done by an insulin sensitivity test based on a parallel infusion of glucose, insulin, and octreotide. Using this test, insulin resistance correlates with the difference of blood glucose rise from baseline to steady state during infusion (14,15). The test reflects insulin-mediated glucose uptake in the peripheral tissues (i.e., mainly skeletal muscle and adipose tissue) and is highly correlated with the results of a hyperinsulinemic-euglycemic clamp (14,16). Quantification of insulin resistance by this test revealed some insulin resistance in every heterozygous individual evaluated. The absolute blood glucose at the end of the test was 12.0 ± 0.85 mmol/l in the FA heterozygous study group vs. 7.1 ± 1.1 mmol/l in the control group ($P = 0.001$) (Fig. 1A). Thus, pronounced insulin resistance (i.e., an increase 5 mmol/l glucose) was observed in 8 of the heterozygous individuals. Together these findings not only reflected an average trend, but it also indicated almost full penetration in those carrying the heterozygous GAA-tract expansion (Fig. 1B), including the parent carrying a premutation as previously described (Fig. 1B). Because the heterozygous individuals were from 2 generations and were consequently partly related, the phenotype observed might be influenced by an independently inherited trait. Therefore,

we excluded consanguinity by performing an additional subgroup analysis to evaluate the 7 remaining heterozygous parents, who were unrelated. Comparing these individuals with the same matched control subjects revealed that the heterozygously expanded parents also had a significantly increased degree of insulin resistance ($P = 0.043$). Not surprisingly, the difference between the parental subgroup versus the matched control subjects was less pronounced, presumably because of numerous factors contributing to insulin resistance in a subpopulation of advanced age.

Taken together, we conclude that a heterozygous expansion of the GAA tract of the *X25/frataxin* gene might be associated with resistance in otherwise healthy individuals. This observation is supported by previous studies that have clearly indicated an increased incidence of diabetes in FA-patients (17,18) together with normal or sometimes increased stimulus-coupled insulin secretion in FA-patients (19–22) and obligate heterozygous carriers (18,22) of the GAA-tract expansion (i.e., >50 GAA repeats). On the other hand, population-based studies on premutations (i.e., >15 but <51 GAA repeats) and their association with common type 2 diabetes are as inconclusive (23–26) as studies on the relevance of these premutations on transcription of the

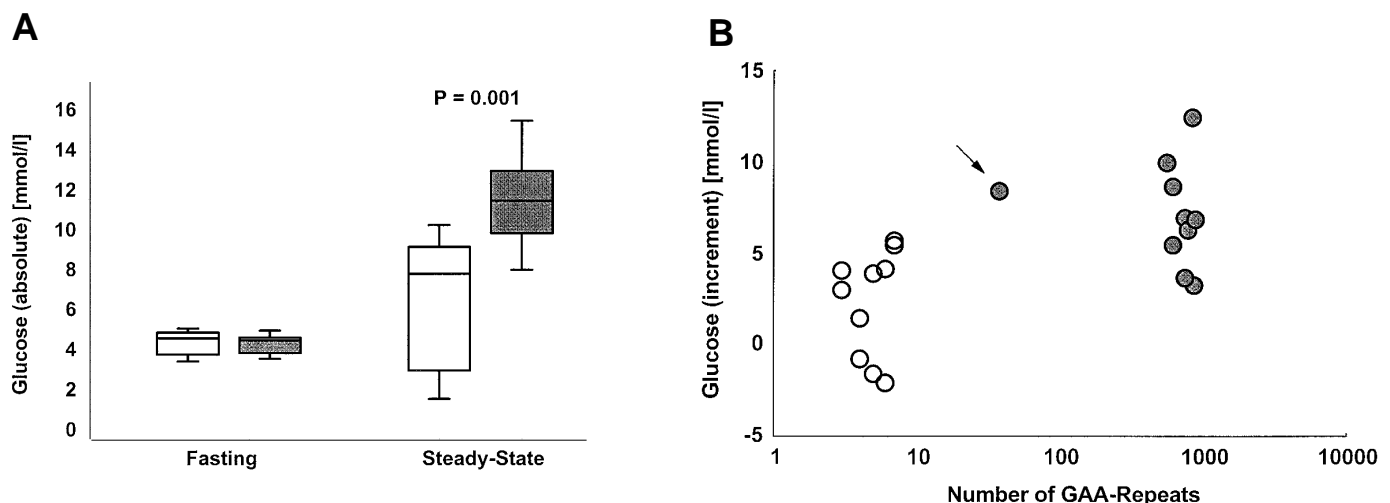


FIG. 1. Heterozygous carriers of the frataxin GAA-repeat expansion are insulin resistant. **A:** An octreotide-based insulin resistance test was performed in first-degree relatives of FA-patients compared with matched control subjects. Briefly, glucose, insulin, and octreotide were infused in parallel for 3 h using previously published concentrations (32). The boxes represent the blood glucose values as follows: □ refers to control subjects and ■ depicts heterozygous carriers. The left pair of boxes represents fasting blood glucose values at the beginning of the test, determined in duplicate for each individual. The right pair of boxes reflects steady-state blood glucose values at the end of the test, averaging 4 measurements at 150, 160, 170, and 180 min after the start of infusion for each individual. (For details on boxplot definitions, refer to RESEARCH DESIGN AND METHODS and [33].) **B:** Clustering between GAA-repeat expansion size (X-axis, logarithmic scale) and the increase of blood glucose during the octreotide-based insulin sensitivity test (Y-axis, linear scale). Control subjects are represented by ○, whereas subjects with a heterozygous GAA expansion are represented by ●. The → indicates the premutated individual as explained in the text.

frataxin gene (11,27,28). In addition, while full expansions of the GAA tract inhibit transcription when evaluated in vitro (2,3,11), the question of whether heterozygous carriers of these GAA expansions show decreased expression of frataxin protein in vivo remains to be evaluated.

In summary, we have demonstrated an association between an intronic GAA expansion of the FA gene *X25/frataxin* and a subtype of insulin resistance. For an underlying mechanism, a deficiency in peripheral ATP synthesis might be discussed, as described in patients with FA by us and others (29,30). Clearly, this study should be considered preliminary because of the small sample size and the fact that the study subjects were related. Thus, further studies will be needed to dissect the metabolic role of frataxin in energy metabolism and in the pathogenesis of mitochondrial subtypes of diabetes.

RESEARCH DESIGN AND METHODS

Genotyping was performed by polymerase chain reaction (PCR)-amplification of the GAA tract within the intron 1 of the *X25/frataxin* gene and subsequent hybridization using a (TTC)₆-probe, as previously described (12). Accuracy of genotyping was ± 30 GAA repeats for the expanded and ± 3 triplets for the normal alleles, as previously determined by us (12) and others (31). Additionally, each set of PCR reactions contained a DNA sample derived from an individual previously known to be heterozygous for a full GAA expansion, to verify the amplification efficiency concerning the larger of both alleles for the specific reaction used (positive control). Furthermore, each DNA sample that tested negative for a larger allele was subsequently tested for heterozygosity of the shorter (normal) alleles to further reduce the possibility of overlooking FA carriers based on the fact that the heterozygosity rate for the shorter (normal) alleles is $>80\%$ (12).

Phenotyping for diabetes was performed by OGTTs according to World Health Organization criteria. Quantification of insulin sensitivity was performed, as previously described (32), using the octreotide-based method as introduced by Pei et al. (15).

Statistical analyses were performed using the software package SPSS for Windows, version 9.0 (SPSS). Significances were determined by 1-way analysis of variance and considered significant when $P < 0.05$. Boxplot graphics were generated using SigmaPlot for Windows, version 5.0 (SPSS), where the box covers the range in between the lower and upper quartiles, the whiskers cover the range in between the 5th and 95th percentiles, and the horizontal bar dividing the box indicates the mean, as suggested previously (33).

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