Rapid Publication

An Association Between NIDDM and a GAA Trinucleotide Repeat Polymorphism in the *X25*/Frataxin (Friedreich's Ataxia) Gene

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Friedreich's ataxia is the most common hereditary ataxia and is frequently associated with disturbances of glucose metabolism. This autosomal recessive disease is caused by the decreased expression of a mitochondrial protein, frataxin, encoded by the X25 gene. Homozygous expansion of a GAA repeat in the first intron of X25 inhibits frataxin expression and is associated with clinical disease. We evaluated whether heterozygous expansions of the triplet repeat in the frataxin gene X25 may be associated with NIDDM in two genetically distinct populations—one in Germany (n = 358) and the other in the U.S. (n = 292)—using a polymerase chain reaction–based assay. Intermediate expansions (10–36 repeats), which are longer than normal but not sufficient for the appearance of the ataxia phenotype, were found in 24.7 and 27.3% of these two NIDDM cohorts compared with 7.6 and 6.3% of the matched control subjects (both P < 0.001). The odds ratios were 3.36 (95% CI 1.72–6.55) for the German group and 4.01 (2.08–7.74) for the U.S. group. Therefore, we conclude that the *X25*/frataxin GAA repeat polymorphism is associated with NIDDM in a frequency higher than any other mutation heretofore described. Further studies are needed to elucidate the possible role of frataxin in the pathogenesis of NIDDM. *Diabetes* 47:851–854, 1998

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bp, base pair; FRDA, Friedreich's ataxia; PCR, polymerase chain reaction.

NIDDM is a genetically and clinically heterogenous disorder (1) characterized by insulin resistance in peripheral tissues combined with impaired insulin secretion of the pancreatic β-cell (2). Investigations of candidate genes for NIDDM have led to the identification of polymorphisms of several genes encoding proteins of insulin action and insulin secretion, but none show a high frequency of association with the disease (3,4).

Friedreich's ataxia (FRDA) is the most common hereditary ataxia (5), with an estimated prevalence of 1 in 50,000 people (6). FRDA is a degenerative disease characterized by progressive ataxia, lack of tendon reflexes, loss of position sense, dysarthria, and hypertrophic cardiomyopathy. Clinically apparent diabetes is seen in ~10% of FRDA patients, and impaired glucose tolerance is present in 30% (7). Reduced early insulin response to intravenous arginine stimulation occurs in almost all FRDA patients, while insulin response to intravenous glucose is generally normal or elevated (7).

FRDA is inherited as an autosomal recessive disease. The FRDA has been mapped to chromosome *9q13-q21* (8) and attributed to a GAA triplet repeat expansion (4) in the intron of the *X25* gene. *X25* encodes a protein, frataxin, which is targeted to the mitochondria and has been suggested to function as an endogenous antioxidant (9). FRDA appears to belong to the family of triplet repeat disorders, including myotonic dystrophy, fragile X syndrome, and Huntington's disease (10). Analysis of the *X25*/frataxin polymorphism in a German population (6) of ataxia-free individuals has demonstrated two distinct groups: one (83%) with unexpanded (normal) repeat size of up to $(GAA)_n = 9$ and another (16%) with intermediate repeats of up to $(GAA)_n = 29$. This compares with the minimum repeat lengths for FRDA carriers of $(GAA)_n = 66$.

To investigate whether intermediate GAA repeat polymorphisms might be associated with NIDDM, we assessed the triplet repeat length in the *X25* gene using a polymerase chain reaction (PCR)-based assay in two large population groups, one in Germany and one in the U.S. In both, we found a significantly increased frequency of intermediate expansions in NIDDM patients compared with matched control subjects, suggesting a possible role for this gene in the pathogenesis of this metabolic disease.

RESEARCH DESIGN AND METHODS

Subjects. Two populations were evaluated. The German group consisted of 186 people with NIDDM (age 62.5 ± 12.6 years, BMI 28.9 ± 5.6 kg/m^2, 93 men, 93 women) and 172 control subjects (age 53.2 ± 15.8 years, BMI 27.1 ± 4.9 kg/m^2, 88 men, 84 women). The U.S. group from the Joslin Diabetes Center consisted of 165 individuals with NIDDM (age 63 ± 6.3 years, BMI 30.3 ± 6.2 kg/m^2, 92 men, 73 women) and 127 control subjects (age 53.2 ± 17.1 years, BMI 25.2 ± 4.1 kg/m^2, 75 men, 52 women). All German subjects were Caucasian residents of Nordrhein-Westfalen, Germany; all U.S. subjects were Caucasian residents of Massachusetts. Control subjects consisted of employees of the participating institutions and spouses of the NIDDM patients.

Phenotyping. Diabetes status was determined by oral glucose tolerance test using World Health Organization criteria and a Beckmann Glucose Analyzer II (Munich, Germany). Ataxia symptoms were excluded with a neurologic examination. Subjects with impaired glucose tolerance, but not diabetes, or ataxia-like symptoms on examination were excluded from the study. Furthermore, NIDDM subjects were only included when diabetes was diagnosed after the age of 20 years and treated with diet or oral antidiabetic agents for at least 2 years after diagnosis of diabetes.

Genotyping. Genomic DNA was extracted from EDTA-stabilized blood. PCR primers and the PCR conditions were as described by Filla et al. (11), except that Taq-Polymerase BioTherm was from GeneCraft (Muenster, Germany). Gel separation was performed under two different conditions. A first agarose gel (1.5%) was used for average size determination. For exact length determination, separation was done on 4% agarose gels at 30 V for ~8 h. Because heterozygous repeat expansions may be difficult to detect, a heterozygous positive control was included in every PCR. For small repeat elongations (as occurs in NIDDM patients), a 50–base pair (bp) ladder (Gibco, Gaithersburg, MD) was used to obtain exact length determination. The estimated error for repeat expansion determination was ± 3 bp for nonexpanded and ± 6 bp for intermediate expanded alleles. The gene was defined as intermediately expanded when it contained >27 bp and <120 bp, as recommended by Epplen et al. (6) and confirmed by our data (Fig. 3).

Statistical analysis. Statistical analyses were performed with Statistical Package for Social Science (SPSS, Munich, Germany) for Windows, release 7.5. NIDDM and control groups were evaluated separately for German and U.S. populations by χ^2 and Mann-Whitney U tests (12) comparing normal and intermediately expanded subgroups. Box plot characteristics are as suggested by Tukey (13).

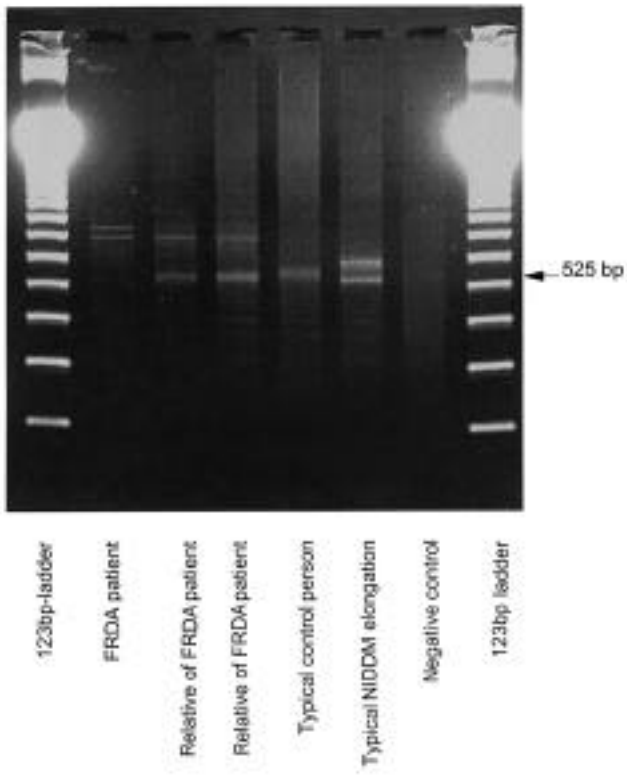

FIG. 1. PCR assay for *X25*/frataxin polymorphism. Ethidium bromide–stained agarose gel (1.5%) showing PCR products generated by amplification of genomic DNA. Each lane represents an individual with different intronic GAA repeat expansions of the *X25*/frataxin gene.

RESULTS

To determine the length of the intronic GAA repeat, genomic DNA samples were evaluated using a PCR-based assay. This assay was expected to generate a product of 525 bp for nonexpanded alleles and appropriately larger alleles for those containing expansions (11). To determine the sensitivity of this assay to detect expanded alleles, genomic DNA from an FRDA patient predicted to carry two expanded alleles and from two first-degree relatives of this patient putatively carrying one expanded allele and one normal allele each were amplified. As shown in Fig. 1, the FRDA patient was shown to carry two fully expanded alleles (6), while the relatives carried one normal and one fully expanded allele each (Fig. 1, *lanes 2–4* from left), easily distinguishable on the low-percentage screening gel (1.5% agarose). As predicted, while no signal was detected in the negative control, DNA samples derived from the 650 study subjects were amplified and evaluated using the same approach. The majority of NIDDM patients and almost all matched control subjects showed a normal allele size (Fig. 1, *lane 5*). However, ~25% of NIDDM patients showed a heterozygous intermediate expansion of the GAA repeat tract (Fig. 1, *lane 6*).

The exact size of the repeat was determined by subsequent high-percentage gels and sequencing. The distribution of intron repeat length was evaluated for the German and U.S. populations separately. The average length for the longer of both alleles was 22.3 bp (SD 23.1) for the German NIDDM patients vs. 9.9 bp (SD 13.3) for the German control subjects and 19.4 bp (SD 21.4) for the U.S. diabetic patients vs. 9.8 bp (SD 9.1) for the U.S. control subjects (Fig. 2). No expansion typical of FRDA [$(GAA)_n$ > 65 or 195 bp] was found. Statistical evaluation using the Mann-Whitney U test (12) indicated a significance of P < 0.001 for the German study and P = 0.003 for the U.S. study when NIDDM patients were compared with control subjects.

Two recent studies have described the prevalence of intermediate expansions in the general population and suggested a cutoff of 27 bp, i.e., $(GAA)_n$ = 9 (6), to distinguish between normal and intermediately expanded alleles. Indeed, the allele distribution in our study reflects the overall prevalence as described by Epplen et al. (6) and Cossee et al. (14) (Fig. 3). In addition, in both populations studied we found that the size of 27 bp distinguished between normal and intermediately expanded groups as previously described. Based on this cutoff size of $(GAA)_n$ = 9, a shift toward intermediate expansions in both German and U.S. NIDDM subgroups was seen (Fig. 3). While in total, 112 intermediately expanded alleles were found among all 650 samples, these intermediate expansions were present in 24.7% (n = 46) of German NIDDM patients vs. 7.6% (n = 13) of German control subjects and in 27.3% (n = 45) of U.S. diabetic patients vs. 6.3% (n = 8) of U.S. control subjects. Evaluation by χ^2 tests revealed high significances, indicated by P values of <0.001 for both German and U.S. populations and an odds ratio of 3.36 (95% CI 1.72–6.55) for the German group and 4.01 (2.08–7.74) for the U.S. group. Further evaluation of the intermediately expanded compared with the nonexpanded subgroups revealed no significant correlation between age, sex, or BMI of patients or control subjects and allele length.

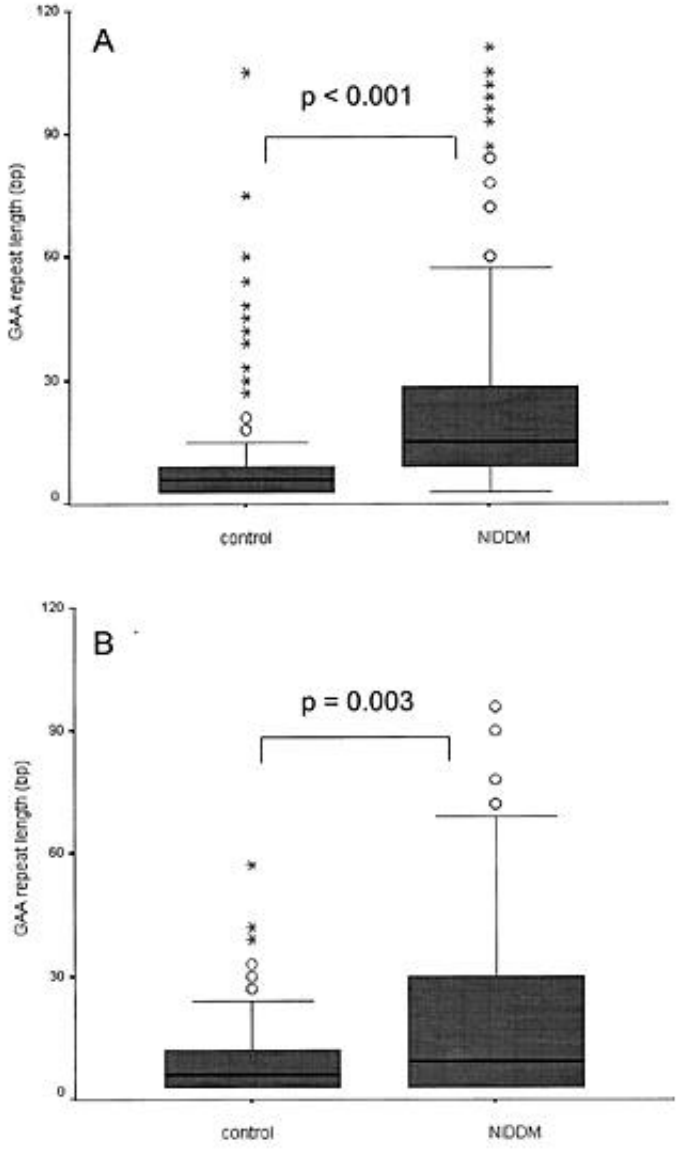

FIG. 2. Intermediate GAA repeat expansion is associated with NIDDM. Box plot graph showing the size of repeat expansions found in two different populations (German [*A*] and U.S. [*B*]), separated for NIDDM patients versus control subjects. The box covers interquartile distance (25th to 75th percentile), bars indicate 1.5-fold range of interquartile distance, circles indicate threefold range of interquartile distance, and stars indicate values outside threefold interquartile distance. Statistical significance was calculated using the Mann-Whitney *U* test.

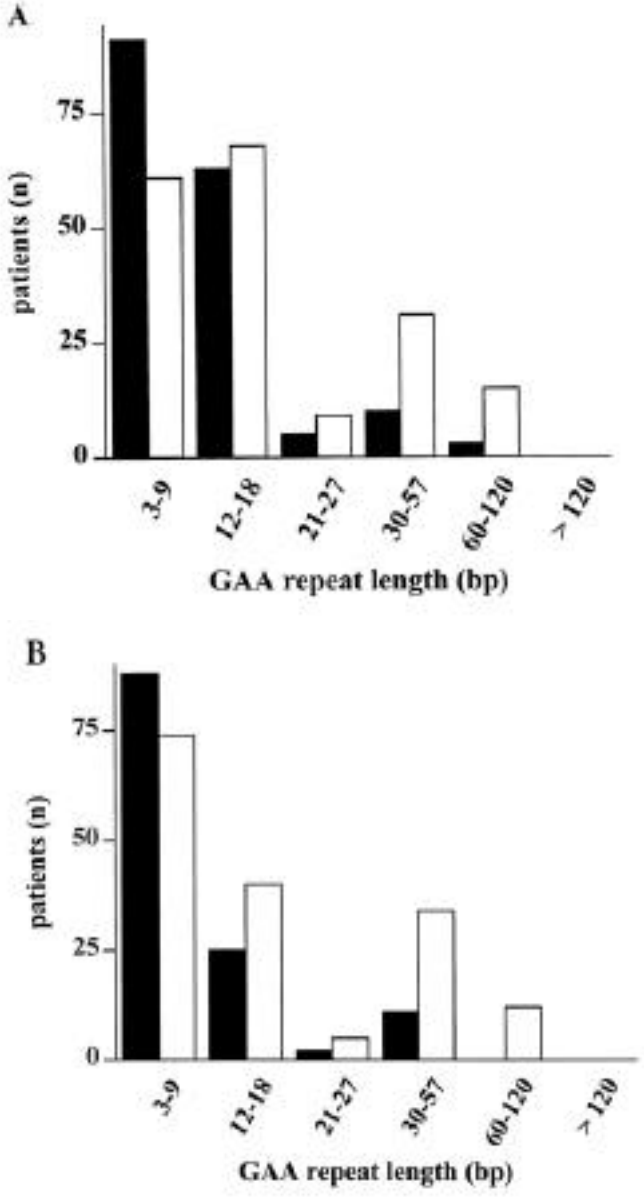

FIG. 3. Allele size distribution. *A*: German subjects (■, control subjects; □, NIDDM patients); *B*: U.S. subjects (■, control subjects; □, NIDDM patients). The *x*-axis indicates groups of repeat lengths, and the *y*-axis indicates number of individuals.

DISCUSSION

While an increased prevalence of diabetes among FRDA patients has been known for decades, the cloning of an intronic GAA repeat in the *X25*/frataxin gene causing FRDA (5) made it possible to evaluate a possible association between NIDDM and this genetic locus by direct assessment of the *X25*/frataxin polymorphism. Our data indicate that an intermediate expansion of this GAA repeat tract may be involved in the pathogenesis in up to 25% of patients with NIDDM. Thus, a heterozygous repeat expansion, shorter than that found in first-degree relatives of FRDA patients, is significantly associated with NIDDM in two separate populations. As indicated by the odds ratios, individuals carrying an intermediately expanded allele have a three- to fourfold increased risk of developing NIDDM, compared with individuals with normal alleles. The GAA repeat lengths found in these diabetic subgroups ranged from 10 to 36. Similar intermediate expansions were rarely found in the control groups, and in cases larger than $(GAA)_n = 14$, these control subjects were younger than 45 years of age, and thus still at a significant likelihood of developing NIDDM in later years (3,4).

Frataxin is a small protein that is targeted to the mitochondria while sharing a conserved NH_2-terminal region with other proteins involved in mitochondrial metabolism (15). Although very little is known about the physiological role of frataxin, an involvement in cellular iron metabolism has been

repeatedly demonstrated (9,16,17). Thus, frataxin might represent an endogenous antioxidant, as supported by additional observations (18).

Recently it has been demonstrated that homozygous expansions of *X25*, as found in FRDA patients, lead to a lack of frataxin expression, presumably affecting neuronal and cardiomyocyte function in these patients (19). Since the level of frataxin expression seems to be inversely correlated with expansion size, we would predict a mild decrease in frataxin expression in the expanded NIDDM group described in this study. Clearly, frataxin deficiency alone is insufficient to cause NIDDM, since the majority of FRDA patients do not suffer from diabetes (7). On the other hand, a deficiency of frataxin due to an intronic repeat expansion could be a highly prevalent genetic cofactor in the pathogenesis of NIDDM.

Although the possible impact of this putative deficiency in frataxin expression on metabolism is at present unknown, inherited tocopherol transport defects causing vitamin E deficiency in humans lead to a phenotype similar to that seen in FRDA patients (18), suggesting that frataxin may be an endogenously produced antioxidant protein. If this is the case, lack of this antioxidant may be involved in β-cell damage and increased lipid peroxidation, two factors known to be involved in the pathogenesis of NIDDM (1). Thus, while it is clear that an intermediate trinucleotide repeat expansion of the *X25*/frataxin gene is associated with NIDDM in a significant fraction of our patients, further studies will be needed to replicate our findings in additional populations and to subsequently elucidate the underlying diabetogenic effect of this gene product.

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E.G. performed parts of the experiment for the fulfillment of the requirements for her MD thesis.

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