*Brief Genetics Report*

# Intermediate Expansions of a GAA Repeat in the Frataxin Gene Are Not Associated With Type 2 Diabetes or Altered Glucose-Induced β-Cell Function in Danish Caucasians

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**A variable expansion of a GAA repeat is present in the first intron of the frataxin gene, also termed *FRDA1* or *X25*. Long repeat lengths (>66 repeats) are present in patients with Friedreich's ataxia, while an intermediate expansion (10–66 repeats) has recently been reported to be highly associated with type 2 diabetes. Using a polymerase chain reaction–based assay, we found that 32.4% (95%CI 29.9–34.9) of 636 Danish Caucasian type 2 diabetic patients were carriers of an intermediate expansion, whereas the frequency was 30.4% (26.4–34.4) among 224 matched glucose-tolerant control subjects ($P = 0.6$). In the control subjects, the values of serum insulin and C-peptide responses during an oral glucose tolerance test were similar between the 69 carriers and 155 noncarriers. Furthermore, we investigated a possible relationship between expansions of the *FRDA1* gene and glucose-induced β-cell function in 338 young Caucasians (33.7% [30.1–37.3] carriers) and in 215 glucose-tolerant subjects (31.0% [26.6–35.4] carriers) with a type 2 diabetic parent. In neither population did the carriers differ from noncarriers according to values of fasting plasma glucose, serum insulin, or C-peptide, acute serum insulin, or C-peptide responses after intravenous glucose. In conclusion, intermediate expansion of the frataxin trinucleotide repeat is not associated with type 2 diabetes or altered glucose-induced insulin secretion in Danish Caucasians. *Diabetes* 48:914–917, 1999**

Long expansions of a GAA trinucleotide (ranging from 66 to >1,700 repeats) in the first intron of the frataxin gene, *FRDA1* or *X25*, which has been located to chromosome 9q13–21.1, are responsible for the spinocerebellar degenerative disease Friedreich's ataxia (FRDA) (1), whereas the normal range of repeats is from 7 to 36 (2). Frataxin is a mitochondrial protein involved in cellular iron metabolism (3). The expression pattern is wide, but the gene is predominantly expressed in tissues with a high metabolic rate. Frataxin mRNA levels are greatly reduced in FRDA patients, presumably leading to reduced function of the respiratory chain (4). The clinical picture of FRDA patients is very heterogeneous, with a strong correlation between the expansion length and the severity of the disease (5). FRDA is often accompanied by type 2 diabetes and cardiomyopathy, and the incidence of both diseases increases with increasing mean allele length. This makes it imaginable that minor expansions of this triplet repeat could lead to smaller reductions in frataxin mRNA, thus contributing to the development of β-cell dysfunction. Recently, it has been demonstrated that intermediate expansions (defined as >9 repeats and <66) are associated with type 2 diabetes in both an American and a German population (6). The objective of the present study was to examine whether intermediate expansions of the *FRDA1* gene were associated with type 2 diabetes or altered β-cell function and insulin sensitivity.

Association studies were performed in 636 Danish Caucasian type 2 diabetic patients recruited from the outpatient clinic at the Steno Diabetes Center and 224 age-matched, unrelated, and glucose-tolerant Danish Caucasian control subjects recruited through the Danish Central Population Register and living in the same area of Copenhagen as the type 2 diabetic patients. Diabetes was diagnosed by World Health Organization criteria. All control subjects underwent a standard 2-h 75-g oral glucose tolerance test (OGTT) (five blood samplings for analysis) with measurements of plasma glucose, serum insulin, and serum C-peptide during the test. For further studies of an effect of intermediary expansions on glucose metabolism, two populations were investigated: *1*) a population-based sample of 338 unrelated Danish Caucasians aged 18–32 years who underwent a tolbutamide-modified intravenous glucose tolerance test (IVGTT) for measurement of acute serum insulin and C-peptide responses and the

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

insulin sensitivity index (7) and 2) a study of 215 glucose-tolerant offspring from 62 Danish Caucasian nuclear families identified through one parent suffering from type 2 diabetes. All offspring underwent an examination with a tolbutamide-modified frequently sampled IVGTT for estimation of acute insulin and C-peptide responses and the insulin sensitivity index. The plasma glucose concentration was analyzed by a glucose oxidase method (Granutest; Merck, Darmstadt, Germany). The concentration of specific insulin (excluding des(31,32)- and intact proinsulin) in serum was measured by enzyme-linked immunosorbent assay (Dako insulin kit K6219; Dako Diagnostics, Ely, U.K.), and the concentration of serum C-peptide was determined using Steno Diabetes Center routine methods. Informed consent was obtained from all studied subjects before participation in the study, and the studies were carried out in accordance with the principles of the Declaration of Helsinki II. The study protocol was approved by the Committee of Ethics in Copenhagen.

The trinucleotide expansions of the first intron of the *FRDA1/X25* gene were studied using a polymerase chain reaction (PCR) assay. Primers were designed to amplify a product of 425 base pairs for the most frequent allele with nine GAA repeats. The forward primer was labeled with one of the fluorescent dyes: Fam, Hex, or Tet. Primer sequences were forward: 5′-ggc tta aac ttc cca cac gtg tt-3′, and reverse: 5′-gct ctg tcg ccc agg ccg gag-3′. PCR was carried out in 10-µl reactions containing 40 ng DNA, 10 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 200 mmol/l of each dNTP, 240 nmol/l of each primer, and 0.14 U Taq polymerase (Perkin Elmer Cetus, Foster City, CA). PCR conditions were as follows: an initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, annealing at 62°C for 30 s, elongation at 72°C for 1 min, and a final elongation step at 72°C for 5 min. Differently labeled PCR products from three different subjects were run in each lane of a Long Ranger polyacrylamide gel on an ABI 377XL. Allele sizes were scored using Genotyper 2.0 (Applied Biosystems, Foster City, CA), with Tamra 500XL as an internal size standard. From 27 patients, a new PCR was performed, and the products were sequenced directly using the protocol of dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) with the unlabeled reverse primer and subjected to electrophoresis on an ABI 377XL. Exact length determination of the differing alleles was possible from a single sequencing reaction.

$\chi^2$ analysis was applied to test for differences in carrier frequencies. A Mann-Whitney test was used for comparison between groups. To control for the influence of sex, age, and BMI on estimates of β-cell function and insulin sensitivity, multiple regression analyses were performed. Non-normally distributed variables were logarithmically transformed. Data are presented as medians (interquartile range). In the study of 215 glucose-tolerant offspring, the key variables were analyzed applying a variance component model (random effects), in which an extra source of variation is allowed to account for the fact that individuals from the same family may be correlated. A $P$ value <0.05 (two-tailed) was considered significant.

In 54 chromosomes, the number of repeats was estimated by sequencing in addition to the fragment analysis. The correlation of results obtained with fragment analysis versus those from direct sequencing was high ($r = 0.956$, $P < 0.001$). The repeat size estimated by fragment analysis, however, was on average scored one-half repeat lower compared with the number of repeats identified by sequencing. The size range of the short and intermediate repeats was 5–42, and the size distribution of alleles in the diabetic and control subjects was very similar (Fig. 1). The carrier frequency of intermediate repeats was 30.4% (26.4–34.4) among control subjects (68 of 224) and 32.4% (29.9–34.9) among type 2 diabetic patients (206 of 636) ($\chi^2 = 0.273$, $P = 0.6$). In the group of young Danish Caucasians and in the group of glucose-tolerant offspring from one diabetic proband, we studied the relationship between expanded alleles and the following variables: fasting plasma glucose, serum insulin, and C-peptide; acute serum insulin and C-peptide responses after an IVGTT (incremental area under the curve from 0 to 8 min); and the insulin sensitivity index (Table 1). There were no differences in any of the listed variables between the group having one or two expanded alleles compared with the group having two short alleles, except for the insulin sensitivity index ($P = 0.013$) in the population of young Danish Caucasians. However, when correcting for the influence of age, BMI, and sex, this difference disappeared ($P = 0.054$). In the cohort of glucose-tolerant offspring from one diabetic proband, there was no difference between groups with respect to any of the measured variables (Table 1). In the population of glucose-tolerant control subjects who participated in the association study, there was no difference between the two groups.

Recently, Ristow et al. (6) reported a three- to fourfold higher prevalence of intermediate expansions (10–36 repeats) in the first intron of the frataxin gene in both German and white American patients with type 2 diabetes. This finding was of interest, since it is well known that ~10% of patients with FRDA (caused by expansions longer than 66 GAA repeats in the frataxin gene) have diabetes and that an additional 20% may have impaired glucose tolerance (5,8). Also, FRDA patients have reduced β-cell function upon intravenous arginine compared with control subjects (8). In the present study, we failed to replicate the findings of Ristow et al. The reason for this is unknown. However, the frequency of intermediate expansions among diabetic patients in our study (32.4%) is close to what was reported in the German (24.7%) and in the U.S. (27.3%) populations (6). The major difference appears to be that our population-based sample of matched glucose-tolerant control subjects has a much higher

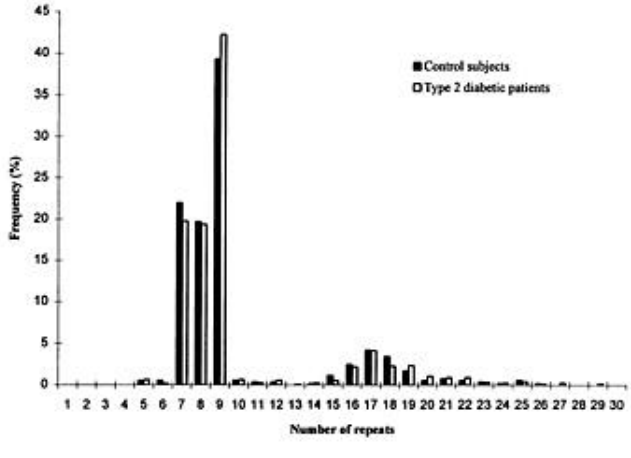


**FIG. 1. Histogram showing the size distribution of GAA triplet repeats in the first intron of the frataxin gene in 224 control subjects and 636 type 2 diabetic patients.**

TABLE 1
Clinical and biochemical data of a population-based sample of 338 young healthy Danish Caucasians, 215 glucose-tolerant offspring of 62 type 2 diabetic probands, and 224 middle-aged glucose-tolerant control subjects

| | Expanded | Nonexpanded | *P* values |
|---|---|---|---|
| Young Danish Caucasians | | | |
| *n* (M/W) | 56/58 | 113/111 | 0.82 |
| Age (years) | 25 (7) | 25 (5) | 0.24 |
| BMI (kg/m$^2$) | 22.4 (4.0) | 23.3 (5.8) | 0.10 |
| Acute serum insulin response (IVGTT) (0–8 min) (min · pmol$^{-1}$ · l$^{-1}$) | 1,677 (1,184) | 2,053 (1,721) | 0.19 (0.48) |
| Acute serum C-peptide response (IVGTT) (0–8 min) (min · pmol$^{-1}$ · l$^{-1}$) | 6,068 (3,772) | 6,390 (4,212) | 0.25 (0.80) |
| Fasting plasma glucose (mmol/l) | 4.9 (0.5) | 5.0 (0.7) | 0.26 (0.32) |
| Fasting serum insulin (pmol/l) | 29 (18) | 32 (25) | 0.08 (0.23) |
| Fasting serum C-peptide (pmol/l) | 443 (138) | 448 (208) | 0.33 (0.48) |
| Insulin sensitivity index (10$^{-5}$ l/(min · pmol$^{-1}$ · l$^{-1}$) | 15 (4) | 11 (10) | 0.01 (0.05) |
| Offspring of diabetic proband | | | |
| *n* (M/W) | 29/34 | 68/84 | 0.86 |
| Age (years) | 39 (12) | 38 (11) | 0.37 |
| BMI (kg/m$^2$) | 24.8 (5.0) | 24.9 (5.3) | 0.20 |
| Acute serum insulin response (IVGTT) (0–8 min) (min · pmol$^{-1}$ · l$^{-1}$) | 1,699 (1,627) | 1,710 (1,542) | 0.46 (0.64) |
| Acute serum C-peptide response (IVGTT) (0–8 min) (min · pmol$^{-1}$ · l$^{-1}$) | 5,414 (3,106) | 5,868 (3,936) | 0.83 (0.74) |
| Fasting plasma glucose (mmol/l) | 5.1 (0.8) | 5.2 (0.5) | 0.20 (0.23) |
| Fasting serum insulin (pmol/l) | 31 (17) | 35 (32) | 0.97 (0.40) |
| Fasting serum C-peptide (pmol/l) | 490 (235) | 515 (188) | 0.25 (0.14) |
| Insulin sensitivity index (10$^{-5}$ l/min · pmol$^{-1}$ · l$^{-1}$) | 11 (8) | 10 (9) | 0.44 (0.12) |
| Glucose-tolerant control subjects | | | |
| *n* (M/W) | 35/34 | 79/76 | 0.89 |
| Age (years) | 53.6 (19.4) | 50.4 (20.1) | 0.07 |
| BMI (kg/m$^2$) | 24.8 (5.4) | 24.8 (4.3) | 0.89 |
| Fasting plasma glucose (mmol/l) | 5.2 (0.7) | 5.0 (0.7) | 0.28 (0.56) |
| Fasting serum insulin (pmol/l) | 35 (28) | 37 (148) | 0.32 (0.36) |
| Fasting serum C-peptide (pmol/l) | 553 (248) | 534 (178) | 0.66 (0.69) |
| AUC glucose 0–120 min (min · mmol$^{-1}$ · l$^{-1}$) | 179 (146) | 154 (169) | 0.10 (0.22) |
| AUC insulin 0–120 min (min · pmol$^{-1}$ · l$^{-1}$) | 20,978 (14,151) | 18,559 (75,165) | 0.94 (0.43) |
| AUC C-peptide 0–120 min (min · pmol$^{-1}$ · l$^{-1}$) | 145,931 (67,322) | 133,313 (63,694) | 0.60 (0.69) |

Data are medians (interquartile range). Subjects are classified according to the presence of an intermediary expansion of the GAA trinucleotide repeat in the first intron of the frataxin gene. *P* values compare carriers of expanded alleles with individuals with two short alleles using a Mann-Whitney test. *P* values in parentheses refer to linear regressions with age, sex, and BMI as dependent variables. With respect to the offspring of a diabetic proband, the variance component model was applied (see METHODS). AUC, area under the curve during an OGTT for the glucose, insulin, or C-peptide responses.

frequency of intermediate expanded alleles of the triplet repeat (30.4%) than the ones reported by Ristow et al. (7.6 and 6.3%) (6). We can exclude the risk of a type II error with a high probability, since the estimated power of our study to detect the same difference in allele frequencies as Ristow et al. is >99.5%. The validation of our chosen method shows that it reliably estimates the number of trinucleotide repeats. In conclusion, intermediate expansions of the frataxin trinucleotide repeat are not associated with type 2 diabetes or altered glucose-induced insulin secretion in Danish Caucasians.

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