

Brief Genetics Report

Variation in the Calpain-10 Gene Affects Blood Glucose Levels in the British Population

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Variation in the calpain-10 gene (*CAPN10*) has been shown to be associated with type 2 diabetes in Mexican-Americans and in at least three Northern European populations. Studies in nondiabetic Pima Indians showed that one of the at-risk DNA polymorphisms, single-nucleotide polymorphism (SNP)-43, in *CAPN10* was associated with insulin resistance, and individuals with the G/G-genotype had significantly higher fasting plasma glucose and 2-h insulin concentrations after a 75-g oral glucose tolerance test (OGTT). We have examined the effect of variation in *CAPN10* on plasma glucose and insulin levels in a group of 285 nondiabetic British subjects after a 75-g OGTT. The results showed that subjects with G/G genotype at SNP-43 had higher 2-h plasma glucose levels than the combined G/A + A/A group ($P = 0.05$). We also examined the SNP-43, -19, and -63 haplotype combination 112/121, which is associated with an approximately threefold increased risk of diabetes. Subjects with the 112/121 haplotype combination ($n = 29$) had increased fasting ($P = 0.004$) and 2-h plasma glucose levels ($P = 0.003$) compared with the rest of the study population after correction for age, sex, and BMI. The 112/121 haplotype combination was also associated with a marked decrease in the insulin secretory response, adjusted for the level of insulin resistance ($P = 0.002$). We conclude that genetic variation in the *CAPN10* gene influences blood glucose levels in nondiabetic British subjects and that this is due, at least in part, to the effects of calpain-10 on the early insulin secretory response. *Diabetes* 51:247–250, 2002

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Additional information can be found in an online appendix at <http://diabetes.diabetesjournals.org>.

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CV, coefficient of variation; EIR, early insulin response; HOMA_{IR}, homeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

Type 2 diabetes is a complex metabolic disorder characterized by defects in hepatic glucose production and insulin secretion and action (1). Genetic factors play an important role in determining susceptibility, presumably by affecting one or more of these processes. We have recently identified the calpain-10 gene (*CAPN10*) as a diabetes susceptibility gene (2). Variation in *CAPN10* is associated with a threefold increased risk of type 2 diabetes in Mexican-Americans and an increased risk of diabetes in Northern European populations (2,3). The gene accounts for ~14% of the diabetes risk in Mexican-Americans, but only 4% in Northern Europeans. Calpain-10 is a nonlysosomal cysteine protease of unknown function that is expressed in many tissues, including pancreatic islets, muscle and adipose tissue, and liver—tissues that play the key roles in controlling glucose homeostasis. Our data also suggest that variation in the levels of calpain-10 determines susceptibility (2). Studies in nondiabetic Pima Indians showed an association of the *CAPN10* single-nucleotide polymorphism (SNP)-43 with calpain-10 mRNA levels in skeletal muscle and insulin resistance, with subjects homozygous for the G allele at SNP-43 having lower levels of calpain-10 mRNA in skeletal muscle and also being more insulin-resistant (4). In addition, during an oral glucose tolerance test (OGTT), they had higher mean fasting plasma glucose ($P = 0.01$, adjusted for age, sex, percentage body fat, and nuclear family membership) and higher mean plasma insulin at 2 h ($P = 0.05$) compared with a combined group consisting of individuals with G/A and A/A genotypes. They also tended to have higher 2-h glucose and fasting insulin levels, although the differences did not reach statistical significance. The aim of this study was to investigate whether variation in the calpain-10 gene predicted blood glucose levels and metabolic traits in a nondiabetic group of British subjects ($n = 285$), of which 145 had a family history of type 2 diabetes.

We typed three DNA polymorphisms in *CAPN10* (SNP-43, SNP-19, and SNP-63) in these 285 nondiabetic subjects, all of whom had undergone a standard 75-g OGTT. We then examined the effect of SNP-43 genotype on plasma glucose levels and other metabolic traits. We also did the same for the SNP-43, -19, and -63 haplotype combinations.

SNP-43 had allele frequencies of G (allele 1) = 0.79 and A (allele 2) = 0.21 in the study population and were in

TABLE 1
Clinical and metabolic characteristics of subjects by SNP-43 genotype

	G/G	G/A+A/A	P
<i>n</i>	177	108	
Age (years)	40 ± 1	40 ± 1	0.8
Sex (M/F)	82/95	47/61	0.7
BMI (kg/m ²)	26.4 ± 0.4	26.3 ± 0.5	1.0
WHR	0.83 ± 0.01	0.84 ± 0.01	0.7
Fasting glucose (mg/dl)	86 ± 1	85 ± 1	0.2
2-h Glucose (mg/dl)	99 ± 3	92 ± 3	0.05
HOMA _{IR}	1.9 (1.7–2.1)	2.1 (1.9–2.4)	0.3
2-h Insulin (μU/ml)	39 (35–45)	39 (33–46)	0.9
EIR (mU/mmol)	22 (20–24)	24 (21–27)	0.3
Adjusted insulin secretory response	11 (9–13)	11 (10–13)	0.7

Data are the means ± SE or geometric mean (95% CI) *P* values are two-sided. WHR, waist-to-hip ratio.

Hardy-Weinberg equilibrium. There was no difference in genotype frequencies between those subjects with and without a family history of type 2 diabetes (the frequencies are listed in Table A1 in the online appendix at <http://diabetes.diabetesjournals.org>). The G/G genotype alone is associated with a 1.5-fold increased risk of diabetes in Mexican-Americans and a 1.8-fold increase in Botnians (appendix to ref. 2). We compared the metabolic characteristics of subjects homozygous for the G allele (*n* = 177) with the remaining subjects (*n* = 108) (i.e., G/A and A/A genotypes) (Table 1). The two groups had similar ages, numbers of men and women, BMIs, and waist-to-hip ratios. The subjects with the G/G genotype had a higher 2-h blood glucose concentration of borderline significance (*P* = 0.05), which remained after correction for age, sex, and BMI (*P* = 0.03). However, there was no impairment of the insulin secretory response, in keeping with the findings of a similar study conducted in nondiabetic German subjects (5).

We then explored whether the haplotype combination that conferred a threefold risk of diabetes (2) predicted increased glucose levels and specific metabolic traits in our sample of nondiabetic subjects. This haplotype is defined by SNP-43, -19, and -63 (SNP-19: allele 1 = two repeats of a 32-bp sequence, allele 2 = three repeats; and SNP-63: C = allele 1, T = allele 2). SNP-19 had the following allele frequencies: allele 1 = 0.37, allele 2 = 0.63. SNP-63 had the following frequencies: C (allele 1) = 0.88, T (allele 2) = 0.12. Both SNPs were in Hardy-Weinberg equilibrium, and genotype and common haplotype frequencies were comparable between subjects with and without a family history of type 2 diabetes (the frequencies are listed in Tables A1 and A3 in the online appendix at <http://diabetes.diabetesjournals.org>). The frequencies of SNP-43, -19, and -63 genotype combinations and the frequencies of the common haplotypes for the whole sample are shown in Tables A2 and A3, respectively, in the online appendix at <http://diabetes.diabetesjournals.org>. Parental haplotype information was not available for our group of subjects. However, it is now clear that only four common haplotypes exist across different populations, including the U.K. (3), and these are as follows: haplotypes 111, 112, 121, and 221; SNP-43, -19, and -63. We were therefore able to infer that subjects in our study sample homozygous for

SNP-43 and heterozygous at SNP-19 and -63 had to be carriers of the high-risk 112/121 haplotype combination previously identified in the Mexican-American and North European populations (2,3).

We first compared the group with the 112/121 haplotype combination (*n* = 29) with the remainder of the sample (*n* = 256) (Table 2). The two groups had similar ages, numbers of men and women, and waist-to-hip ratios. BMI was higher in the group with the 112/121 haplotype, although the difference did not reach statistical significance (28.0 ± 1.0 vs. 26.1 ± 0.3 kg/m²; *P* = 0.09). The group with the 112/121 haplotype combination had a significantly higher 2-h glucose concentration, with a tendency to higher fasting glucose and 2-h insulin levels. This was associated with a borderline significant decrease (*P* = 0.05) in the early insulin response (EIR) and an increase (*P* = 0.06) in basal insulin resistance (measured with the homeostasis model assessment of insulin resistance [HOMA_{IR}]). We then adjusted the insulin secretory response for the level of insulin resistance akin to the previously described disposition index (6). Comparison between groups showed that the adjusted insulin secretory response was markedly decreased (*P* = 0.005) in the subjects with the 112/121 haplotype combination (Table 2). The differences in fasting (*P* = 0.004) and 2-h (*P* = 0.003) blood glucose levels, EIR (*P* = 0.02), and adjusted insulin secretory response (*P* = 0.002) remained after correction for age, sex, and BMI.

To take into account the potential confounding effects of family relationship in those subjects with a family history of type 2 diabetes, we randomly selected one relative from sibships in which there was more than one nondiabetic relative. In this way, 11 of the 14 relatives with the 112/121 haplotype combination were selected, as well as 68 of the 129 relatives with the other haplotype combinations. Because all of the subjects with no family history of diabetes were unrelated, all of these subjects were retained. We then conducted the same comparisons between the subjects with the 112/121 haplotype combination (*n* = 26) and those with the other haplotype combinations (*n* = 196), as shown in Table 3. As before, those with the 112/121 haplotype combination had higher fasting glucose and 2-h glucose and 2-h insulin levels, with a tendency to higher HOMA_{IR} and lower EIR values. Again,

TABLE 2
Clinical and metabolic characteristics of subjects by CAPN10 haplotype combination (SNP-43, -19, and -63)

	112/121	All others	P
<i>n</i>	29	256	
Age (years)	43 ± 2	40 ± 1	0.29
Sex (M/F)	12/17	114/142	0.9
BMI (kg/m ²)	28.0 ± 1.0	26.1 ± 0.3	0.09
WHR	0.83 ± 0.02	0.83 ± 0.01	0.9
Fasting glucose (mg/dl)	90 ± 3	85 ± 1	0.03
2-h Glucose (mg/dl)	112 ± 7	92 ± 2	0.01
HOMA _{IR}	2.4 (1.9–3.1)	1.9 (1.7–2.1)	0.06
2-h Insulin (μU/ml)	51 (38–68)	37 (34–42)	0.04
EIR (mU/mmol)	18 (14–23)	24 (22–26)	0.05
Adjusted insulin secretory response	7 (5–11)	13 (12–14)	0.005

Data are presented as the means ± SE or geometric mean (95% CI). *P* values are 2-sided. WHR, waist-to-hip ratio.

TABLE 3
Clinical and metabolic characteristics of subjects by *CAPN10*-haplotype combination (SNP-43, -19, and -63) after correction for family relationships

	112/121	All others	<i>P</i>
<i>n</i>	26	196	
Age (years)	43 ± 2	40 ± 1	0.3
Sex (M/F)	12/14	93/103	0.7
BMI (kg/m ²)	28.2 ± 1.0	25.9 ± 0.3	0.08
WHR	0.83 ± 0.02	0.83 ± 0.01	0.8
Fasting glucose (mg/dl)	89 ± 3	83 ± 1	0.04
2-h Glucose (mg/dl)	111 ± 8	91 ± 2	0.02
HOMA _{IR}	2.4 (1.8–3.2)	1.9 (1.6–2.0)	0.05
2-h Insulin (μU/ml)	54 (40–73)	36 (34–42)	0.02
EIR (mU/mmol)	19 (14–25)	23 (22–26)	0.08
Adjusted insulin secretory response	8 (5–11)	13 (12–14)	0.007

Data are presented as the means ± SE or geometric mean (95% CI). *P* values are 2-sided. WHR, waist-to-hip ratio.

the adjusted insulin secretory response remained lower ($P = 0.007$) in the subjects with the 112/121 haplotype combination (Table 3). The differences in fasting ($P = 0.005$) and 2-h ($P = 0.005$) blood glucose levels, EIR ($P = 0.05$), and adjusted insulin secretory response ($P = 0.002$) remained after correction for age, sex, and BMI. Therefore, the metabolic differences between the two haplotype groups were independent of family relationships.

We conclude that variation in the calpain-10 gene is a determinant of glucose levels in nondiabetic British subjects. In agreement with Horikawa et al. (2), the predictive risk appears to extend beyond SNP-43 and includes SNP-19 and SNP-63. Our study also highlights a potential mechanism for the increased blood glucose levels and increased risk of type 2 diabetes. There was a minor impairment of the EIR, combined with a tendency to increased insulin resistance, in those subjects with the high-risk haplotype combination. However, the impairment of the insulin response became more apparent after adjustment for the ambient level of insulin resistance. This suggests that calpain-10 influences pancreatic β -cell function, although the mechanism by which it modulates β -cell function is unknown. Nondiabetic British subjects with the 112/121 haplotype combination also tended to be insulin-resistant, although this was of borderline statistical significance (HOMA_{IR} $P = 0.06$, 2-h insulin $P = 0.04$, both 2-sided), similar to nondiabetic Pima Indians homozygous for the G allele at SNP-43 (4).

In summary, variation in the calpain-10 gene is associated with measures of insulin secretion and insulin action in nondiabetic subjects and may thus affect susceptibility to type 2 diabetes by multiple mechanisms.

RESEARCH DESIGN AND METHODS

Study population. The study sample comprised 285 nondiabetic subjects (aged 20–65 years) of U.K./Irish ancestry. The sample was enriched with subjects at increased risk of developing type 2 diabetes because 145 were nondiabetic first-degree relatives of type 2 diabetic patients. The nondiabetic relatives originated from 58 pedigrees; 38 contributed just one relative, whereas the rest provided more than one. Within the 58 pedigrees, there were 79 separate family sibships. All of the type 2 diabetic probands to the 58 pedigrees were recruited from the Newcastle Diabetes Service. The remaining 140 nondiabetic subjects did not have a family history of diabetes and were all unrelated. These subjects were recruited from a randomly selected subgroup from the same background population as the type 2 diabetic pedigrees.

Informed written consent was obtained from all subjects, and the study approved by the Newcastle and North Tyneside Joint Ethics Committee.

Clinical studies. All subjects underwent a 75-g OGTT and anthropometric assessment. Glucose concentrations were measured by the glucose oxidase method (interassay coefficient of variation [CV] 3.5%), and serum insulin concentrations were measured by specific-enzyme immunoassay (interassay CV 4.8% at 34 pmol/l) (Dako Diagnostics, Ely, U.K.). HOMA_{IR} was determined from fasting plasma glucose and insulin levels as an index of basal insulin resistance (7). The EIR was determined as $\Delta 30-0$ min insulin/ $\Delta 30-0$ min glucose and has been shown to correlate closely with insulin secretion determined by intravenous glucose tolerance test (8). The adjusted insulin secretory response was calculated as the product of the EIR and 1/HOMA_{IR}. Insulin levels, HOMA_{IR}, EIR, and adjusted insulin secretory response data were log₁₀ transformed to normalize distributions, and comparisons between groups were made using *t* test and analysis of covariance. In the light of the multiple comparisons, the threshold for clear statistical significance was taken as $P = 0.01$.

Genotyping. Genomic DNA isolated from peripheral blood lymphocytes was genotyped for the following *CAPN10* polymorphisms: SNP-43, *CAPN10*-g.4852G/A; SNP-19, *CAPN10*-g.7920indel32bp; and SNP-63, *CAPN10*-g.16378C/T. SNP-43 genotyping was carried out using an amplification refractory mutation system–polymerase chain reaction (PCR), which involved two separate PCRs (for both the G and A alleles) in parallel and generated a 230-bp PCR product. Primer sequences were as follows: the common primer 5'-GGCTGGCTGGTGACATCAGTG-3' and the G and A allele primers 5'-GCTTAGCCTCACCTTCAATC-3' and 5'-GCTTAGCCTCACCTTCAATT-3', respectively. PCR conditions were: denaturation at 94°C for 5 min, 30 cycles at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 10 min. As an internal control for efficient PCR amplification, we included primers designed to amplify exon 16 of the hexokinase II gene, which produces a 351-bp product. The PCR products were separated on a 2% NuSieve agarose gel and visualized by ethidium bromide staining. The G and A alleles of SNP-43 were designated as alleles 1 and 2 in haplotypes including this DNA polymorphism. SNP-19 is a two-allele insertion/deletion (indel) polymorphism consisting of two or three copies of a 32-bp repeat sequence. This polymorphism was typed using the forward and reverse primers 5'-GTTTGGTTCTCTTCAGCGTGGAG-3' and 5'-CATGAACCTG GCAGGGTCTAAG-3', respectively. The PCR products were separated on a 3% NuSieve agarose gel. Allele 1 (two repeats) is 155 bp, and allele 2 (three repeats) is 187 bp. SNP-63 genotyping was carried out using a mismatch PCR method (provided by Dr. Marju Orho-Melander, Malmo University Hospital, Lund, Sweden), which creates a *HhaI* site with the common C allele. The forward and reverse primers were 5'-AAGGGGGGCCAGGGCCTGACGGGG GTGGCG-3' and 5'-AGCACTCCAGCTCTGATC-3', respectively. The 192-bp PCR product was digested at 37°C for 2 h to produce a 162-bp and 30-bp product in the presence of the C allele (allele 1). The T allele (allele 2) is not cleaved by *HhaI* and gives a product of 192 bp.

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