Original Article

Susceptibility to Type 1 Diabetes Is Associated With ApoCIII Gene Haplotypes

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Type 1 diabetes is a disease of β-cell destruction leading to insulin deficiency. Genes for type 1 diabetes have been identified; however, much of the genetic risk remains unexplained. Genetic variation within the apolipoprotein CIII (apoCIII) gene alters apoCIII levels, which are increased in type 1 diabetes and induce β-cell apoptosis. We therefore hypothesize haplotypes within the apoCIII gene are associated with type 1 diabetes. DNA from 584 type 1 diabetic patients and 591 control subjects were genotyped for six single nucleotide polymorphisms (SNPs) in the apoCIII gene (C-641A, C-482T, T-455C, C1100T, C3175G, and T3206G). Two alleles of a haplotype block (promoter SNPs + C3175G) were associated with type 1 diabetes. The A-T-C-C allele frequency was higher in type 1 diabetes (0.19 vs. 0.16, $P = 0.05$), and the C-C-T-C allele was reduced in type 1 diabetes (0.60 vs. 0.65, $P = 0.04$). The odds ratio (OR) for A-T-C-C allele increased with 0, 1, and 2 copies (OR of 1.00, 1.24, and 1.60, respectively; $P = 0.05$) and decreased for the C-C-T-C allele (1.00, 0.97, and 0.73, respectively; $P = 0.03$). This haplotype block contains an insulin response element. Screening for this haplotype may identify at-risk individuals, and this pathway may offer a target for prevention of type 1 diabetes. *Diabetes* 55: 834–838, 2006

Type 1 diabetes is an autoimmune disease characterized by β-cell destruction leading to insulin dependency. Genetic susceptibility plays a major role in the etiology of type 1 diabetes. Specific genetic markers in the HLA region lead to increased susceptibility to type 1 diabetes (1). Other genes outside the HLA region have been associated with type 1 diabetes and autoimmunity (2–4). A substantial portion of the genetic risk of type 1 diabetes remains unexplained.

Apolipoprotein CIII (apoCIII) is a glycoprotein that is primarily associated with triglyceride-rich lipoproteins (5). ApoCIII protein levels are increased in young children with type 1 diabetes (6). A recent study has demonstrated that serum from type 1 diabetic patients leads to calcium-dependent β-cell apoptosis that was inhibited by antibodies to apoCIII (7). In vitro apoCIII-mediated β-cell apoptosis was also shown to be dose dependent.

The apoCIII gene is part of the apo AI/CIII/AIV/AV gene cluster on chromosome 11q23-q24 (8). Common genetic variants in the apoCIII gene have been shown to have biological effects (rev. in 9). Of particular interest are two common promoter variants (C-482T and T-455C) within a negative insulin response element (10). These substitutions have been shown to blunt the inhibition of expression of the apoCIII gene by insulin (11). Common polymorphisms within the apoCIII gene are in linkage disequilibrium with a promoter haplotype block and a coding region haplotype block (12). The current report addresses a specific a priori hypothesis that haplotypes in the apoCIII gene are associated with an increased susceptibility to type 1 diabetes.

RESEARCH DESIGN AND METHODS

Patients with type 1 diabetes were recruited from the Barbara Davis Center for Childhood Diabetes and from local diabetes clinical practices (13). Control subjects who had never been diagnosed with diabetes were enrolled from among the spouses and friends of the participants with diabetes. All participants were 19–56 years of age at the time of enrollment and had no history of coronary heart disease. Participants with diabetes were diagnosed before the age of 30, or had positive antibodies or a clinical course consistent with type 1 diabetes, and were on insulin therapy within 1 year of diagnosis. The current study was limited to non-Hispanic Caucasian participants who provided DNA for genetic analysis ($n = 584$ with type 1 diabetes and $n = 591$ control subjects) (Table 1). Study participants were members of the Coronary Calcium in Type 1 Diabetes (CACTI) Study (12). All study participants provided informed consent, and the study protocol was approved by the institutional review board.

Genetic analysis. Genomic DNA was extracted from leukocytes after erythrocyte lysis by salt deproteinization. ApoCIII single nucleotide polymorphisms (SNPs) were determined within a multilocus assay by amplification with biotinylated primers and hybridization to immobilized sequence-specific oligonucleotides, as previously described by Cheng et al. (12). Oligonucleotide specificity was confirmed using genomic targets of known genotypes by restriction fragment–length polymorphism analysis or by sequencing. Six SNPs were genotyped within the apoCIII gene: three promoter SNPs (C-641A, C-482T, and T-455C) and three coding region SNPs (C1100T, C3175G, and T3206G). Six SNPs were genotyped within the apoCIII gene, three promoter SNPs (C-641A, C-482T, and T-455C) and three coding regions SNPs (C1100T, C3175G, and T3206G) chosen for putative functional effects and to include promoter variants in the negative insulin response element (C-482T and T-455C). The negative insulin response element is a promoter sequence that downregulates gene expression with insulin (11,14).

Statistical analysis. Allele frequencies were determined by counting, and Hardy-Weinberg equilibrium was tested using χ^2 analysis. Linkage disequilibrium between markers was assessed by D′ (14). Haplotypes were estimated by the expectation-maximization algorithm (15). Haplotype blocks were constructed based on D′ in control subjects, with a D′ of ≥0.80 for pairs of markers representing a haplotype block. D′ values for the SNP pairs were similar in both control subjects and patients with type 1 diabetes. Initial tests

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apo, apolipoprotein; SNP, single nucleotide polymorphism.

TABLE 1
Characteristics of the study population

	Type 1 diabetic subjects	Control subjects	*P*
Age (years)	36.9	40.0	<0.001
M/F (%)	44.8/55.2	52.9/47.1	<0.01
Age at diagnosis (years)	13.2	—	—
Duration of diabetes (years)	23.6	—	—

of genotype and haplotype associations with case-control status used maximum likelihood estimations. The magnitude of significant associations was determined by odds ratios (OR) calculated from contingency tables. The influence of the number of copies of an allele (0, 1, or 2) on the presence of type 1 diabetes was assessed by trend tests. For each allele showing statistically significant trends, a cumulative OR and 95% CIs were calculated. Cumulative ORs represent the change in risk associated with the incremental increase in the number of alleles. The specific a priori hypothesis that haplotypes in the apoCIII gene are associated with type 1 diabetes was tested at an α-level of 0.05 (two tailed).

RESULTS

All genotype distributions for SNPs in the apoCIII gene were consistent with Hardy-Weinberg equilibrium. Allele frequencies in the control subjects were similar to what has been reported in other Caucasian populations (Table 2) (12,16). Allele frequencies for all three promoter SNPs were significantly greater in subjects with type 1 diabetes. There was no significant difference in allele frequencies between case and control subjects in the coding region SNPs in the apoCIII gene (Table 2).

Based on D′, two overlapping haplotype blocks were defined (Fig. 1). One haplotype block consists of the 5′ promoter SNPs and C3175G, whereas the second haplotype block consists of all three coding region SNPs. In this population, C3175G is in strong linkage disequilibrium with all SNPs and is therefore represented in both haplotype blocks.

Haplotype analyses indicate that within haplotype block 1, the A-T-C-C allele is significantly more common in subjects with type 1 diabetes compared with control subjects (haplotype allele frequency of 0.19 vs. 0.16 in case and control subjects, respectively; $P = 0.05$) (Table 3). In contrast, the most common allele of this haplotype (C-C-T-C) is protective for type 1 diabetes (0.60 vs. 0.65, respectively; $P = 0.004$). Alleles of the 3′ haplotype block showed no association with diabetes status (Table 4).

TABLE 2
Allele frequencies for genetic markers in the apoCIII gene in type 1 diabetic and control subjects

ApoCIII SNPs	Type 1 diabetic subjects	Control subjects	*P*
C-641A	0.39 ± 0.01	0.35 ± 0.01	0.04
C-482T	0.28 ± 0.01	0.24 ± 0.01	0.05
T-455C	0.39 ± 0.01	0.35 ± 0.01	0.05
C1100T	0.26 ± 0.01	0.25 ± 0.01	NS
C3175G	0.09 ± 0.01	0.09 ± 0.01	NS
T3206G	0.35 ± 0.01	0.35 ± 0.01	NS

Data are allele frequencies ± SE.

To determine the magnitude and nature of the association for the two alleles of haplotype block 1 with type 1 diabetes, individuals were assigned genotypes based on a probability of ≥0.99 of having a specific haplotype allele. In both patients with type 1 diabetes and control subjects, 98% of individuals were able to be assigned genotypes (571 of 584 for type 1 diabetes, and 578 of 591 for control subjects).

For the A-T-C-C allele, the OR significantly increased as the number of alleles increased (OR 1.00, 1.24, and 1.60 for 0, 1, and 2 copies, respectively; $P = 0.05$) (Table 5). The overall cumulative OR for the A-T-C-C allele was 1.29 (1.01–1.65). The OR for the C-C-T-C allele decreased with the number of copies of this allele (1.00, 0.97, and 0.73 for 0, 1, and 2 copies, respectively; $P = 0.03$). The overall cumulative OR for the C-C-T-C allele was 0.78 (0.62–0.97). Homozygosity for the common C-C-T-C allele, compared with all other genotypes, was associated with a significant decrease in risk of type 1 diabetes (OR 0.75, 95% CI 0.58–0.95, $P = 0.02$).

DISCUSSION

Type 1 diabetes is, at least in part, genetically determined. The current study identified a haplotype block in the promoter region of the apoCIII gene that is associated with type 1 diabetes. The A-T-C-C allele of haplotype block 1 (SNPs C-641A, C-482T, T-455C, and C3175G) within the apoCIII gene is associated with a significant increase in type 1 diabetes. The risk of type 1 diabetes increases with

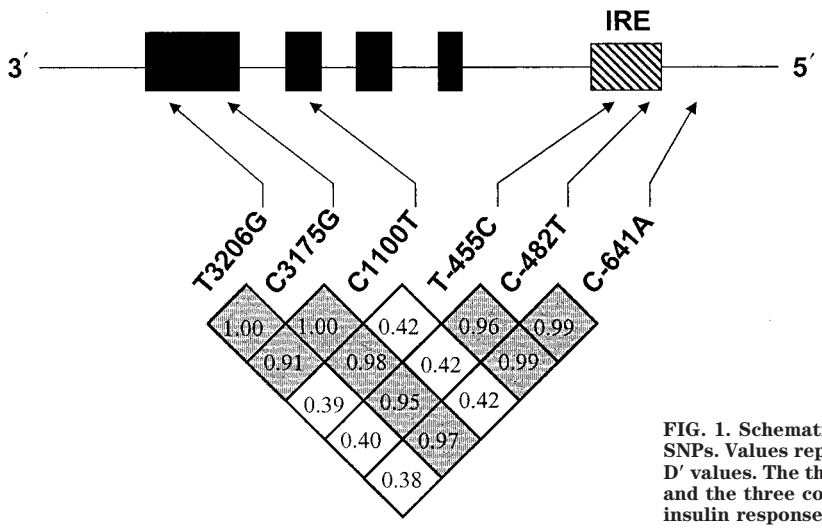

FIG. 1. Schematic of the apoCIII gene and linkage disequilibrium between SNPs. Values represent D′s. Shaded areas denote haplotype blocks based on D′ values. The three promoter SNPs plus C3175G identify haplotype block 1, and the three coding region SNPs identify haplotype block 2. IRE, negative insulin response element in the promoter region of the apoCIII gene.

TABLE 3
ApoCIII haplotype allele frequencies in type 1 diabetic and control subjects

ApoCIII haplotype block 1	Type 1 diabetic subjects	Control subjects	*P*
A-C-C-C	0.11	0.11	NS
A-C-C-G	0.004	0.003	NS
A-C-T-C	0.002	0.002	NS
A-C-T-G	—	—	NS
A-T-C-C	0.19	0.16	0.05
A-T-C-G	0.08	0.08	NS
A-T-T-C	0.006	0.003	NS
A-T-T-G	0.002	—	NS
C-C-C-C	0.001	—	NS
C-C-C-G	—	—	NS
C-C-T-C	0.60	0.65	0.04
C-C-T-G	0.0001	0.001	NS
C-T-C-C	—	—	NS
C-T-C-G	0.002	0.002	NS
C-T-T-C	—	—	NS
C-T-T-G	—	—	NS

ApoCIII haplotype block 1: haplotype consists of C-641A, C-482T, T-455C, and C3175G listed in order (5′ to 3′).

the number of copies of this allele, with an overall cumulative OR of 1.29 (1.01–1.65). The most common allele (C-C-T-C) of this haplotype block is protective for type 1 diabetes. Homozygosity for this allele is associated with a 25% lower risk of type 1 diabetes.

Based on the current study, it is not possible to ascertain the functional genetic variant(s) within this haplotype block. It may be one of the SNPs used to identify this apoCIII haplotype, or it may be another genetic variant in linkage disequilibrium with this haplotype. The current study examined an a priori hypothesis that haplotypes *within* the apoCIII gene were associated with type 1 diabetes. This was based on previous studies demonstrating that apoCIII was elevated in patients with type 1 diabetes (6) and that apoCIII induced β-cell apoptosis (7). Taking the results of the current study within the context of these previous reports, it is most likely the functional variant(s) responsible for this association is within the apoCIII gene.

Two of the promoter variants (C-482T and T-455C) are within a negative insulin response element (10). This region is known to regulate transcription of the apoCIII gene (17). Both of these substitutions attenuate the decrease in gene transcription associated with insulin (11). It is possible that one or both of these functional elements may account for this apoCIII haplotype being associated with type 1 diabetes. ApoCIII protein levels are increased in the plasma of patients with type 1 diabetes (6). ApoCIII has been shown to promote calcium-dependent pancreatic β-cell apoptosis (7). The attenuated ability of the negative insulin response element to reduce apoCIII gene expression because of either the apoCIII C-482T or T-455C variant would lead to greater apoCIII plasma levels. This could then lead to an increase in apoCIII-mediated β-cell destruction and stimulate the pathophysiological process leading to type 1 diabetes. This may promote a cycle of β-cell loss, decreased insulin secretion, and enhanced apoCIII production.

We propose a model of the etiology of type 1 diabetes in which the apoCIII gene may play an important role in progressive β-cell loss (Fig. 2). The current study demonstrates that a haplotype block that includes genetic variants within the regulatory region of the apoCIII promoter is associated with type 1 diabetes. This leads to an increase in apoCIII levels and β-cell apoptosis. The reduction in β-cell number leads to a decrease in insulin and further increases in apoCIII through lack of attenuation of gene expression by the negative insulin response element. This cycle could continue until the β-cells fail to produce adequate insulin for glucose homeostasis, leading to the clinical manifestation of type 1 diabetes. It is also proposed that the negative insulin response element within the promoter region of the apoCIII gene may play an important role in propagating β-cell destruction in the context of other genetic factors that lead to decreased insulin and type 1 diabetes. This suggests that apoCIII levels would be increased prior to the onset of clinical type 1 diabetes and insulin deficiency. Thus, the modest level of susceptibility to type 1 diabetes associated with variations in the apoCIII gene observed in the current study may be moderate compared with the overall effect of the apoCIII gene negative insulin response element.

Direct in vivo effects of apoCIII promoter variants appear variable. To our knowledge, the impact of apoCIII haplotypes on apoCIII levels has not been investigated. Most studies have focused on the role of apoCIII in modulating lipids levels. ApoCIII C-482T was associated with lipids in some (18–20) but not all studies (21). This promoter variant alters insulin and glucose response to an oral glucose load (22). ApoCIII T-455C was also related to insulin and glucose response to oral glucose (22). In

TABLE 4
ApoCIII 3′ haplotype allele frequencies in type 1 diabetic and control subjects

ApoCIII 3′ haplotype block	Type 1 diabetic subjects	Control subjects	*P*
C-C-G	0.10	0.12	NS
C-C-T	0.64	0.63	NS
C-G-G	—	0.001	NS
C-G-T	—	—	NS
T-C-G	0.15	0.15	NS
T-C-T	0.02	0.01	NS
T-G-G	0.09	0.09	NS
T-G-T	0.00001	—	NS

ApoCIII 3′ haplotype block: haplotype consists of C1100T, C3175G, and T3206G listed in order (5′ to 3′).

TABLE 5
Risk estimates (ORs) and 95% CI for haplotype block 1 alleles associated with type 1 diabetes

	OR (95% CI)	*P* value (trend test)	Cumulative OR (95% CI)
A-T-C-C allele			
Reference (0 copies)	1	0.05	1.29 (1.01–1.65)
Heterozygote (1 copy)	1.24 (0.95–1.61)		
Homozygote (2 copies)	1.60 (0.75–3.56)		
C-C-T-C allele			
Reference (0 copies)	1	0.03	0.78 (0.62–0.97)
Heterozygote (1 copy)	0.97 (0.67–1.43)		
Homozygote (2 copies)	0.73 (0.49–1.08)		

Cumulative OR represents the overall risk associated with an incremental increase in the number of copies of the allele.

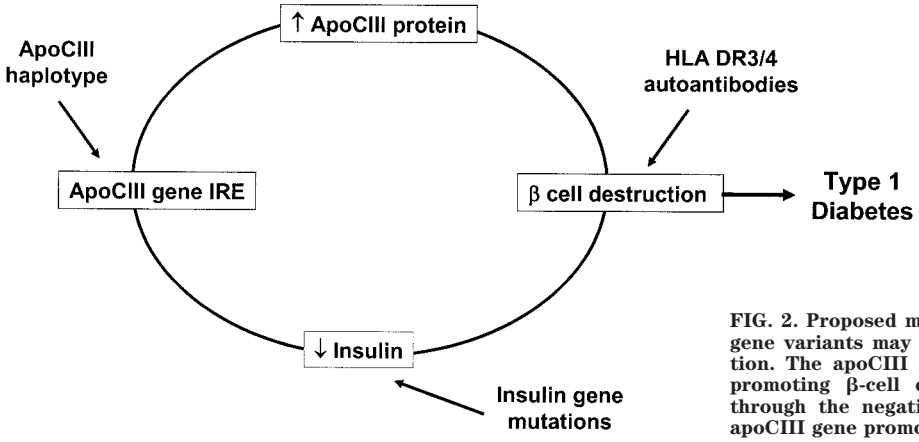

FIG. 2. Proposed model of the etiology of type 1 diabetes. ApoCIII gene variants may act directly by apoCIII-mediated β-cell destruction. The apoCIII gene may also represent a secondary factor in promoting β-cell destruction associated with decreased insulin through the negative insulin response element (IRE) within the apoCIII gene promoter.

patients with long-standing type 1 diabetes (diabetes duration of ≥18 years), apoCIII T-455C showed no relationship with apoCIII or lipid levels; however, apoCIII was related to lipid levels and HbA_{1c} (23). The impact of the promoter haplotype in the apoCIII gene on the initiation and early development of type 1 diabetes, or the development of autoantibodies, has not been previously studied.

Given the role of apoCIII on lipid levels, we considered the impact of ascertaining study participants free of coronary disease on the results of this study. The promoter SNPs in the high-risk haplotype attenuate the decrease in gene expression associated with insulin (11,14). This would lead to greater apoCIII levels and possibly lipid changes associated with an increase in coronary disease. Thus, the impact of excluding individuals with coronary disease, if any, would be to reduce the true magnitude of the association between this apoCIII haplotype and type 1 diabetes. In this study population, we did not observe any relationship between apoCIII haplotypes and subclinical coronary disease as measured by coronary calcium (P = 0.69).

Screening for this common apoCIII gene haplotype may be important in the prevention of type 1 diabetes in high-risk individuals. If the promoter variants within the insulin response element are indeed the functional elements, then early insulin therapy may help prevent the cycle of increased apoCIII levels, β-cell apoptosis, decreased insulin secretion, more apoCIII, and further β-cell destruction that ultimately leads to clinical disease. This pathophysiological process may represent an important target for early intervention in the prevention of type 1 diabetes.

Genetic susceptibility to type 1 diabetes has been clearly linked to the HLA-DR3/4 (1). The current study population has not yet been characterized with respect to HLA type. The apoCIII gene may act independently or perhaps synergistically with respect to HLA type. ApoCIII may be an important factor stimulating β-cell destruction irrespective of the mechanism of initial reduction in insulin secretion.

The current study examined a specific a priori hypothesis that haplotypes in the apoCIII gene are associated with type 1 diabetes. A common apoCIII haplotype block in the promoter region of the apoCIII gene is associated with type 1 diabetes. Independent replication of this finding will be important. Two substitutions of this haplotype block are within a negative insulin response element. This apoCIII gene haplotype block is associated with susceptibility to type 1 diabetes and may represent an important genetic marker for the early pathophysiological process that leads to type 1 diabetes.

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