

No Association Between the Friedreich's Ataxia Gene and NIDDM in the French Population

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Recent advances in molecular genetics have allowed the identification of genetic anomalies associated with some monogenic forms of NIDDM, such as maturity-onset diabetes of the young (MODY) or maternally inherited diabetes associated with deafness (MIDD). However, these genes do not account for a great part of the genetic determinism of the common forms of NIDDM. A candidate gene approach studying the genes or chromosomal areas involved in syndromes associated with diabetes, such as ataxia telangiectasia (1) or Friedreich's ataxia (FRDA) (2), may contribute to the identification of susceptibility genes for NIDDM.

FRDA is an autosomal recessive disorder characterized by a progressive degeneration of the central and peripheral nervous systems. In most cases, it is caused by an expansion of a GAA triplet (in general, from 200 to >900 GAA repeats) in the first intron of the *X25* gene located on chromosome 9q13, encoding the protein frataxin. The frataxin gene is a good candidate gene for NIDDM for several reasons. First, diabetes or impaired glucose tolerance is present in 30% of FRDA subjects. Several alterations in insulin secretion and insulin sensitivity have been described in FRDA. A reduced insulin response to arginine stimulation was reported in FRDA patients whatever their glucose tolerance, while FRDA patients with diabetes, as well as first degree relatives, showed insulin resistance (3,4). Second, frataxin plays a crucial role in mitochondrial metabolism and mitochondrial DNA stability. FRDA gene mutations cause a multiple Fe-S-dependent respiratory chain enzyme deficiency, probably triggered by an oxidative stress secondary to an iron overload in the mitochondrial matrix (5). Based on knowledge of the role of mitochondrial oxidative phosphorylation in the β -cell metabolism, β -cell function may be abnormal in FRDA, as in other mitochondrial diseases. Third, a recent study showed that although the clinical features of FRDA are correlated with the size of the GAA repeat expansion, this was not the case

for diabetes (6). Thus, it could be possible that small GAA expansions in the frataxin gene contribute to the development of diabetes in subjects free from FRDA. Furthermore, in a recent study, Ristow et al. (7) reported an association between NIDDM and the GAA repeat length in the FRDA gene in German and U.S. populations.

To examine whether the GAA polymorphism could be linked to NIDDM in patients with no classical symptoms of FRDA, we compared the distribution profile of the alleles in NIDDM patients and nonaffected individuals and performed family linkage analysis using markers located in the vicinity of the FRDA gene.

Two independent association studies were conducted by genotyping NIDDM patients and control subjects for the GAA triplet expansion in intron 1 of the FRDA gene. Population A comprised 182 unrelated NIDDM individuals selected from a well-described collection of French multiplex NIDDM pedigrees, and the control group consisted of 111 unrelated normoglycemic spouses from NIDDM families (8). The clinical profiles of the NIDDM and control groups were, respectively: age 61 ± 10.2 and 57 ± 20 years ($P = 0.0005$), BMI 27.4 ± 4.8 and 23 ± 2.7 kg/m² ($P = 0.0001$), fasting glucose 10.1 ± 3.9 and 5.2 ± 0.5 mmol/l ($P = 0.0001$), and male sex 54 and 45%. The mean age at diagnosis of NIDDM was 42 ± 10 years. Population B, recruited from the diabetes department of Necker Hospital (Paris), comprised 161 patients with overt NIDDM (60% men, 51% with a family history of diabetes, mean age at diagnosis 48 ± 12 years, BMI 28.5 ± 5 kg/m², 75% obese, 37% treated with insulin) and 62 control subjects. FRDA intron 1 triplet expansion genotyping was analyzed using primers and polymerase chain reaction (PCR) conditions described by Filla et al. (9), with one of the primers labeled with a fluorophore. We used separation on polyacrylamide gels on an ABI 377 automated sequencer and analysis with the Genescan 2.1 and Genotyper 2.0 computer software programs (Perkin Elmer, Foster City, CA). The number of repeats was deduced from allele sizes obtained through genotyping analysis (size product = $500 + 3n$; n = number of GAA repeats) and checked by direct sequencing for 32 random samples (data not shown). Through sequencing of those samples, we have detected a 6-base pair (bp) TAATAA deletion in a (TAA)₄ tract 165 bp downstream of the GAA repeat. This deletion, found at the same frequencies in our NIDDM and control groups, corresponded in each case to individuals with a size product of 515 bp. Thus, they seem to have five GAA repeats, but sequencing analysis revealed that those individuals had, in fact, seven GAA repeats and the

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Received for publication 27 February 1998 and accepted in revised form 1 July 1998.

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

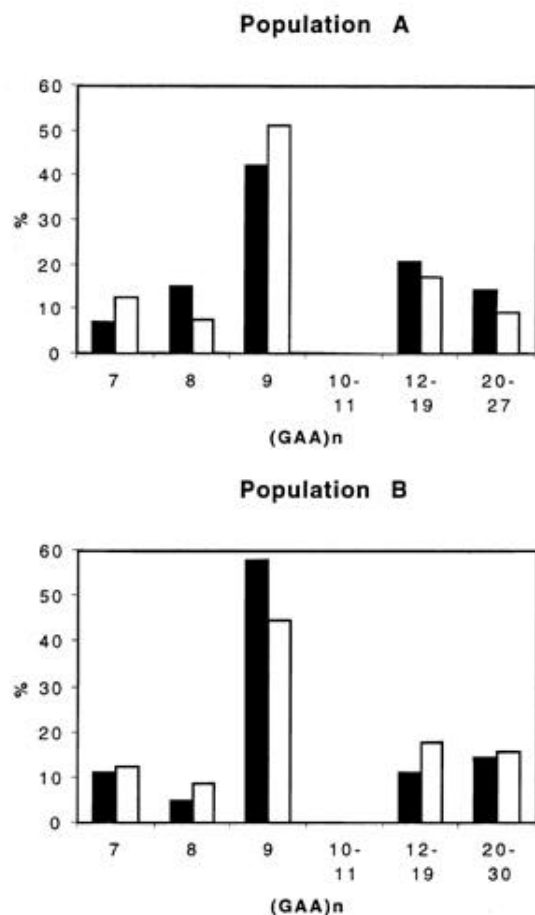


FIG. 1. GAA repeat expansion in the first intron of the frataxin gene in NIDDM and control subjects in the two populations. The x-axis indicates the number of GAA repeat expansions of the longer allele of each individual. The rare alleles longer than 12 repeats were pooled in two groups: 12–19 and 20–30 bp. The y-axis indicates the percentage of each allele in both groups. ■, control subjects; □, NIDDM patients.

deletion. Therefore, alleles with 515 bp size were assigned the seven repeats instead of five.

The GAA repeat length ranged from 7 to 27 in population A and from 7 to 30 in population B. The average number of repeats was 11.5 ± 5.1 in the NIDDM group and 12.5 ± 5.6 in the control group of population A and 12.4 ± 5.9 in the NIDDM group and 11.6 ± 5.5 in the control group of population B. The general distribution profile of the polymorphic GAA repetitive tract revealed two distinct classes of alleles (Fig. 1). The first class (class I) comprised alleles with 7–9 repeats, and the second (class II) alleles with 12–30 repeats (no alleles were found with 10 or 11 repeats), corresponding respectively to the 7–10 and 17–29 groups described by Epplen et al. (10).

No association was found between NIDDM and the class (I or II) of the longer of both alleles or the genotype (I/I, I/II, II/II) of the subjects calculating χ^2 ratios. Moreover, there was no difference in the absolute number of GAA repeats of the longer allele in patients with NIDDM compared with control subjects (data not shown).

The association study on population B was performed in parallel on 2% agarose gel after PCR amplification of a 228 + 3n bp product (n = number of GAA triplets). The primers were 5'-GGGATTGGTGCCAGTGCTTAAAAGTTAG-3' and 5'-

CGACACCACGCCCGGCTAAC-3', and PCR conditions were as follows: 94°C for 10 min, then 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and finally 72°C for 10 min. The distribution into classes I and II was the same for NIDDM patients and control subjects, but the definite size of the alleles could not be determined accurately using these experimental conditions.

In the NIDDM patients of populations A and B, no association was found between the class of the longer of both alleles or the genotype of the subjects and BMI or age at diagnosis of the disease. To test whether there was an interaction between GAA repeat length and obesity (BMI >27 kg/m²) in determining the risk of diabetes, logistic regression analyses were done in population A with the diabetic status as the categorical dependent variable (NIDDM versus control groups) and with age, sex, BMI, and the repeat allele classes as independent variables. This analysis confirmed no association between the diabetic status and the allele class either in dominant (I/I vs. I/II + II/II) or recessive (I/I + I/II vs. II/II) models (data not shown).

Linkage studies at the FRDA locus were performed through genotyping microsatellites located near the FRDA gene (D9S15 and D9S166) in 149 NIDDM French families, using automated-fluorescent based procedures (11). Families came from the group of 172 families previously described (12), from which we withdrew families with bilineal transmission of diabetes, resulting in 638 individuals with 392 NIDDM patients. Sib-pair analysis was carried out with the computer program SIBPAL of the SAGE package (13).

No excess in allele sharing was found with markers D9S15 (0.5 ± 0.28 , NS) and D9S166 (0.48 ± 0.3 , NS) among 271 affected sib pairs. These results suggest that the FRDA locus does not play a major role in susceptibility to NIDDM in the French population. Similar results were obtained in Caucasian families from Utah (14).

In conclusion, our results do not support a role for the polymorphic FRDA gene in the development of the common form of NIDDM in the French population. The discrepancy between our results and those of Ristow et al. (7) may be explained by the different methods used in the two studies, although we could not exclude the role of genetic heterogeneity of NIDDM. Further studies in other populations are needed to clarify this issue.

ACKNOWLEDGMENTS

This study was supported by the Centre Hospitalier Universitaire de Lille and the Conseil Régional Nord-Pas-De-Calais Grant.

We would like to thank Karine Clément and Christian Dina for help with statistical calculations.

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