The Exon 1 Cys7Gly Polymorphism Within the Betacellulin Gene Is Associated With Type 2 Diabetes in African Americans

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In vitro and in vivo studies suggest a role for betacellulin in islet neogenesis and regeneration. Since abnormalities in β-cell function play a role in the development of type 2 diabetes, a mutation in the betacellulin gene could potentially contribute to the development of type 2 diabetes. Using RT-PCR, we initially determined that betacellulin was expressed in 9- to 24-week-old human fetal pancreas. We then screened the betacellulin gene for mutations in subjects with type 2 diabetes and identified seven polymorphisms in segments encompassing the 5′ untranslated region (G-233C, A-226G), exon 1 (TGC19GGC, Cys7Gly), exon 2 (CTC130TTC, Leu44Phe), exon 4 (TTG370ATG, Leu124Met), intron 2 (T-31C), and intron 4 (C-4T). These polymorphisms were genotyped in an expanded set of diabetic case and control subjects. Among African Americans ($n = 334$), the frequency of the Gly7 allele in exon 1 was 31.9% in diabetic case subjects compared with 45.1% in nondiabetic control subjects ($P = 0.0004$). Allele frequencies for the other polymorphisms did not differ significantly between African-American case and control subjects. Additionally, there were no significant differences in allele frequencies between case and control subjects among the Caucasian sample ($n = 426$) for any of the seven polymorphisms, including the Gly7 variant. Further studies will be needed to understand the different roles that betacellulin polymorphisms play in susceptibility to type 2 diabetes in Caucasians and African Americans. *Diabetes* **54:1179–1184, 2005**

Betacellulin is a member of the epidermal growth factor (EGF) family and interacts with the erb-B family of tyrosine kinase receptors (1–3). It was initially identified in conditioned media of mouse pancreatic β-cell tumors and found to be a potent mitogen for retinal pigmented epithelial and vascular smooth muscle cells (4). Human betacellulin localizes to chromosome 4q13-q21. Betacellulin cDNA is 1.3 kb and translates into a 178–amino acid polypeptide, with the mature polypeptide being 80 amino acids (5). In humans, betacellulin mRNA is found at highest levels in pancreas and small intestine (6).

In vitro and in vivo studies suggest a role for betacellulin in islet neogenesis and regeneration (7–14). In INS-1 insulinoma cells and islet cell clusters from human fetal pancreas, betacellulin stimulates an increase in DNA content and cell number (7). In AR42J cells, the addition of betacellulin results in upregulation of insulin, pancreatic polypeptide, glucokinase, and GLUT2 (8). However, only 3% of cells stain for insulin. When both activin A and betacellulin are added to the AR42J cells, 10% of cells stain for insulin (8). The activin A/betacellulin-treated cells also increase insulin secretion when stimulated with tolbutamide, potassium chloride, and glucagon-like peptide 1. Similarly, pancreatic duodenal homeobox factor-1–transfected IEC-6 cells (rat intestinal crypt-like cells) develop the capacity to secrete insulin after treatment with betacellulin (9). In human undifferentiated fetal pancreatic cells, betacellulin has a mitogenic effect (10). The effects of betacellulin have also been tested in alloxan- (11) and streptozotocin-treated (14) mice and in streptozotocin-treated (13) and 90% pancreatectomized rats (12). In these studies, animals receiving recombinant human betacellulin had greater improvement in glucose tolerance, most likely through an increase in β-cell volume, compared with those that did not receive betacellulin (11–14). As such, betacellulin appears to play a role in increasing the number of β-cells and differentiation of cells toward a β-cell phenotype.

Because abnormalities in β-cell function play a role in the development of type 2 diabetes, a mutation in the betacellulin gene could potentially result in a decreased number of or abnormally functioning β-cells and thereby contribute to the development of type 2 diabetes. To determine if mutations in the betacellulin gene play a role in the development of type 2 diabetes, we screened subjects with type 2 diabetes for the presence of mutations.

RESEARCH DESIGN AND METHODS

Betacellulin in fetal pancreas. Human pancreatic tissue from 11 fetuses age 9–24 weeks (weeks 9, 10, 12, 14, 15, 16, 17, 18, 21, 23, and 24) was obtained through Advanced Bioscience Resources (Alameda, CA). Informed consent for the tissue donation was obtained by the procurement center. Gestational age was determined by crown rump length or fetal foot length. Tissue was sent overnight on ice in RPMI media. On arrival, the specimen was dissected free of nonpancreatic tissue, snap frozen in liquid nitrogen, and stored at −70°C. Total RNA was prepared using RNAqueous (Ambion, Austin, TX) according to
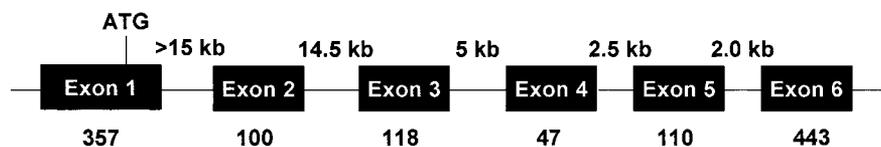
FIG. 1. Genomic structure of betacellulin gene. The number of nucleotides in each exon is shown below each exon. The estimated size of each intron in kilobases is shown above each intron.

the manufacturer's directions. Reverse transcription was performed using AMV reverse transcriptase (Promega), 10 IU/μl, with random primers. Before reverse transcription, contaminating DNA was digested with DNase (Boehringer Mannheim) at 37°C for 30 min. PCR was performed using 5′-TGGCAG ATGGGAATTCCACC-3′ as the upstream primer and 5′-GCATCTCCCTTTGA TGCAGTA-3′ as the downstream primer. The primers were designed so that the PCR product spanned the second intron. Therefore, a product from cDNA (158 bp) could be distinguished from a product from gDNA (~14 kb). RT-PCR products were run on a 3% agarose gel and visualized with ethidium bromide.

Determination of genomic structure and estimation of intron sizes. A cDNA betacellulin probe was generated and used to screen a human bacterial artificial chromosome (BAC) genomic library (Genome Systems, St. Louis, MO). Two BACs were identified (clones 20063 and 20064) that had homology to the betacellulin probe. Using primers derived from the betacellulin cDNA sequence, the clones were sequenced using dideoxy sequence analysis and an ABI 377 sequencer. By comparing the sequence data with those of the published betacellulin cDNA sequence, the intron-exon junctions were determined for exons 2–6. Neither BAC contained the 5′ untranslated region (UT), first exon, or complete first intron. Therefore, the structure of this portion of the gene was determined by screening the human genome database (www.ncbi.nlm. nih.gov).

Once the genomic structure was established, the size of the introns was determined using gDNA and long PCR (LA-PCR) (TaKaRa Biomedicals, Otsu, Japan) with primers designed to produce PCR products that spanned each intron. Products were subjected to agarose gel electrophoresis and stained with ethidium bromide.

Single-strand conformational polymorphism analysis of the betacellulin gene. All protocols were approved by the University of Maryland and the Johns Hopkins University Bayview Campus Institutional Review Boards and were performed after written informed consent had been obtained from subjects. For the single-strand conformational polymorphism (SSCP) analysis and sequencing studies, type 2 diabetic subjects ($n = 91$) were recruited from a Baltimore retirement center, the University of Maryland Joslin Diabetes Center, and the Johns Hopkins Weight Management Center (63 ± 14 years old, age of diabetes onset 53 ± 16 years, BMI 32.3 ± 8.1 kg/m^2, 43% female). Subjects were defined as having diabetes if they were currently using medication for diabetes or had an elevated fasting (≥7mmol/l) or 2-h glucose level (>11 mmol/l) on a 75-g oral glucose tolerance test.

Genomic DNA was isolated from whole blood using the QIAamp DNA Blood Minikit (Qiagen, Valencia, CA) according to the manufacturer's directions. For SSCP analysis, DNA was amplified generating PCR products (Table 1 in the online appendix [available at http://diabetes.diabetesjournals.org]) that encompassed the coding region for exons 1–5, intron-exon splice junctions, and 294 bp of 5′UT. The PCR products were radiolabeled by the addition of [α-^{32}P]deoxycytidine triphosphate to the PCR mixture. Denatured PCR products were loaded onto a polyacrylamide gel (MDE [mutational detection enhancement]; AT Biochemicals, Malvern, PA) and subjected to electrophoresis at 4–6 watts for 18–20 h under four gel conditions: with and without 10% glycerol at 4°C and 25°C. SSCP variants were subjected to dideoxy sequence analysis with an ABI 377 automated sequencer (Foster City, CA). Exon 6 contains 3′UT and was screened for polymorphisms directly by dideoxy sequence analysis.

Genotyping for the betacellulin polymorphisms. Subjects with and without type 2 diabetes were genotyped for each of the seven polymorphisms identified in the betacellulin gene. A total of 144 Caucasian and 185 African-American subjects with type 2 diabetes were recruited from the University of Maryland Joslin Diabetes Center. A total of 282 Caucasian and 149 African-American nondiabetic control subjects were recruited from the University of Maryland Baltimore campus (primarily health care workers, research personnel, and students) and local workplace sites via flyers and health fairs.

Inclusion in the control group was based solely on race and subjects self-reporting of the absence of a history of diabetes.

The Leu44Phe and intron 2 T-31C polymorphisms were genotyped by PCR-fluorescent primer single nucleotide polymorphism (SNP) detection (AcycloPrimer-FP SNP Detection Kit; PerkinElmer, Boston, MA) according to the manufacturer's directions. The 5′UT G-233C, 5′UT A-226G, Leu124Met, and intron 4 C-4T polymorphisms were genotyped by pyrosequencing (PSQ 96MA System; Pyrosequencing, Uppsala, Sweden) according to the manufacturer's directions. The Cys7Gly polymorphism was detected using PCR restriction fragment–length polymorphism analysis using the restriction enzyme SmaI. (PCR primers used for each analysis can be found in Table 2 in the online appendix). The overall replication rate based on repeat genotyping of at least 10% of samples was 97.2%.

Statistical analysis. We initially compared allele frequencies between case and control subjects and then used logistic regression to determine the effect of genotype on type 2 diabetes, controlling for age and sex. For our primary analysis, we tested the association between diabetes status and genotype assuming an additive genetic model. We also tested, as secondary analyses, the presence of an association under dominant and recessive genetic models. All analyses were stratified by race and carried out using the SAS statistical package (SAS User's Guide, Version 8; SAS Institute, Cary NC). Measures of linkage disequilibrium (r^2 and $D′$) between SNPs were computed using ZAPLO (15). For each pair of SNPs for which there was substantial evidence for linkage disequilibrium ($r^2 > 0.5$ and/or $D′ > 0.8$), we used HAPLO-SCORE (16) to estimate and compare haplotype frequencies between case and control subjects. Significance levels for the global test of whether haplotype distributions differed between case and control subjects were computed by permutation. When significant, frequencies of specific haplotypes were compared between case and control subjects, also by permutation.

RESULTS

Betacellulin in fetal pancreas. Studies suggest that betacellulin may play a role in islet neogenesis (7–14). Betacellulin has been identified previously in 16-week-old human fetal pancreas (17). However, the time at which betacellulin first appears in human fetal pancreas has not been established. Therefore, RT-PCR was performed on total RNA from human fetal pancreas ages 9–24 weeks. A clear signal for betacellulin was present in each specimen. The presence of betacellulin throughout early pancreatic development suggests that betacellulin may play an integral role in pancreatic ontogeny. Based on the presence of betacellulin early in pancreatic development (17) and studies suggesting a role in pancreatic regeneration (8,9,11–14), betacellulin is a candidate gene for diabetes.

Genomic structure of betacellulin. To screen the betacellulin gene for mutations, we first determined its genomic structure. Using a cDNA betacellulin probe, two BACs were identified as having betacellulin sequence. These BACs were sequenced using primers based on the cDNA sequence, and we were able to establish the presence of five exons. However, neither BAC contained the published 5′UT or remaining 5′ coding sequence. Therefore, we searched the human genome database (www.ncbi.nlm.nih. gov) and were able to identify a clone (gb/ac018935) that
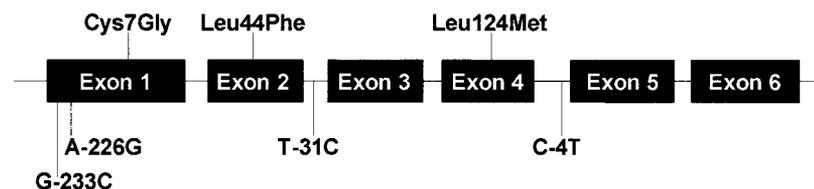

FIG. 2. Polymorphisms in the betacellulin gene.

TABLE 1
Characteristics of type 2 diabetic subjects versus control subjects

	Caucasians		African Americans	
	Type 2 diabetes	Control	Type 2 diabetes	Control
n	144	282	185	149
Sex (% female)	47.9	66.2	63.2	75.0
Age (years)	56.9 ± 10.0	40.7 ± 13.4	54.4 ± 12.4	43.1 ± 12.3
BMI (kg/m^2)	34.4 ± 7.9	26.9 ± 5.8	35.1 ± 9.5	30.6 ± 7.8
Age at diabetes onset (years)	48.1 ± 11.3	—	45.0 ± 13.3	—
Treatment (%)				
Diet	5.8	—	8.0	—
Oral hypoglycemic agents	64.4	—	52.2	—
Insulin	12.3	—	19.3	—
Insulin + oral hypoglycemic agents	17.4	—	20.5	—

Data are means ± SD unless otherwise indicated.

contained this region of betacellulin sequence. Using this clone, we were able to confirm that no additional introns were present in this region. Shown in Fig. 1 is the betacellulin intron-exon genomic structure based on the human genome database and the BAC studies. The betacellulin gene is composed of six exons. The first five exons contain coding sequence and the last exon is made up of the 3′UT. Using human gDNA, long PCR was performed across introns, revealing intron sizes ranging from 2.0 to 14.5 kb for introns 2–5. Using long PCR, we were unable to establish the size of the first intron. The clone identified through the human genome database contained part, but not all, of the first intron and established that the intron was >15 kb. The size of the introns is consistent with later versions of the human genome database. As expected, the genomic structure of betacellulin is similar to the structure of EGF and other members of the EGF family (18).

Polymorphism detection. Either SSCP or direct sequence analysis was used to scan exons 1–6, splice junctions, and 294 bases of the 5′UT for polymorphisms from at least 50 subjects with type 2 diabetes. Seven variant patterns were identified. Sequence analysis revealed polymorphisms in segments encompassing the 5′UT (G-233C, A-226G), exon 1 (TGC19GGC, Cys7Gly), exon 2 (CTC130TTC, Leu44Phe), exon 4 (TTG370ATG, Leu124Met), intron 2 (T-31C), and intron 4 (C-4T) (Fig. 2). Cys7Gly in exon 1 is found in the signal peptide. Although it involves a cysteine, it is not one of the critical six cysteines that characterize members of the EGF family of proteins. In mouse and bovine, this amino acid is also a glycine. The Leu44Phe polymorphism is located in the

mature betacellulin peptide. Because the phenylalanine is a relatively large amino acid compared with leucine, this substitution could potentially affect secondary structure of the molecule. Leu124Met is found in the transmembrane portion of the protein, and the leucine is conserved among other species (18).

Genetic association studies. We genotyped African-American and Caucasian control and type 2 diabetic subjects for the seven variants in betacellulin. Characteristics of the case and control subjects are shown in Table 1. Over 94% of the Caucasian and 92% of the African-American type 2 diabetic subjects were treated with oral hypoglycemic agents and/or insulin therapy. Control subjects were on average substantially leaner and 10–15 years younger than diabetic case subjects.

The observed genotype frequencies for the seven polymorphisms did not differ significantly from those predicted under Hardy-Weinberg equilibrium. Because of differences in allele frequencies between the African-American and Caucasian subjects, the two groups were analyzed separately (Tables 2 and 3). In African Americans, the frequency of the Gly7 allele in exon 1 was significantly higher among nondiabetic control subjects than among diabetic case subjects (45.1 vs. 31.9%, $P = 0.0004$ [allelic test], $P = 0.001$ [genotype test], adjusted for age and sex; Table 2). This association was virtually unchanged when the control group was restricted to those over the age of 40 years ($n = 90$) (51.1 vs. 31.9%, $P = 0.0002$). There was no association of the Cys7Gly variant in exon 1 with BMI in the African-American control group (data not shown). None of the other six polymorphisms were associated with diabetes in

TABLE 2
Allele frequency for African-American type 2 diabetic subjects

		Minor allele frequency		
Polymorphism	Major/minor allele	Type 2 diabetes ($n = 185$)	Control ($n = 149$)	Age- and sex-adjusted P value*
Cys7Gly (exon 1)	T/G	0.32 (0.27–0.37)	0.45 (0.39–0.51)	0.001
Leu44Phe (exon 2)	C/T	0.09 (0.06–0.12)	0.10 (0.07–0.14)	0.49
Leu124Met (exon 4)	T/A	0.22 (0.18–0.26)	0.24 (0.19–0.29)	0.68
5′UT G-233C	G/C	0.42 (0.36–0.47)	0.46 (0.40–0.52)	0.08
5′UT A-226G	A/G	0.25 (0.20–0.30)	0.20 (0.15- 0.25)	0.14
Intron 2 T-31C	C/T	0.32 (0.27–0.37)	0.29 (0.24–0.35)	0.55
Intron 4 C-4T	C/T	0.23 (0.19–0.28)	0.23 (0.18- 0.28)	0.71

Data are means (95% CI). *P value for genotype effect obtained from logistic regression assuming additive genetic model.

TABLE 3
Allele frequency for Caucasian type 2 diabetic subjects

Polymorphism	Major/minor allele	Minor allele frequency		Age- and sex-adjusted P value*
		Type 2 diabetes ($n = 144$)	Control ($n = 282$)	
Cys7Gly (exon 1)	T/G	0.13 (0.09–0.18)	0.15 (0.12–0.18)	0.74
Leu44Phe (exon 2)	C/T	0.0 (0.0–0.02)	0.002 (0.0–0.01)	—
Leu124Met (exon 4)	T/A	0.29 (0.24–0.34)	0.31 (0.27–0.35)	0.58
5′UT G-233C	G/C	0.17 (0.12–0.23)	0.15 (0.12–0.18)	0.09
5′UT A-226G	A/G	0.37 (0.31–0.43)	0.34 (0.30–0.38)	0.35
Intron 2 T-31C	C/T	0.42 (0.36–0.48)	0.41 (0.38–0.45)	0.74
Intron 4 C-4T	C/T	0.28 (0.23–0.34)	0.29 (0.25–0.33)	0.71

Data are means (95% CI). *P value for genotype effect obtained from logistic regression assuming additive genetic model.

African Americans, even when considering dominant and recessive genetic models. Additionally, none of the polymorphisms, including the Gly7 variant, were associated with diabetes in Caucasians (Table 3).

We hypothesized that in subjects susceptible to the development of type 2 diabetes, polymorphisms in betacellulin might manifest as an earlier onset of diabetes or as subjects requiring insulin therapy instead of diet or oral hypoglycemic medication to control blood glucose. Therefore, we assessed whether there were differences in the age of onset of diabetes or the type of diabetes treatment (diet, oral hypoglycemic, insulin, or oral hypoglycemic + insulin) in type 2 diabetic subjects with and without the seven variants. No significant differences by genotype were found for these characteristics for the seven

variants in either African Americans or Caucasians (data not shown).

There was substantial linkage disequilibrium among the two SNPs in the 5′UT, Cys7Gly SNP in exon 1, and Leu44Phe SNP in exon 2, with pairwise D' values ranging from 0.43 to 0.89 in both the African-American and Caucasian subgroups (Fig. 3). Those SNPs that were in strong linkage disequilibrium ($r^2 > 0.5$ and/or $D' > 0.8$) were tested for pairwise haplotype associations. In African Americans, the global test for differences in haplotype frequencies was significant for all tested haplotypes containing the Cys7/Gly7 allele in exon 1. In each case, haplotypes containing the Cys allele at this locus were observed significantly more frequently among case subjects than among control subjects (Table 4). In Cauca-
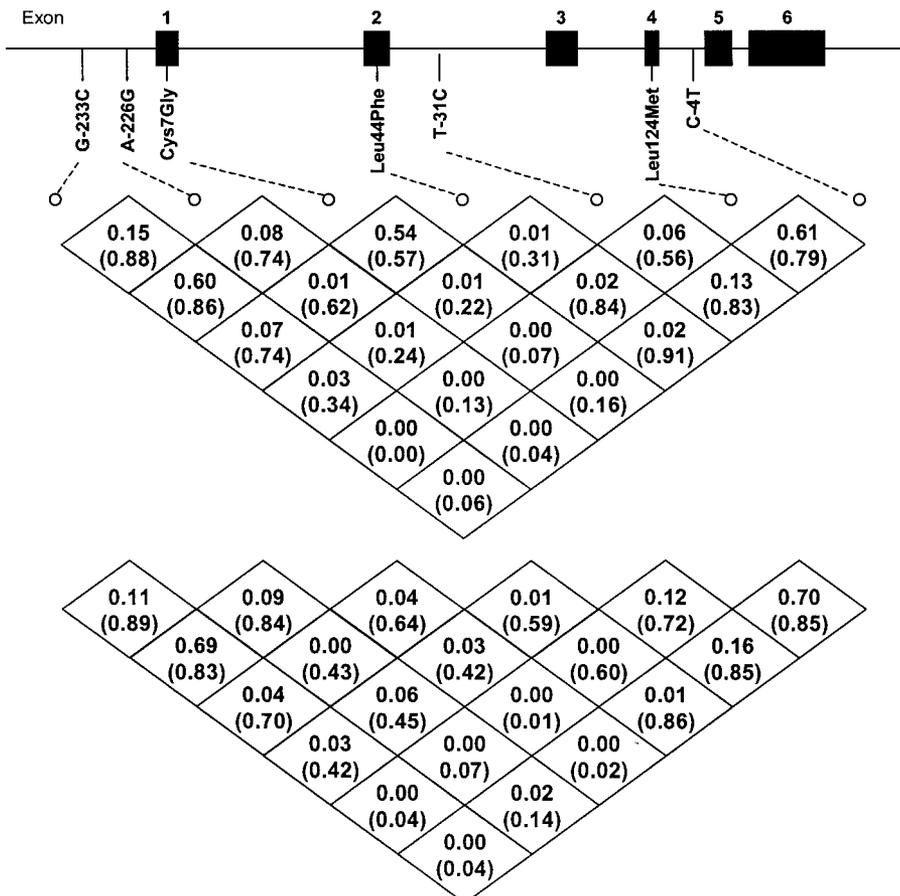

FIG. 3. Linkage disequilibrium in African Americans (upper) and Caucasians (lower). Linkage disequilibrium measures between SNPs are provided (r^2 values on top and D' in parentheses).

TABLE 4
Haplotype analysis

	Case subjects (%)	Control subjects (%)	P
African Americans			
Cys7Gly/5′UT G-233C			
(global $P = 0.0005$)			
Cys7/5′UT G-233	0.54	0.51	0.3
Cys7/5′UT C-233	0.13	0.04	<0.0001
Gly7/5′UT G-233	0.04	0.03	0.7
Gly7/5′UT C-233	0.29	0.42	0.0009
Cys7Gly/5′UT A-226G			
(global $P = 0.01$)			
Cys7/5′UT A-226	0.47	0.36	0.02
Cys7/5′UT G-226	0.21	0.20	0.3
Gly7/5′UT A-226	0.28	0.44	0.0003
Gly7/5′UT G-226	0.30	0.006	0.2
Cys7Gly/Leu44Phe			
(global $P = 0.002$)			
Cys7/ Leu44	0.63	0.52	0.004
Cys7/ Phe44	0.05	0.02	0.3
Gly7/ Leu44	0.28	0.37	0.009
Gly7/ Phe44	0.04	0.08	0.1
Caucasians			
Cys7Gly/5′UT G-233C			
(global $P = 0.00001$)			
Cys7/5′UT G-233	0.80	0.85	0.14
Cys7/5′UT C-233	0.07	0.01	0.00009
Gly7/5′UT G-233	0.03	0.004	0.004
Gly7/5′UT C-233	0.10	0.14	0.14

sians, a significant difference in haplotype frequencies was observed for the Cys7Gly/5′UT G-233C haplotype (global *P* value = 0.00001), although no single allele was consistently associated with type 2 diabetes. For the other variants in linkage disequilibrium, haplotype analyses did not demonstrate significant associations of haplotype with diabetes status.

DISCUSSION

As we better understand the pathophysiology of type 2 diabetes, there is a growing appreciation of the role of the pancreas in its development. In animal models, mutations in genes such as *IPF-1* (19), *IRS-2* (20), and *NeuroD1* (21) lead to developmental abnormalities in β-cells, which in turn lead to the development of diabetes. In humans, heterozygosity for a mutation in the transcription factor IPF-1 (Pro63fsdelC) leads to the development of early-onset type 2 diabetes (maturity-onset diabetes of the young [MODY]-4) (22). Similarly, mutations in other MODY genes (glucokinase, *HNF1α*, *HNF1β*, and *HNF4α*) lead to abnormal insulin secretion and diabetes (23,24).

In vitro and in vivo studies suggest that betacellulin fits into this category of β-cell development/function genes in which mutations lead to abnormal β-cell number or function, decreased insulin secretion, and, thereby, type 2 diabetes. A role for betacellulin in pancreatic ontogeny is supported by our studies in human fetal pancreas. Previously, Miyagawa et al. (17) reported that betacellulin was present in 16-week-old human fetal pancreas; however, it was not known when expression began. Our studies demonstrate that betacellulin is present in human fetal pancreas as early as 9 weeks and is present through at least 24

weeks of development. These studies imply that betacellulin plays a role in pancreatic development. Additional studies will be necessary to better localize betacellulin in the fetal pancreas and to determine its specific role during development.

Here, we describe seven polymorphisms identified in the betacellulin gene including three coding mutations, two intron mutations, and two 5′UT mutations. The Cys7 allele in exon 1 is significantly associated with type 2 diabetes in African Americans. We did not explicitly adjust for multiple comparisons in our study, although the allele frequency difference between case and control subjects was large (31.9 vs. 45.1%) and the nominal level of significance very high. The Cys7Gly polymorphism in exon 1 is found in the signal peptide and potentially could affect the ability of betacellulin to insert properly into the cell membrane. Further in vitro studies will be necessary to determine differences in the function of the betacellulin Cys7 allele compared with the Gly7 allele.

It is noteworthy that we detected the association of the Gly7 variant in the African-American but not Caucasian samples. This difference could indicate that the association is either a false-positive or that the polymorphism is not functional but is merely marking (in the African-American sample only) a nearby functional variant. On the other hand, African Americans tend to be more insulin resistant than Caucasian populations (25). As a result, they need higher levels of insulin to compensate for the insulin resistance to maintain euglycemia. If, by way of decreased β-cell number or function, having the Cys7 allele decreases insulin production or secretion, then the effects of this polymorphism on the development of type 2 diabetes would be greatest in those with the highest levels of insulin resistance. Thus, the effects of a betacellulin mutation may only manifest when the system is stressed (e.g., obesity and insulin resistance) and insulin requirements rise. As such, the manifestations of this polymorphism on the development of type 2 diabetes would be more evident in African Americans than Caucasians. Clinical studies measuring insulin secretion and insulin resistance in African Americans and Caucasians with and without the Cys7 allele will be necessary to prove this hypothesis.

There are several limitations of our study. First, the mean age of our control group is substantially younger than that of the case group. Thus, some of the members of the control group may be misclassified as they may develop type 2 diabetes as they get older. However, we believe that this age difference does not alter our results, since the difference in allele frequencies between case and control subjects is still maintained even in the subset of control subjects ≥40 years or age, and statistical significance of the association remains high. Second, we did not perform fasting or oral glucose tolerance tests in the control group to confirm that they were euglycemic. Here again, there are issues related to misclassification of subjects. However, if we included subjects in the control group that should be in the diabetes group, we would expect that any differences found between the two groups would be diluted and therefore statistical significance would be diminished. Finally, the allele frequency of the Cys7Gly variant in exon 1 is much lower in diabetic and control Caucasians than in African Americans. Because

we have not documented the number of grandparents who are African American, it is possible that the lower prevalence of the Gly7 variant in the African-American diabetic subjects is due to a greater admixture of Caucasian genes than in the control population. We do not believe that this scenario is likely as we would expect to see that all of the variants had this trend toward Caucasian allele frequencies and would also expect to see other variants with significant differences between African-American case and control subjects. Because we did not find this to be the case, we believe that the difference in allele frequency for the Cys7Gly variant between African-American case and control subjects is real and not due to sampling error.

In conclusion, we have identified seven polymorphisms in the human betacellulin gene. The Cys7 allele is associated with type 2 diabetes in African Americans. Further studies will be needed to assess the effects of the polymorphism on insulin secretion and the basis for the racial differences on its effect on susceptibility to type 2 diabetes.

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