

Altered β -Cell Characteristics in Impaired Glucose Tolerant Carriers of a GAA Trinucleotide Repeat Polymorphism in the Frataxin Gene

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Friedreich's ataxia is associated with GAA trinucleotide repeat expansions in the frataxin gene. In the general population, these trinucleotide expansions are variable in length, and three types of expansions are seen: short, intermediate, and long repeats. Friedreich's ataxia patients are generally homozygous for the long repeats and exhibit diabetes as pronounced comorbidity (1,2). Ristow et al. (3) recently reported an association between the intermediate-length normal allele in the frataxin gene and type 2 diabetes.

We have investigated in 94 subjects with impaired glucose tolerance (IGT) as to whether the length of the GAA trinucleotide repeat polymorphism in the frataxin gene associates with parameters reflecting β -cell function. A hyperglycemic clamp at 10 mmol/l glucose for 3 h was used to quantify β -cell characteristics. Carriers of one or two intermediate repeat alleles ($n = 32$) had a 50% higher median first-phase insulin response to glucose than the noncarriers. Furthermore, they needed less time to reach peak insulin. An analysis of the distribution of the various repeat lengths in elderly type 2 diabetic ($n = 179$) and control subjects ($n = 183$), with the same age and ethnic background, did not provide evidence for an association of the intermediate-length repeat allele with type 2 diabetes in Dutch Caucasians.

The GAA trinucleotide repeat in the first intron of the frataxin gene has previously been linked to Friedreich's ataxia, a progressive neurological disorder (4). Almost all of the Friedreich's ataxia patients have very large repeat expansions on both alleles, the average repeat length varying from 200 to >1,700 repeats (4). Normal individuals carry repeat expansions, which can be divided into two classes: short-length normal alleles with 1–10 repeat units (allele frequency

~83%) and intermediate-length normal alleles with 11–36 repeat units (allele frequency ~17%) (4). About 40% of all Friedreich's ataxia patients have diabetes or IGT (2). The glucose intolerance in Friedreich's ataxia patients has been ascribed to insulin resistance and β -cell defects (2,5). A recent study by Filla et al. (6) showed that in subjects with Friedreich's ataxia, diabetes is associated with the length of the GAA repeat. Furthermore, Ristow et al. (3) reported an association between the intermediate-length normal allele in the frataxin gene and type 2 diabetes, with approximately a four-fold increase in occurrence of the intermediate allele in the diabetic group compared with the control group (3).

We have investigated whether the length of the GAA trinucleotide repeat in the frataxin gene associates with parameters reflecting β -cell function. We studied 94 subjects with IGT. These subjects are at a high risk of developing type 2 diabetes (7), but the adverse effects of hyperglycemia on β -cell function and insulin sensitivity have not yet developed (8). Therefore, these subjects enable the study of early dysfunction in insulin action and β -cell function in relation to repeat length variations in the frataxin gene. A hyperglycemic clamp at 10 mmol/l glucose for 3 h was used to quantify β -cell characteristics.

Short-length normal alleles were present on both alleles in 62 subjects (66%). In 32 (34%) cases, we observed intermediate-length normal alleles. In the latter group, 8 of 32 subjects carried the intermediate-length normal repeat on both alleles. The remaining 24 subjects had one short-length normal and one intermediate-length normal allele. Long repeats were absent. The observed allele frequency for the intermediate-length repeat (21%) was comparable with those found in other Caucasian populations (3,4,9). When the data of the hyperglycemic clamps were evaluated, carriers of the intermediate repeats on one or both alleles had a 50% higher median first-phase insulin response compared with carriers of the short-length normal alleles (14.0 [7.2–23.5] vs. 9.0 [3.3–15.5] $\mu\text{U} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$; $P = 0.01$, Table 1). Analysis of covariance showed that the association was independent of differences in age, sex, BMI, and insulin sensitivity at 10 mmol/l glucose (estimated from the hyperglycemic clamp data). We also noted a shorter time until the peak insulin level in carriers of intermediate-length repeats. When subjects were classified according to "time-to-insulin peak" (35%, 0–5 min, 34% 5–20 min, 31% >20 min), there was a significant inverse association between the presence of the intermediate-length

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I, plasma insulin response; IGT, impaired glucose tolerance; M, amount of glucose infused.

repeat allele (i.e., one or two intermediate-length alleles) and time until the insulin peak (0–5 min, 56% of intermediate-length repeat carriers, 5–20 min, 30% and >20 min, 21%, respectively, $\chi^2 = 7.4$, $df = 2$, $P = 0.03$). Other parameters related to β -cell function, such as peak height, second-phase insulin response, and proinsulin-to-insulin ratios, were not significantly different between carriers and noncarriers (Table 1). No significant differences were observed between the genotypes in relation to fasting and postload glucose levels, BMI, HbA_{1c}, lipids, and proinsulin levels (Table 1). Furthermore, we investigated whether the number of intermediate-length repeat alleles (i.e., 0, 1, or 2) was associated with indicators of β -cell function, but no significant relationship was observed (data not shown).

The observed hypersecretion of insulin during glucose stimulation and shorter time until the insulin peak in carriers of the intermediate-length repeat likely reflects an altered β -cell response to glucose, although insulin hypersecretion caused by insulin resistance cannot be fully excluded. Whether this altered insulin secretion pattern represent a disadvantage or in fact just shows to be beneficial compared with the carriers of two short-length alleles remains to be elucidated. A follow-up of the participants will provide insight.

The carrier frequency of the intermediate-length repeat in a population-based sample of elderly type 2 diabetic and control subjects was 31% (55 of 179) and 36% (65 of 183), respectively, which is not significantly different ($\chi^2 = 0.94$, $df = 1$; $P = 0.33$). Also, no differences in diabetes-related parameters (such as fasting and 2-h glucose levels, insulin levels, HbA_{1c}, and BMI) were seen between carriers of short- and intermediate-length alleles (data not shown). β -cell function and insulin action were not assessed in these subjects. Also, a recent study in the French population did not provide support for the association with type 2 diabetes (9). The origin of the difference with the results reported by Ristow et al. (3) is unclear, but one should realize that genetic association studies are vulnerable to genetic admixture (10). To reduce the risk of artifactual association, we have validated the observed frequencies in the Hoorn study in another population-based study in the Netherlands, known as the Rotterdam Study. We observed no significant differences between both populations (data not shown).

Previously, it has been shown that triplet expansions in several genes, expressed in muscle, do not differ between type 2 diabetic and control subjects (11), suggesting that trinucleotide repeat expansions in general are not a major factor contributing to the development of type 2 diabetes. In addition, the similar distribution of the frataxin repeat alleles in both the type 2 diabetic and control groups and the lower frequency of a positive family history we observed in the IGT subjects (Table 1) also argues against a major contribution of the intermediate-length repeat in the frataxin gene to the development of type 2 diabetes in Dutch Caucasians. It might be, however, that the intermediate-length repeat allele contributes to the polygenic origin of type 2 diabetes, e.g., by epistatic mechanisms, in susceptible individuals.

The frataxin protein is located in the mitochondria, where it might influence mitochondrial metabolism due to iron accumulation and increased sensitivity to oxidative stress (4). Some of the clinical features of Friedreich's ataxia are also characteristic for mitochondrial diseases. Little is known about the effect of variations in the trinucleotide repeat

length on frataxin activity. Recent data using recombinant DNA constructs containing different GAA repeat numbers suggest that the presence of the intermediate-length repeat may have functional consequences (12,13). A change in β -cell function caused by mutations in mitochondrial DNA has been implicated in the pathogenesis of some diabetic subtypes, such as maternally inherited diabetes and deafness (MIDD) (14,15). It seems plausible that changes in frataxin expression result in alterations in mitochondrial functioning and, thereby, in insulin secretion.

In conclusion, our data suggest that the intermediate-length repeat expansion in the frataxin gene affects insulin secretion from the β -cell, resulting in a 50% higher first-phase insulin secretion and a shorter time to reach peak insulin level. The mechanism underlying this hypersecretion remains unclear. As both phenomena can be related to the milieu interieur of the β -cell, it is unlikely that they have occurred by chance as result of multiple hypothesis testing (10). The lower frequency of a positive family history of diabetes in the IGT subjects and similar frequency of the intermediate-length alleles in type 2 diabetic and normoglycemic control subjects suggest that the intermediate-length repeat in the frataxin gene is unlikely to enhance the risk of development of type 2 diabetes in Dutch Caucasians.

RESEARCH DESIGN AND METHODS

Subjects. IGT subjects were selected from a random sample ($n = 12,093$) of inhabitants of the town Hoorn in the Netherlands. All subjects, aged between 45 and 75 years, were invited for a fasting blood glucose determination. Responders (55% with fasting glucose values >5.5 mmol/l ($n = 3,147$)) were invited for a subsequent 75-g oral glucose tolerance test. Subjects with 2-h plasma glucose >7.8 mmol/l ($n = 554$) underwent a second oral glucose tolerance test and those with a mean 2-h plasma glucose value between 8.6 and 11.1 mmol/l were included in the present study. These individuals ($n = 94$) were examined by the hyperglycemic clamp technique at a constant level of 10 mmol/l glucose for 3 h (16). All participants gave written informed consent, and the protocol was approved by the appropriate medical ethics committees.

Hyperglycemic clamp. All subjects underwent a hyperglycemic clamp in the fasting state in a supine position (16). Baseline samples were drawn for glucose and proinsulin determination. Hyperglycemia was induced by a priming infusion of 20% glucose (150 mg/kg). Blood glucose was measured at 5-min intervals and adjusted, when necessary, with a variable glucose infusion to maintain a constant blood glucose concentration of 10 mmol/l. Samples for insulin determination were taken at 2.5-min intervals during the first 10 min of the clamp and at 10-min intervals during the next 170 min. The first-phase insulin response was calculated as increment in the area under the curve during the 0- to 10-min time period ($\mu\text{U} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$). Second-phase insulin response was the mean insulin concentration during the 150- to 180-min time period ($\mu\text{U/ml}$). Insulin sensitivity (M/I) was calculated as the amount of glucose infused (M) ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) divided by the plasma insulin response (I) ($\mu\text{U/ml}$) (16). Subjects with a plasma insulin response <4.2 $\mu\text{U/ml}$ were excluded for this estimation (17). Time-to-peak insulin represents the time to reach the peak in insulin level after glucose stimulation.

Biometry and laboratory analyses. Height, weight, and waist and hip circumferences of all participants were measured, and BMI (kg/m^2) and waist-to-hip ratio were calculated from these data. Plasma glucose levels were measured by the glucose dehydrogenase method (Merck, Darmstadt, Germany). Specific insulin and intact proinsulin were measured by immunoradiometric assays (Medgenix Diagnostics, Fleures, Belgium and Dako, Cambridgeshire, U.K., respectively). Other relevant clinical data were obtained by standard procedures and/or questionnaires. Hypertension was defined as diastolic blood pressure ≥ 95 and/or systolic blood pressure ≥ 160 mmHg, respectively, and/or the use of blood pressure-lowering medication.

Association study. We determined the repeat length in patients with type 2 diabetes ($n = 179$) and normoglycemic control subjects ($n = 183$) from the Hoorn study and the Rotterdam study, which are both population-based studies in the Netherlands (18,19). Subjects participating in this study were randomly selected from the initial cohorts. All participants previously underwent an oral glucose tolerance test, as described (18–20). Characteristics of both groups: age 65 ± 7 and 61 ± 7 years, BMI 27 ± 4 and 26 ± 3 kg/m^2 , sex 50 and 48% men for type 2 diabetes and normoglycemic subjects, respectively, $P < 0.05$ for age and BMI.

TABLE 1
Clinical characteristics in categories of frataxin repeat size

	Frataxin GAA trinucleotide repeat size		
	Short-length	Intermediate-length	P value
<i>n</i>	62	32	
Age (years)	56 ± 8	59 ± 6	0.10
BMI (kg/m ²)	28.9 ± 4.0	27.5 ± 3.5	0.09
Sex (% M/F)	48	50	0.88
Waist/hip ratio	0.93 ± 0.10	0.93 ± 0.07	0.84
Hypertension (%)	64	50	0.18
Positive family history of diabetes (%)	52	29	0.04
Fasting glucose (mmol/l)	6.6 ± 0.6	6.5 ± 0.6	0.24
2-h OGTT glucose (mmol/l)	9.6 ± 0.7	9.5 ± 0.7	0.80
HbA _{1c} (%)	5.8 ± 0.7	5.6 ± 0.5	0.16
Fasting insulin (μU/ml)	11.8 (9.7–16.7)	10.5 (8.7–16.5)	0.65
Fasting proinsulin (μU/ml)	0.93 (0.63–1.40)	0.88 (0.65–1.43)	0.49
Fasting proinsulin/insulin ratio	0.07 (0.06–0.11)	0.08 (0.06–0.15)	0.20
Time-to-peak insulin (min)	8 (5–30)	5 (5–8)	0.10
Peak insulin (μU/ml)	37.8 (23.7–53.3)	42.7 (24.8–58.3)	0.50
First-phase insulin (μU · ml ⁻¹ · min ⁻¹)	9.0 (3.3–15.5)	14.0 (7.2–23.5)	0.01
Second-phase insulin (μU/ml)	46.2 (31.8–76.8)	49.2 (40.2–81.0)	0.57
Insulin sensitivity (100 mg · kg ⁻¹ · min ⁻¹ · μU ⁻¹ · ml)	8.5 (5.7–13.0)	9.5 (5.1–13.1)	0.82

Data are means ± SD or medians (interquartile range). Frataxin GAA repeat size: short-length normal (1–10 repeats) and intermediate-length normal (11–40 repeats). Difference between short- and intermediate-length normal repeat sizes was tested by analysis of variance (ANOVA). Variables with a skewed distribution were log transformed before ANOVA.

DNA analysis. DNA was extracted from peripheral blood samples using standard procedures. The GAA trinucleotide repeat polymorphism length in intron 1 of the frataxin gene was determined by a polymerase chain reaction–based assay with primers GAA-F and GAA-R as described by Campuzano et al. (1).

Statistical analysis. Statistical analyses were performed using the SPSS 7.0 package (SPSS, Chicago). Carriers and noncarriers were compared using either *t* tests or analysis of covariance, when appropriate. Variables with a skewed distribution were logtransformed before analysis to normalize the residuals. A χ^2 test was used to analyze the distribution of intermediate-length repeats over the different classes of time until the insulin peak. Multiple analysis of variance and regression analysis was performed to adjust for possible confounding. Differences in allele distribution between type 2 diabetes and control groups were tested by the χ^2 method.

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