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An apolipoprotein CIII haplotype protective against hypertriglyceridemia is specified by promoter and 3′ untranslated region polymorphisms

MARILYN DAMMERMAN*, LODEWIJK A. SANDKUIJL†, JEFFREY L. HALAAS*, WENDY CHUNG*, AND JAN L. BRESLOW*‡

*Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399; and †Department of Clinical Genetics, Dijkzigt University Hospital, Rotterdam, The Netherlands

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ABSTRACT **Five DNA polymorphisms were detected in the promoter of the apolipoprotein CIII gene of a type III hyperlipidemic subject with severe hypertriglyceridemia (HTG). The polymorphic sites were $C^{-641} \rightarrow A$, $G^{-630} \rightarrow A$, $T^{-625} \rightarrow$ deletion, $C^{-482} \rightarrow T$, and $T^{-455} \rightarrow C$, with the previously reported base at each site designated allele 1 and the variant base designated allele 2. The sites were in strong linkage disequilibrium with each other and with a polymorphic *Sst* I site in the apolipoprotein CIII 3′ untranslated region whose presence (S2 allele) has previously been shown to be associated with HTG. The distribution of haplotypes of the form −625 −482 *Sst* I among 78 normolipidemic adults and 79 adults with severe HTG was estimated by maximum likelihood analysis. The 211 haplotype was estimated to be 3.8-fold more common in normal subjects than in HTG subjects (estimated proportions, 0.186 and 0.049, respectively). This haplotype was associated with reduced HTG risk (relative risk, 0.28; $P = 0.005$) when compared with other haplotypes lacking the *Sst* I site (S1 allele). The 222 haplotype was estimated to be present on 48 of the 54 S2-containing chromosomes observed and was associated with increased risk for HTG (relative risk, 3.14; $P < 0.0025$). These results support the existence of apolipoprotein CIII promoter/*Sst* I haplotypes conferring either protection against or susceptibility to severe HTG.**

Genetic predisposition to hypertriglyceridemia (HTG) is relatively common in the population (1) and elevated triglycerides (TGs) have been found in many studies to be a risk factor for coronary heart disease (2). Many patients with HTG have syndrome X, which is also characterized by reduced high density lipoprotein cholesterol (HDL-C), insulin resistance, hypertension, obesity, and increased incidence of myocardial infarction (3). In very rare individuals, severe HTG is due to homozygosity for a mutation in the gene for lipoprotein lipase, which hydrolyzes TG in chylomicrons and very low density lipoprotein (VLDL) particles, or in the gene for apolipoprotein (apo) CII, a lipase cofactor. In the vast majority of cases, the genetic basis of HTG is unknown.

ApoCIII, a 79-aa glycoprotein, is synthesized by the liver and small intestine and is a major constituent of chylomicrons and VLDL particles. The human apoCIII gene has been mapped to chromosome 11q23.3 (4), where it is flanked by the genes for apoAI and apoAIV (5). Several lines of evidence have implicated apoCIII, and specifically overexpression of this protein, in primary HTG. A strong positive correlation has been observed between plasma TG and apoCIII levels across a wide range of TG values (6). Turnover studies have demonstrated elevated apoCIII synthetic rates in HTG, and the TG-lowering drug fenofibrate, together with a low-fat diet, reduces synthesis and increases catabolism of apoCIII (7). Overexpression of the human apoCIII gene is sufficient to cause HTG in transgenic mice (8). In these animals, plasma TGs are proportional to human apoCIII levels (8) and, among mice with differing levels of apoCIII expression, as little as 40% additional apoCIII results in a doubling of TGs (T. Hayek and J.L.B., unpublished results). The *in vivo* function of apoCIII is poorly understood. ApoCIII inhibits lipoprotein lipase *in vitro* (9) and may play a role in hepatic uptake of TG-rich particles and their remnants (10). In transgenic mice, elevated apoCIII levels cause displacement of apoE from VLDL particles and decreased VLDL removal from plasma (11). Association studies have implicated the apoAI/CIII/AIV locus in apoCIII overexpression and HTG. The presence of a polymorphic *Sst* I site (S2 allele) in the apoCIII 3′ untranslated region (UTR) is associated with HTG in Caucasians (ref. 12; for review, see ref. 13) and Arabs (14). In one study, healthy carriers of S2 had higher apoCIII levels than noncarriers (15).

The apoCIII gene region between approximately position −1000 and the transcriptional start site is required for maximal apoCIII expression in cultured cells and contains both positive and negative transcriptional elements (16, 17). We postulated that this region may encompass sites of relatively common genetic variation in the population. DNA sequence analysis of the apoCIII promoter[§] from a type III hyperlipidemic subject with severe HTG revealed five polymorphisms relative to the published sequences (16, 17). The distribution of two of these polymorphisms in normal subjects and in subjects with a fasting TG level of ≥1000 mg/dl was determined by allele-specific oligonucleotide (ASO) hybridization. The relationship of the promoter polymorphisms to the variant *Sst* I site was also analyzed.

MATERIALS AND METHODS

Human Subjects. Blood was drawn from lipid-clinic patients and their spouses and other family members seen at the Rockefeller University Hospital, as well as from members of the University community, for DNA isolation (18) and lipid profiles. A normolipidemic group ($n = 78$) and a group with severe HTG ($n = 79$), both composed of unrelated adult Caucasians, were selected for genotyping. The normal group was constituted so as to be similar to the HTG group with respect to age and gender. The normal group ranged from 21

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Abbreviations: apo, apolipoprotein; HTG, hypertriglyceridemia; TG, triglyceride; HDL, high density lipoprotein; VLDL, very low density lipoprotein; C, cholesterol; UTR, untranslated region; ASO, allele-specific oligonucleotide; PEPCK, phospho*enol*pyruvate carboxykinase.

‡To whom reprint requests should be addressed.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L12590).

to 76 years old, with a mean age of 49.0 years old, while the HTG group ranged from 18 to 82 years old, with a mean age of 48.4 years old. Each group included 30 women. Multiple lipid determinations were available for 29 of the normal subjects and 69 of the HTG subjects. For HTG subjects, values after treatment were not designated as such in the database. For this reason, only the maximum values for TGs, total cholesterol, and low density lipoprotein cholesterol and only the minimum value for HDL-C were recorded for each normal and HTG subject for purposes of this study.

The normal group consisted of subjects whose maximum fasting TG, total cholesterol, and low density lipoprotein cholesterol values were less than or equal to the 75th percentile for age and gender (19). Maximum TG levels ranged from 35 to 172 mg/dl, with a mean maximum of 84 ± 29 mg/dl (mean ± SD). Males with a minimum HDL-C level of <35 mg/dl or females with an HDL-C of <40 mg/dl were excluded from the normal group, as were subjects known to have a history of myocardial infarction, to have used lipid-lowering drugs, or to have a first-degree relative with HTG or a history of myocardial infarction at <55 years old. The HTG group consisted of subjects with a maximum fasting TG level of ≥1000 mg/dl, excluding known cases of lipoprotein lipase deficiency. The criterion of a TG level of ≥1000 mg/dl was chosen to select for subjects with a genetic predisposition to HTG and to exclude subjects whose elevated TG was due to secondary causes such as alcohol abuse. Maximum TG ranged from 1000 to 9833 mg/dl, with a mean maximum of 2124 ± 1513 mg/dl (mean ± SD). All subjects had maximum values greater than the 95th percentile for multiple lipid parameters (85% for total cholesterol, 100% for VLDL-C, and 32% for low density lipoprotein cholesterol) and 84% had minimum HDL-C values less than the 5th percentile. The HTG group included 11 subjects with the apoE2/2 phenotype and a lipid profile consistent with type III hyperlipidemia but with a considerably higher TG level [mean maximum, 1501 ± 682 mg/dl (mean ± SD)] than is typical (mean, ≈700 mg/dl) for this disorder (20). The apoCIII gene 5′ regulatory region of one of these subjects, a 46-year-old female with the palmar xanthomas pathognomonic of type III disease, was subjected to DNA sequence analysis; this subject was included in the case/control analysis. DNA from 99 other subjects not included in the normal or HTG groups was also used for ASO and in some cases restriction fragment length polymorphism studies.

DNA Sequence Analysis. The apoCIII region between positions −1022 and +151 (refs. 17 and 21) was amplified by polymerase chain reaction (PCR) using the primers 5′-CTGAATTCCCTGCTGCGGCTTCAC-3′ (sense strand) and 5′-ATGAATTCAGGCTTCCTTAGCTCTAGCAA-3′ (antisense), which contain engineered *Eco*RI sites. PCRs were carried out in 100 μl containing 100 pmol of each primer, all four dNTPs (each at 0.2 mM), 16.6 mM ammonium sulfate, 67 mM Tris·HCl (pH 8.8), 6.7 mM $MgCl_2$, 10 mM dithiothreitol, 10% (vol/vol) dimethyl sulfoxide, bovine serum albumin (170 μg/ml), and 2.5 units of *Taq* polymerase (22). Denaturing was at 92°C for 1 min, annealing was at 68°C for 2 min, and extension was at 72°C for 3 min for a total of 40 cycles. Products were cloned and sequenced.

ASO Hybridization and *Sst* I Typing. Amplification of the sequence from positions −699 to −188 was achieved with primers 5′-GGCAAAGGCCTCGGGCTCTGAGCGGCCTT-3′ (sense) and 5′-GCCAGCCCTGCAGCCCAGATGAGCTCAGGA-3′ (antisense). PCR conditions were as described above with the exception of annealing, which was at 72°C. ASO hybridization was as described (23). Pairs of ^{32}P-labeled probes and empirically optimized final wash conditions for the five polymorphic sites are shown in Table 1. A total of 173 unrelated Caucasians were genotyped for the five promoter markers, including 46 members of the normolipidemic group and 54 members of the HTG group. The other normal and HTG subjects were genotyped for only the −625 and −482 promoter markers.

Table 1. Allele-specific oligonucleotide hybridization assay

Polymorphism	Allele	Probe sequence	Strand	Wash temperature
−641	1	5′-TCCCCCTGAGTGTAG-3′	AS	45°C
	2	5′-CTACACTAAGGGGGA-3′	S	37°C
−630	1	5′-CCGCCGCCTCCC-3′	AS	37°C
	2	5′-GGGAGGCAGCGG-3′	S	45°C
−625	1	5′-GCGGTGGGGCAC-3′	S	45°C
	2	5′-GCGGGGGGCACA-3′	S	50°C
−482	1	5′-TGATGCCCGGTCTTC-3′	S	57°C
	2	5′-TGATGCCTGGTCTTC-3′	S	50°C
−455	1	5′-CTGGGGGATGTTTGG-3′	AS	50°C
	2	5′-CTGGGGGGTGTTTGG-3′	AS	50°C

Polymorphic bases are underlined. AS, antisense strand; S, sense strand. The final wash was in 6× SSC (900 mM NaCl/90 mM sodium citrate)/0.05% sodium pyrophosphate for 5 min.

Amplification of apoCIII exon 4 and 3′ UTR (positions 2858–3409, ref. 21), achieved using primers 5′-GATTCCTGCCTGAGGTCTCAGGGCTGTCGT-3′ (sense) and 5′-CCTGGAGTCTGTCCAGTGCCCACCCACA-3′ (antisense), was as described above, with the following modifications. Reactions were carried out in 25 μl in 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, and 10% dimethyl sulfoxide, with annealing at 72°C. *Sst* I (10 units) was then added directly to the reaction mixture (24), which was incubated 6–8 hr. Products were resolved on 1.2% agarose gels.

Statistical Analysis. Both deviation from Hardy–Weinberg equilibrium and allele or haplotype distributions for normal versus HTG subjects were assessed by χ^2 tests (1 df) or, when appropriate, by Fisher's exact test. The haplotype of interest was defined as one allele and the remaining haplotypes were pooled to form a second allele. The Bonferroni correction was applied for multiple tests. Tests for Hardy–Weinberg equilibrium in the normal and HTG groups, as well as in the combined data set, yielded no significant deviation from equilibrium. Yule's coefficient of association (25) was used to quantify linkage disequilibrium between marker pairs: $Q = |(ad - bc)/(ad + bc)|$, where a is haplotype 11, b is haplotype 12, c is haplotype 21, and d is haplotype 22. The relative risk conferred by a given allele or haplotype was assigned by calculation of the odds ratio, as follows: $R = ad/bc$, where a is allele 1 in the normal group, b is allele 1 in the HTG group, c is allele 2 in the normal group, and d is allele 2 in the HTG group. Maximum likelihood haplotype distributions were estimated as described by Hill (26). Likelihood calculations assumed Hardy–Weinberg equilibrium, allowed linkage disequilibrium to differ between normal and HTG subjects, and assumed no interaction between the two alleles or haplotypes carried by an individual.

RESULTS

Identification of Promoter Polymorphisms and Haplotypes. DNA sequence analysis was carried out on the apoCIII gene region between positions −970 and +113 from a type III hyperlipidemic subject. A portion of the sequence is shown in Fig. 1. Relative to the sequence reported in ref. 17, the following five variations were detected: $C^{-641} \rightarrow A$, $G^{-630} \rightarrow A$, $T^{-625} \rightarrow$ deletion, $C^{-482} \rightarrow T$, and $T^{-455} \rightarrow C$. PCR products matching the published sequence and products with the variant allele at each of the five sites were observed. The previously reported allele and the variant allele at each site were designated alleles 1 and 2, respectively. Three five-marker promoter haplotypes were commonly observed

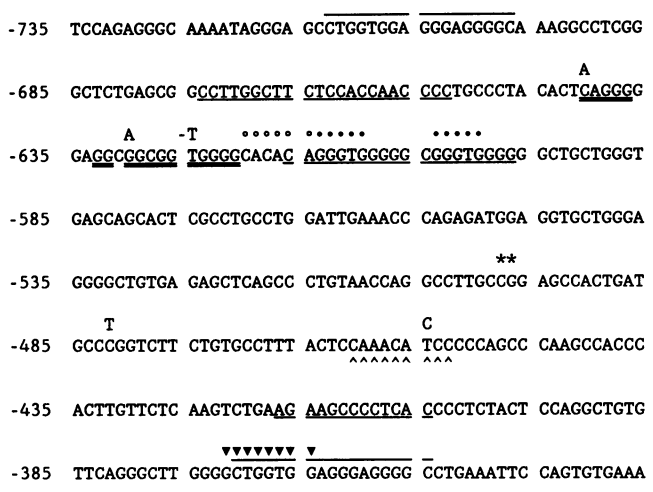

FIG. 1. Partial DNA sequence of the apoCIII promoter in which five polymorphisms were detected. The numbered rows represent haplotype 1; the nucleotides above the rows represent haplotype 2. Haplotype 1 corresponds to the sequence of ref. 17 with the exception of the insertions designated with asterisks, which do not appear to represent polymorphisms. A 17-bp perfect direct repeat flanking the five polymorphisms is overlined. The 3′ repeat overlaps a perfect chi sequence, indicated by inverted triangles. DNase I footprints corresponding to rat liver nuclear protein binding sites (17) are underlined. Beginning at position −641, and encompassing three of the polymorphisms, a sequence highly related to the adjacent footprint beginning at position −616 is indicated by double underlining of the 17 of 21 identical nucleotides. The polymorphic region and the footprint are joined by a CACACA sequence, indicated by open circles, which is a component of the phospho*enol*pyruvate carboxykinase (PEPCK) gene glucocorticoid response element (27). This is followed by two CACCC sequences (positions −614 and −604, antisense strand), indicated by solid circles. In the tryptophan oxygenase gene, the CACCC box interacts positively with the glucocorticoid receptor binding site in an orientation-independent manner (28). A sequence (positions −461 to −453) similar to the insulin/phorbol ester-responsive sequence of the PEPCK gene (29) is indicated by carets. The apoCIII sense strand is similar (7 of 10 bp are identical in haplotype 1; 8 of 10 bp are identical in haplotype 2) to the antisense strand of the PEPCK element, which is active in both orientations (29).

among Caucasian subjects, as shown in Table 2. The previously published sequence was designated haplotype 1, while the variant sequence differing at all five sites was designated haplotype 2. Sequencing of the region between positions −650 and −450 from 11 unrelated subjects revealed an additional haplotype, designated haplotype 3, which consists of the variant allele at four of the five sites, with the previously reported C allele at site −482 (Table 2).

To facilitate typing of a large number of subjects for the five polymorphisms, an ASO hybridization assay was developed for each site. Mendelian segregation of the three common promoter haplotypes was verified in 37 members of eight families, who were also typed for the *Sst* I polymorphism. Among the 173 unrelated Caucasians typed for the five promoter polymorphisms, the genotype at site −625 predicted the genotypes at sites −641 and −630 in all subjects, indicating that these sites were completely concordant. The −625 genotype predicted the −455 genotype in 169 of 173 subjects. Two rare promoter haplotypes were unambiguously detected among the four subjects discordant for sites −625 and −455; these were 22221 and 11112.

Table 2. Common apoCIII promoter haplotypes

	Base				
Haplotype	−641	−630	−625	−482	−455
1	C	G	T	C	T
2	A	A	Deletion	T	C
3	A	A	Deletion	C	C

Allele and Haplotype Distributions in Normal and HTG Subjects. Seventy-eight normolipidemic subjects and 79 subjects with a fasting TG level of ≥1000 mg/dl were typed for the −625, −482, and *Sst* I markers, with genotype and allele distributions shown in Table 3. At site −625, allele frequencies were similar among normal and HTG subjects. At site −482, a marginally significant enrichment of allele 2 was observed among HTG subjects relative to normal subjects. This site, however, is in strong linkage disequilibrium with the *Sst* I site, a known marker for HTG. The S2 allele, with a frequency among normal subjects of 0.083, was enriched 3.1-fold among the HTG subjects and conferred a relative risk of 3.85 ($P < 0.0001$). There were six S2S2 subjects and all had HTG. As shown in Table 3, strong linkage disequilibrium was observed for each of the three pairwise combinations of sites.

The observed distribution of three-marker genotypes among normal and HTG subjects is shown in Table 4. Maximum likelihood estimates of the proportions of three-marker haplotypes of the form −625 −482 *Sst* I are shown in Table 5. The 211 haplotype was estimated to be 3.8-fold more common among normal subjects than among HTG subjects (estimated proportions, 0.186 and 0.049, respectively). The estimated proportion of total haplotypes accounted for by the 111 combination was essentially the same in normal and HTG subjects, while the estimated proportion accounted for by the 221 haplotype did not differ significantly in the two groups. The 211 haplotype was found to confer a relative risk of 0.28 when compared with the two other S1-containing haplotypes ($P < 0.0025$ or $P = 0.005$ after correction for three tests). A subset of S1 carriers thus appeared to be at decreased risk of HTG as a result of carrying the −625/2 −482/1 promoter haplotype. There were four 211 homozygotes and all were normolipidemic. The 211 haplotype was observed in Caucasian subjects with Hispanic, Italian, Greek, Jewish, Eastern European, German, English, and Irish surnames, suggesting that this haplotype is widely dispersed in the Caucasian population.

The 222 haplotype was estimated to be present on 48 of 54 S2-containing chromosomes observed and was also seen in subjects with surnames representative of a variety of Caucasian ethnic groups. The 222 haplotype conferred a relative risk of 3.14 as compared with the 111 and 221 haplotypes ($P < 0.0025$). The rarity of the 112 and 212 haplotypes precluded an assessment of HTG risk associated with these combinations involving S2. Elimination of the 11 type III hyperlipidemic (E2/2) subjects from the HTG group did not significantly affect the estimated proportion of the 211 or 222 haplotypes in that group.

Of special interest were potential carriers of both the protective 211 haplotype and the high-risk 222 haplotype. One affected subject was observed among the five subjects with the corresponding 2/2 1/2 1/2 genotype (Table 4). This was significantly different from the distribution of the 1/2 1/2 1/2 and 2/2 2/2 1/2 genotypes, which may also subsume the 222 haplotype, but not in combination with the 211 haplotype. Among carriers of these genotypes, 26 of 34 subjects were affected ($P = 0.025$).

DISCUSSION

Five polymorphisms have been identified in the apoCIII promoter, at positions −641, −630, −625, −482, and −455. Relative proportions of haplotypes of the form −625 −482 *Sst* I were estimated in 79 Caucasians with fasting TG levels ≥1000 mg/dl and in 78 normolipidemic controls. The haplotype distribution observed in the HTG group was significantly different from that observed in the normal group as a

Table 3. Genotype and allele frequencies for the −625, −482, and *Sst* I markers

	Frequency		Relative		
	Normal subjects	HTG subjects	risk	χ^2	*P* value
−625 genotype			—	0.07	NS
1/1	0.346 (27)	0.329 (26)			
1/2	0.462 (36)	0.481 (38)			
2/2	0.192 (15)	0.190 (15)			
−625 allele			—	2.79	NS
1	0.577 (90)	0.570 (90)			
2	0.423 (66)	0.430 (68)			
−482 genotype			—	4.77	NS
1/1	0.577 (45)	0.443 (35)			
1/2	0.372 (29)	0.418 (33)			
2/2	0.051 (4)	0.139 (11)			
−482 allele*			1.72	4.14	<0.05
1	0.763 (119)	0.652 (103)			
2	0.237 (37)	0.348 (55)			
Sst I genotype			—	16.13	<0.0005
1/1	0.833 (65)	0.557 (44)			
1/2	0.167 (13)	0.367 (29)			
2/2	0.000 (0)	0.076 (6)			
Sst I allele*			3.85	15.89	<0.0001
1	0.917 (143)	0.741 (117)			
2	0.083 (13)	0.259 (41)			

Seventy-eight normal subjects and 79 HTG subjects were studied. Pairwise linkage disequilibria: −625 and −482, $Q = 1$ in normal and HTG subjects; −625 and *Sst* I, $Q = 0.894$ in normal subjects and $Q = 1$ in HTG subjects; −482 and *Sst* I, $Q = 0.962$ in normal subjects and $Q = 0.944$ in HTG subjects. $Q = 0$, no disequilibrium; $Q = 1$, complete disequilibrium. NS, not significant. Numbers in parentheses indicate number of subjects with each genotype or number of alleles of each type.
*Markers for which allele frequencies differed significantly in normal versus HTG subjects.

result of the rarity of the 211 haplotype and the enrichment of the 222 haplotype among HTG subjects. The 211 haplotype was associated with a relative risk of 0.28 ($P = 0.005$), whereas S2-containing haplotypes were associated with a relative risk of 3.85 ($P < 0.0001$). The S2 allele was rarely found in haplotypes other than 222. This finding supports the existence of an HTG susceptibility haplotype at the apoAI/CIII/AIV locus, as postulated (30) on the basis of data from other polymorphisms in the region. The frequency of the S2 allele in the normal group, 0.08, was the same as that reported (13) for a group of normal Caucasian participants in the Framingham Heart Study. S2 frequency among HTG subjects, 0.26, was also consistent with other studies (13).

The distribution of the 2/2 1/2 1/2 genotype among control and HTG subjects was significantly different from that of the 1/2 1/2 1/2 and 2/2 2/2 1/2 genotypes, which may also subsume the 222 haplotype. The five subjects with the 2/2 1/2 1/2 genotype were likely to carry both the protective 211 haplotype and the high-risk 222 haplotype, although the 212 and 221 haplotype combination could not be entirely excluded; this was highly unlikely for controls, among whom the 212 haplotype had an estimated proportion of 0.0. The reduced frequency of the 2/2 1/2 1/2 genotype among affected subjects suggests that the protective 211 haplotype may act in a dominant manner.

Table 4. Three-marker genotype frequencies in normal and HTG subjects

Markers			Frequency	
−625	−482	*Sst* I	Normal subjects	HTG subjects
1/1	1/1	1/1	0.333 (26)	0.329 (26)
1/2	1/2	1/1	0.218 (17)	0.127 (10)
1/2	1/1	1/1	0.179 (14)	0.076 (6)
1/2	1/2	1/2	0.064 (5)	0.253 (20)
2/2	1/2	1/2	0.051 (4)	0.013 (1)
2/2	1/1	1/1	0.051 (4)	0.000 (0)
2/2	2/2	1/2	0.038 (3)	0.076 (6)
2/2	1/2	1/1	0.038 (3)	0.013 (1)
2/2	2/2	1/1	0.013 (1)	0.013 (1)
1/1	1/1	1/2	0.013 (1)	0.000 (0)
2/2	2/2	2/2	0.000 (0)	0.051 (4)
1/2	1/1	1/2	0.000 (0)	0.025 (2)
2/2	1/1	2/2	0.000 (0)	0.013 (1)
2/2	1/2	2/2	0.000 (0)	0.013 (1)

Seventy-eight normal subjects and 79 HTG subjects were studied. Numbers in parentheses indicate number of subjects with each genotype.

It has previously been proposed that the apoAI/CIII/AIV locus may encompass sequences conferring reduced risk, as well as elevated risk, for lipid-related diseases, based on the finding that in Mayan Indians the presence of a polymorphic *Xmn* I site 2.5 kb 5′ to apoAI is associated with reduced cholesterol whereas the presence of the *Sst* I site is associated with elevated TG (31). A deficiency allele with respect to apoCIII levels has been postulated in Mayans (32). In the homozygous state, the proposed deficiency allele was estimated to confer a 4-fold reduction in apoCIII levels relative to the homozygous normal state and was associated with reduced TG and total cholesterol (32).

The molecular bases of the protection against HTG associated with the 211 haplotype and the increased risk associated with the 222 haplotype are unknown. These haplotypes may be in linkage disequilibrium with other functionally important polymorphisms in the apoAI/CIII/AIV locus or may directly affect apoCIII gene expression, as has been demonstrated for a polymorphism in the human apoAI promoter (33). The promoter polymorphisms could affect the level of transcription, either constitutively or in response to various physiologic stimuli, whereas the 3′ UTR *Sst* I site could affect mRNA stability. The possibility of a functionally significant interaction between the apoCIII promoter and 3′ UTR has been proposed (34).

The apoCIII region between positions −691 and −411, encompassing the five polymorphisms, is required for maximal apoCIII expression in HepG2 cells, with deletion of this

Table 5. Proportions of three-marker promoter/*Sst* I haplotypes in normal and HTG subjects as estimated by maximum likelihood analysis

Haplotype			Proportion				
−625	−482	*Sst* I	Normal subjects	HTG subjects	Relative risk	χ^2	*P* value
1	1	1	0.570	0.570			
2	1	1*	0.186	0.049	0.28†	9.92	0.005†
2	2	1	0.161	0.122			
1	1	2	0.007	0.000			
2	1	2	0.000	0.033			
2	2	2*	0.076	0.226	3.14‡	9.81	<0.0025

Seventy-eight normal subjects and 79 HTG subjects were studied.
*Haplotypes whose estimated proportions differed significantly between normal and HTG subjects.
†As compared with the other two S1-containing haplotypes, 111 and 221; $P < 0.0025$ prior to correction for three tests.
‡As compared with haplotypes 111 and 221.

region resulting in a 13-fold decrease in transcription (17). Binding sites for proteins present in rat liver nuclear extracts have been demonstrated in the region by DNase I footprint analysis (17), as shown in Fig. 1. The region between positions −620 and −600 contains sequences that in other genes are components of the glucocorticoid response unit, a transcription-inducing element (27, 28). The −455 polymorphism lies within a sequence (Fig. 1) highly similar to an element in the PEPCK gene (P. Gruber and T. Leff, personal communication), which in that context plays a dual role in the modulation of transcription. The PEPCK element is required for maximal glucocorticoid induction of the gene but also mediates insulin- and phorbol ester-induced suppression of glucocorticoid-stimulated transcription (29). Many subjects with elevated TG, including many of the HTG subjects in this study, have syndrome X. A role for insulin in modulating apoCIII gene expression would constitute a link between two important components of syndrome X, abnormal glucose metabolism and HTG.

As shown in Fig. 1, the five apoCIII promoter polymorphisms are flanked by a 17-bp perfect direct repeat (T. Leff, personal communication). The 3′ member of the repeat pair overlaps, by 7 of 8 bp, a perfect chi sequence (GCTGGTGG, position −372). Chi sequences are strong promoters of homologous recombination in λ phage (35) and in eukaryotes have been implicated in immunoglobulin heavy chain class switching (36) and in a possible gene conversion event in the β-globin gene in β-thalassemia (37).

In this study, two apoCIII promoter/*Sst* I haplotypes were observed to influence risk status for HTG, with carriers of the 211 haplotype at decreased risk and carriers of the 222 haplotype at increased risk. While multiple genetic and environmental factors are likely to influence plasma TG levels, our data suggest that the apoAI/CIII/AIV locus plays an important role in both resistance and susceptibility to severe HTG.

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