New genetic variants in the apoA-I and apoC-III genes and familial combined hyperlipidemia

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Abstract **Linkage and association between the apolipoprotein (apo) A-I/C-III/A-IV gene region on chromosome 11 and familial combined hyperlipidemia (FCHL) has been observed previously. Using sequence analysis two new allelic variants were identified, C_{317}-T in intron 2 of the apoA-I gene and C_{1100}-T in exon 3 of the apoC-III gene. These variants were studied in 30 FCHL probands, 159 hyperlipidemic relatives, 327 normolipidemic relatives, and 218 spouses. The allele frequencies of both variants were significantly different in probands and spouses ($P < 0.002$ and $P < 0.001$, respectively), with increased frequency of the minor alleles in the probands. The minor genotypes (TT) were associated with elevated plasma triglyceride and apoC-III. Both variants were in strong, although not complete, linkage disequilibrium with each other and with the *Msp*I site in the promoter region of the apoA-I gene and the *Sst*I site in the 3′ untranslated region of exon 4 of the apoC-III gene. Haplotypes based on these four variants were constructed and the distributions of haplotype combinations were significantly different ($P < 0.0001$). Two distinct haplotypes predisposing to FCHL were found: 2-2-1-2 and 1-2-2-2 (*Msp*I, C_{317}-T; *Sst*I, C_{1100}-T). The haplotype combinations carrying one of these high risk alleles are associated with elevated lipid levels in probands and in spouses. While these effects can be attributed to the presence of the M2 and S2 minor alleles, these results suggest that the importance of specific allelic haplotypes may be greater than single genotypic effects.** —Groenendijk, M., R. M. Cantor, T. W. A. De Bruin, and G. M. Dallinga-Thie. **New genetic variants in the apoA-I and apoC-III genes and familial combined hyperlipidemia.** ***J. Lipid Res.*** **2001.** 42: **188–194.**

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Familial combined hyperlipidemia (FCHL) is the most commonly inherited disorder of lipid metabolism. Individuals characterized with FCHL have elevated levels of plasma triglycerides and/or cholesterol. FCHL was originally assumed to be an autosomal dominant trait (1), but multiple genes may account for the variable expression of hyperlipidemia observed in FCHL, although its primary etiology remains unknown (2, 3).

Several studies have suggested that variations in the apolipoprotein (apo) A-I/C-III/A-IV gene cluster on chromosome 11 are associated with altered lipid and (apo) lipoprotein levels (4–12), although other studies were unable to confirm these findings (13–17). We have previously reported linkage of FCHL to this gene region and association with small nuclear proteins (SNP) in noncoding regions of this gene family (18). Two polymorphic sites in this gene cluster were examined in a sample of Dutch FCHL families: *Msp*I, 5′ of the apoA-I gene, and *Sst*I in the untranslated region of exon 4 of the apoC-III gene. A combination of haplotypes based on minor alleles in transconfiguration (M2-S1/M1-S2) was significantly more frequent in affected, hyperlipidemic subjects and was associated with significantly elevated plasma cholesterol, triglycerides, and apoC-III concentrations as compared with the wild-type combination of haplotypes. The observed association and linkage of these two markers in FCHL may also be explained by their linkage disequilibrium with a yet-unidentified locus elsewhere within or outside the apoA-I/C-III/A-IV gene cluster. To test other new variants, as possible contributors to the FCHL phenotype, two additional polymorphic sites, located in the insulin response element (IRE) of the apoC-III promoter region at positions −455 and −482, were studied. Inclusion of these IRE markers in the haplotypes did not improve their genetic informativeness (19). A variation in the apoC-III gene (C_{1100}-T) was associated with FCHL in German FCHL families (20). The minor T-allele had a significantly higher frequency in FCHL patients compared with control subjects and a gene-dosage effect was observed with plasma triglyceride levels (20) and significantly elevated plasma apoA-I, apoC-III, and triglyceride levels were observed in Spanish FCHL patients carrying the T_{1100} allele (21). This genetic variation was also associated with atherosclerosis (22). The C_{1100}-T polymor-

Abbreviations: FCHL, familial combined hyperlipidemia; SSCP, single-strand conformation.

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phism is in strong allelic association with the *Msp*I variant (20). The rare allele of the *Sst*I polymorphism is found on the same chromosome as the T_{1100} allele (C. F. Xu, Department of Medicine, University College London Medical School, UK, unpublished data). Haplotypes based on the *Msp*I and C_{1100}-T sites and another variant in the apoC-III gene (T_{3206}-G) were studied in FCHL families, but cosegregation of the minor alleles of these variants in the apoAI/CIII/AIV gene cluster with FCHL was not supported (20), suggesting that other genetic or environmental factors play a role in the etiology of FCHL (23). Our aim was to search for additional sequence variations in the apoC-III and apoA-I genes that might explain the observed linkage between these genes and FCHL in this population. Single-strand conformation polymorphism (SSCP) analysis revealed the presence of a C_{317}-T variant in the apoA-I gene and a C_{1100}-T variant in the apoC-III gene. They were tested in 30 FCHL families, including 30 probands, 159 hyperlipidemic relatives, 327 normolipidemic relatives, and 218 spouses, to assess association with FCHL.

MATERIALS AND METHODS

Subjects

Thirty unrelated Dutch Caucasian FCHL index patients were recruited from the Lipid Clinic of the University Medical Center Utrecht (Utrecht, The Netherlands). These subjects met the criteria described previously (1, 24, 25), including *a*) a primary hyperlipidemia with varying phenotypic expression, including fasting plasma cholesterol, triglycerides, and apoB equal to or greater than the age-sex-specific 90th percentile; *b*) at least one first-degree relative with a hyperlipidemic phenotype different from that of the proband; *c*) a positive family history of premature coronary artery disease, defined as myocardial infarction or cerebrovascular disease before the age of 60 years in at least one blood-related subject or in the index patient; and *d*) absence of xanthomas. Exclusion criteria included diabetes, familial hypercholesterolemia [absence of isolated elevated plasma low density lipoprotein (LDL) cholesterol levels, and tendon xanthomas], and type III hyperlipidemia (apoE2/E2 genotype). All subjects gave informed consent. The Human Investigation Review Committee of the University Medical Center Utrecht (The Netherlands) approved the study protocol. An attempt was made to collect the index patients, all available relatives, and their spouses without any selection bias. Hyperlipidemic relatives (n = 159) were assigned the FCHL phenotype when they met the following criteria: elevated plasma total cholesterol and/or triglycerides above the age-sex-specific 90th percentiles and apoB above the 75th age-sex-specific percentile. There were 327 normolipidemic relatives. The spouse group (n = 218) represents an environment-, nutrition-, and age-matched control group.

Lipid analysis

Venous blood was drawn after an overnight fast of 12–14 h and abstention from alcohol use for at least 48 h. Plasma was prepared by immediate centrifugation for analysis. Lipids and apolipoproteins were quantified according to methods described elsewhere (4, 24, 26).

DNA analysis

DNA was isolated from 10 ml of ethylenediaminetetraacetic acid (EDTA)-augmented blood according to standard procedures (27) and amplified by the polymerase chain reaction (PCR) technique in a thermal cycler apparatus (Pharmacia, Uppsala, Sweden). For exon 3 of the apoC-III gene the following primers were developed: 5′-TCCAATGGGTGGTCAAGCAG-3′ (sense) and 5′-TTCCATTGTTGGGATCTCAC-3′ (antisense). The PCR was performed in 50 μl, containing 200 ng of genomic DNA, a 0.6 mM concentration of each dNTP, bovine serum albumin (150 μg/ml), 200 ng of each primer, 10% dimethyl sulfoxide, 67 mM Tris-HCl (pH 8.8), 6.7 mM magnesium chloride, 10 mM 2-mercaptoethanol, 16.6 mM ammonium sulfate, 6.7 M EDTA, and 1.5 units of AmpliTaq polymerase (Perkin-Elmer, Norwalk, CT). The PCR conditions were as follows: denaturation for 4 min at 94°C, 33 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 3 min at 72°C. The nonradioactive SSCP technique was performed under nondenaturing conditions, using the Phastsystem (Pharmacia) as described by Orita, Sekiya, and Hayashi (28). One microliter of PCR product was mixed with 1 μl of denaturing solution (98% formamide, 0.05% bromphenol blue, and 0.05% xylene cyanol) and denatured for 4 min at 94°C. Samples were loaded on a precast 12.5% polyacrylamide gel (Pharmacia) and electrophoresed under the following conditions: 400 V, 10 mA, 2.5 W, 15°C, 250 V·h. Gels were silver stained according to a standard protocol, including sodium thiosulfate (2.5%) to reduce background staining. PCR products displaying an abnormal SSCP pattern were reamplified and sequenced by the dideoxy termination method, using a pUC-sequencing kit (Boehringer Mannheim, Indianapolis, IN) and ^{35}S-labeled α-dATP. Sequencing products were analyzed on 8% polyacrylamide, 6 M urea gels. One variation was found in exon 3: C_{1100}-T. This substitution represents an *Mae*III restriction site. *Mae*III (10 units) and specific restriction buffer (Pharmacia) were added to 20 μl of PCR. The incubations were performed at 37°C for at least 3 h. The products were resolved on 3% agarose gels. Alleles were defined as 1 or 2 on the basis of the absence or presence of the restriction site. For the amplification of intron 2 of the apoA-I gene the following primers were developed: 5′-CTTCTGCAT GCTGAAGGCAC-3′ (sense) and 5′-CAGTCTGGCTTCAACATC ATC-3′ (antisense). PCR conditions were as described above, with the exception that extension was for 1 min. SSCP and sequence analysis were performed as described above. One variation was found: C_{317}-T. This substitution resulted in a loss of the restriction site for the enzyme *Hae*III. Restriction with *Hae*III was performed as described for the *Mae*III.

Statistical methods

Statistical analyses were carried out with the SPSS program (SPSS, Chicago, IL). Results are expressed as means ± SEM. Frequencies of the polymorphisms were estimated by allele counting. Deviations from the Hardy-Weinberg equilibrium were tested using the χ^2 goodness-of-fit test. Analysis of variance (ANOVA) was performed to conduct a measured genotype analysis. Comparisons between plasma lipid and apolipoprotein traits for the observed genotypes were tested in independent samples of probands and spouses, using Student's *t*-test. Pairwise linkage disequilibrium between the polymorphic markers in spouses was tested by Fisher's exact test (29).

RESULTS

The clinical and biochemical characteristics of the 30 FCHL probands, their hyperlipidemic and normolipidemic relatives, and spouse control subjects are summarized in **Table 1**. Higher levels of total plasma cholesterol,

TABLE 1. Characteristics of the studied population

Variable	Probands	HL Relatives	NL Relatives	Spouses
Number	30	159	327	218
Age (years)	52 ± 2	42 ± 1	36 ± 1	48 ± 1
Gender (M/F)	21/9	73/86	180/147	90/128
BMI (kg/m^2)	26.1 ± 0.5	25.5 ± 0.3	23.8 ± 0.2	25.1 ± 0.3
Waist-hip ratio	0.94 ± 0.01	0.87 ± 0.01	0.84 ± 0.01	0.85 ± 0.01
ApoA-I (mg/dl)	129 ± 7	138 ± 2	134 ± 1	141 ± 2
ApoB (mg/dl)	142 ± 7	131 ± 2	90 ± 1	102 ± 2
ApoC-III (mg/dl)	15.1 ± 2.9	12.4 ± 0.4	8.1 ± 0.2	8.6 ± 0.2
Chol (mM)	9.52 ± 0.98	7.11 ± 0.11	5.20 ± 0.05	5.69 ± 0.07
HDL-chol (mM)	1.00 ± 0.05	1.15 ± 0.03	1.21 ± 0.02	1.25 ± 0.02
LDL-chol (mM)	4.85 ± 0.36	4.61 ± 0.11	3.35 ± 0.05	3.76 ± 0.07
TG (mM)	8.78 ± 3.09	2.99 ± 0.23	1.43 ± 0.03	1.59 ± 0.07

Values are expressed as means ± SEM; HL, hyperlipidemic; NL, normolipidemic; BMI, body mass index; Chol, cholesterol; TG, triglycerides.

TABLE 2. Plasma TG, apoC-III, and apoA-I levels and C_{1100}-T genotypes in FCHL family members

	CC	CT	TT
Plasma TG			
Probands	3.5 ± 0.5 (11)	13.2 ± 6.7 (13)	8.9 ± 4.7 (6)
Spouses	1.5 ± 0.1 (130)	1.6 ± 0.1 (78)	2.5 ± 1.0 (10)[a]
Plasma apoC-III			
Probands	13.8 ± 1.7	17.1 ± 7.0	13.6 ± 2.2
Spouses	8.4 ± 0.3	8.9 ± 0.4	9.4 ± 1.1
Plasma apoA-I			
Probands	135 ± 16	123 ± 8	130 ± 7
Spouses	139 ± 2	142 ± 3	142 ± 6

Values are expressed as means ± SEM (n). *P* values were calculated using analysis of variance. TG, Triglycerides.
[a] $P < 0.01$.

triglycerides, LDL cholesterol, apoB, and apoC-III, lower levels of high density lipoprotein (HDL) cholesterol, and increased waist-hip ratio and body mass index characterized the hyperlipidemic relatives and probands, compared with the normolipidemic relatives and spouse control subjects. The normolipidemic relatives were younger than the hyperlipidemic relatives and probands, and it is possible that in normolipidemic individuals the FCHL phenotype had not been fully expressed. There was a preponderance of male probands (70%) and normolipidemic relatives (55%), whereas in the spouses and hyperlipidemic relatives the number of females was increased (respectively, 59% and 54%). The observed genotype frequencies of the C_{1100}-T variant in the apoC-III gene and the newly identified C_{317}-T variant were consistent with the Hardy-Weinberg equilibrium.

The minor allele at each variant was significantly more frequent in probands than spouses (C_{317}-T, $P < 0.001$; C_{1100}-T, $P < 0.002$) (**Fig. 1,** solid columns). As expected, the higher frequency of the minor alleles was also observed in the hyperlipidemic and normolipidemic relatives. Using a χ^2 test of proportions, significant differences in genotype frequencies of both variants were found between probands and spouses (C_{317}-T, $P < 0.001$; C_{1100}-T, $P < 0.002$, data not shown). The frequencies of the homozygous TT carriers are given in Fig. 1 (shaded columns). Using a measured genotype approach, significant differences in plasma triglyceride levels were found among spouses with different C_{1100}-T genotypes (**Table 2**, $P < 0.01$). Among probands, CT and TT carriers had higher mean plasma triglyceride levels. Hyperlipidemic and normolipidemic relatives homozygous for the T_{1100} allele had higher mean plasma triglyceride levels than CT or CC car-

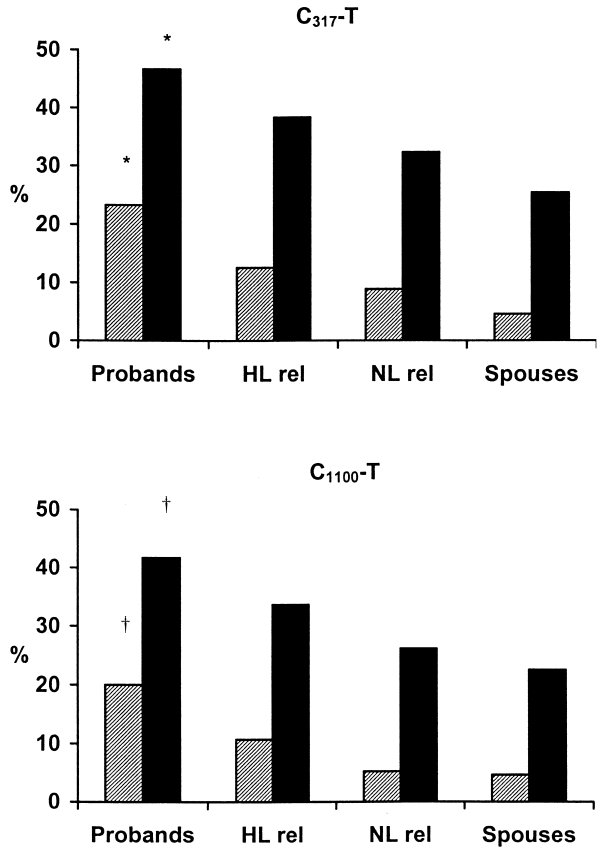

Fig. 1. Genotype and allele frequencies of the minor alleles of C_{317}-T and C_{1100}-T. Shaded columns, TT genotype; solid columns, T allele; HL rel, hyperlipidemic relatives; NL rel, normolipidemic relatives. χ^2 test: * $P < 0.001$, † $P < 0.002$ versus spouses.

TABLE 3. Plasma TG, apoC-III, and apoA-I levels and C_{317}-T genotypes in FCHL family members

	CC	CT	TT
Plasma TG			
Probands	3.3 ± 0.5 (9)	9.1 ± 5.4 (14)	15.2 ± 7.4 (7)
Spouses	1.5 ± 0.1 (127)	1.6 ± 0.1 (81)	2.9 ± 1.0 (10)[a]
Plasma apoC-III			
Probands	13.8 ± 2.0	17.1 ± 6.4	13.2 ± 1.9
Spouses	8.3 ± 0.3	8.8 ± 0.4	10.8 ± 1.2[b]
Plasma apoA-I			
Probands	133 ± 19	124 ± 8	133 ± 5
Spouses	140 ± 2	143 ± 3	130 ± 8

Values are expressed as means ± SEM (n). *P* values were calculated using analysis of variance.
[a] $P < 0.01$.
[b] $P < 0.05$.

TABLE 4. Pairwise linkage disequilibrium in spouses

	*Msp*I	C_{317}-T	*Sst*I
C_{317}-T	0.0001		
*Sst*I	0.2110	0.0001	
C_{1100}-T	0.0001	0.0001	0.0001

P values using Fisher's exact test.

TABLE 5. Haplotype frequencies in FCHL families

M-H-S-m	Probands (N = 60)	HL Relatives (N = 318)	NL Relatives (N = 654)	Spouses (N = 436)
1-1-1-1	0.50[a]	0.58	0.65	0.69
1-2-2-2	0.17[b]	0.07	0.07	0.05
2-2-1-2	0.23[c]	0.23	0.16	0.12
1-1-2-2	—	0.02	0.01	0.01
2-2-1-1	0.03	0.02	0.03	0.01
1-1-1-2	0.02	0.01	0.02	0.04
1-2-1-1	0.03	0.06	0.07	0.07
1-2-1-2	—	—	0.01	0.01
2-1-1-2	—	0.01	0.01	0.01
1-2-2-1	—	—	—	0.01
1-1-2-1	0.02	—	—	—

M, *Msp*I; H, *Hae*III; S, *Sst*I; m, *Mae*III. Fisher's exact test of proportions (overall), $P < 0.002$.
[a] χ^2 test: $P < 0.01$ versus spouses.
[b] χ^2 test: $P < 0.001$ versus spouses.
[c] χ^2 test: $P < 0.05$ versus spouses.

riers. Using the same approach, no differences in plasma apoC-III and plasma apoA-I concentrations were observed (Table 2). The C_{317}-T variant shows similar results for plasma triglyceride levels. Individuals carrying at least one T_{317} allele had higher plasma triglycerides (**Table 3,** $P < 0.01$). Plasma apoC-III levels were significantly elevated in spouses carrying the minor allele (Table 3, $P < 0.05$). No significant differences were observed in plasma apoA-I levels between the different genotypes (Table 3). Using Fisher's exact test, pairwise linkage disequilibrium was calculated (**Table 4**). The variants were in linkage disequilibrium with each other and with the *Msp*I and *Sst*I alleles. As shown previously, the *Msp*I and *Sst*I were not in linkage disequilibrium (19).

Haplotypes using the *Msp*I, C_{317}-T, *Sst*I, and C_{1100}-T variants were constructed to identify any differences in distribution between FCHL probands and spouses. **Figure 2** shows a schematic representation of the gene cluster with the approximate positions of the four variants. **Table 5** shows the occurring haplotypes (11 observed out of 16 theoretical possible haplotypes). The frequency distributions of these haplotypes were significantly different between probands and spouses ($P < 0.002$). The wild-type haplotype 1-1-1-1 (in the order: *Msp*I, C_{317}-T, *Sst*I, C_{1100}-T) had the lowest observed frequency in the FCHL probands (50%), and increased in frequency when going to hyperlipidemic relatives (58%), normolipidemic relatives (65%), and spouses (69%) ($P < 0.01$, probands vs. spouses). On the other hand, the 1-2-2-2 haplotype had an increased frequency in probands (17% vs. 5% in spouses, $P < 0.001$) as well as the 2-2-1-2 haplotype (23% vs. 12%, $P < 0.05$). The remaining haplotypes occurred rarely (Table 5). The most frequently occurring haplotype combinations are summarized in **Table 6**. The distribution was significantly different between probands and spouses ($P < 0.0001$). The wild-type combination of haplotypes decreased gradually from 48% in spouses to 40% in normolipidemic relatives, 30% in hyperlipidemic relatives, and 27% in probands. The haplotype combination in which the T_{317} and T_{1100} alleles colocalized with the *Msp*I minor allele, 1-1-1-1/2-2-1-2, and the combination in which they colocalized with the S2 minor allele, 1-1-1-1/1-2-2-2, both had a higher frequency in probands when compared with spouses (respectively, 13% and 23% vs. 8% and 18%). In probands, an increase in frequency of the 2-2-1-2/1-2-2-2 combination was observed (10%) when compared with spouses (0.9%). Combinations of haplotypes in which at least one minor allele of the C_{317}-T or C_{100}-T variant was present and the *Msp*I and *Sst*I minor alleles were absent, were combined and presented as "1-2-1-2/1-2-1-2." The frequencies of the haplotypic combinations increased gradually from 3% in probands to 10% in hyperlipidemic relatives, 11% in normolipidemic relatives, and 15% in spouses.

The association of plasma traits and these combinations of haplotypes are reported in **Tables 7** and **8**. Using ANOVA, no significant association was observed; however, in spouses the 1-1-1-1/1-2-2-2 combination resulted in higher plasma apoC-III concentrations and the 1-1-1-1/2-2-1-2 combination resulted in increased plasma apoA-I levels, compared with the wild-type combination. Only two spouses had the com-

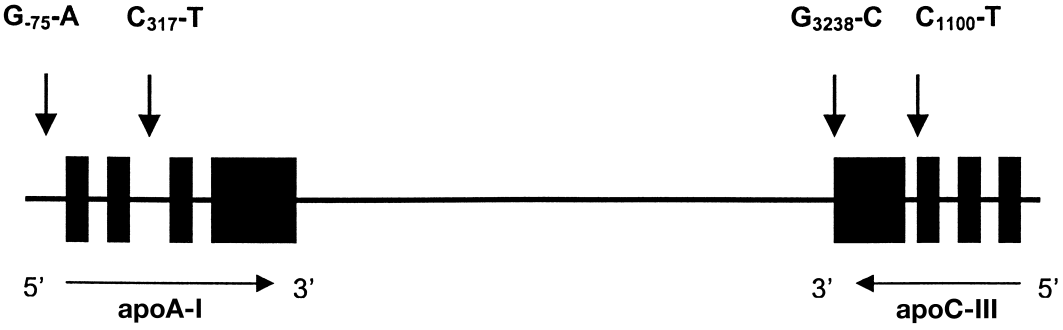

Fig. 2. Schematic representation of the apoA-I/C-III/A-IV gene cluster with the four polymorphisms used in this study.

TABLE 6. Frequently occurring haplotype combinations

M-H-S-m/M-H-S-m	Probands (N = 30)	HL Relatives (N = 159)	NL Relatives (N = 327)	Spouses (N = 218)
1-1-1-1/1-1-1-1	0.27	0.30	0.40	0.48
1-1-1-1/1-2-2-2	0.13	0.04	0.10	0.08
1-1-1-1/2-1-2-2	0.23	0.33	0.24	0.18
2-2-1-2/1-2-2-2	0.10	0.06	0.02	0.01
"1-2-1-2/1-2-1-2"	0.03	0.10	0.11	0.156

M, *Msp*I; H, C_{317}-T; S, *Sst*I; m, C_{1100}-T; "1-2-1-2/1-2-1-2, all combinations with T_{317},T_{1100}, M1, and S1 alleles. Fisher's exact test of proportions, $P < 0.0001$.

bination 2-2-1-2/1-2-2-2 and they had higher plasma cholesterol, LDL cholesterol, triglyceride, apoB, and apoC-III concentrations. No differences were observed in comparing the "1-2-1-2/1-2-1-2" combination of haplotypes with the wild-type combination (Table 7). No significant differences were observed with the 1-1-1-1/2-2-1-2 and 2-2-1-2/1-2-2-2 combinations of haplotypes in probands, but plasma cholesterol levels were, respectively, 30% and 60% higher as compared with wild-type probands and the triglyceride concentrations were about four times higher. There was only one proband having the "1-2-1-2/1-2-1-2" combination (Table 8).

DISCUSSION

FCHL was reported to be the most common familial form of hyperlipidemia in young survivors of myocardial infarction (1). Although this disease was first identified many years ago and clinical and biochemical aspects have been studied, its genetic basis is largely unknown (2, 30–32). Because the FCHL phenotype is heterogeneous, several candidate genes exist. One of these modifier genes is the apoA-I/C-III/A-IV gene cluster. The involvement of apoC-III in the metabolism of triglyceride-rich particles has been shown in studies with transgenic mice overexpressing the human apoC-III gene, which induced hypertriglyceridemia (33, 34). The *Sst*I polymorphism in the 3′ untranslated region of the apoC-III gene is frequently associated with hypertriglyceridemia (4, 5, 7–9, 11). The precise role of apoA-I is not yet fully known, but it is a major component of HDL, which serves as acceptor for surface components of very low density lipoproteins (VLDL) and chylomicrons during their catabolism. Because it is an effective cofactor of lecithin:cholesterol acyltransferase, apoA-I is involved in the regulation of the reverse cholesterol transport from peripheral tissues to the liver (35). The *Msp*I polymorphism in the promoter region of the apoA-I gene was associated with elevated plasma apoA-I and HDL cholesterol levels (36, 37) and triglyceride and apoB concentrations (4). Linkage and association studies of the gene cluster revealed evidence of its involvement in FCHL (4, 18–20, 38). However, some linkage studies do not support these findings (13, 39) and provide no evidence that this gene cluster contains the primary mutation causing FCHL (4, 18, 19). Nevertheless, there is evidence that these genes act as modifiers in lipid metabolism.

In the present study two newly identified genetic variants in the apoA-I and apoC-III genes were studied. The *Msp*I and *Sst*I markers were included in their haplotypes in order to assess whether they explain the apparent increased susceptibility to FCHL of the high risk combination of haplotypes (18, 19). Both the C_{317}-T and the C_{1100}-T variant alleles were about 1.6 times more frequent in FCHL probands when compared with the spouse control group. The frequency of the T_{1100} genotype in probands was identical to the estimate given in other studies (20, 21). The rare T allele was associated with elevated plasma triglyceride (20, 21), but no gene dosage effect was observed. In an association study carried out with patients who had survived a myocardial infarction, the frequency of the T_{1100} allele was similar to that of healthy individuals (22). No associations were found between lipid levels and this polymorphism in patients, although in healthy individuals the levels of triglycerides were higher in T_{1100} carriers. In addition, they showed that postinfarction patients with specific variations in the apoB gene and the lipoprotein lipase gene, and the C_{1100}-T variant, have a higher risk for the development of atherosclerosis, which cannot be explained by effects on plasma lipid or apolipoprotein levels. Individuals homozygous for the T_{317} allele had higher plasma triglyceride and apoC-III concentrations. No effect on plasma apoA-I was observed, indicating that this polymorphism in intron 2 of the apoA-I gene did not directly affect apoA-I transcription. In the present study we investigated whether the two newly identified variants resulted in a more specific high risk haplotype, and whether they were in strong linkage disequilibrium with the *Msp*I and *Sst*I markers. Furthermore, we were inter-

TABLE 7. Association of specific combinations of haplotypes and plasma traits in spouses

	1-1-1-1/1-1-1-1	1-1-1-1/1-2-2-2	1-1-1-1/2-2-1-2	2-2-1-2/1-2-2-2	"1-2-1-2/1-2-1-2"
N	105	17	39	2	33
Chol (mM)	5.5 ± 0.1	6.1 ± 0.2	5.9 ± 0.1	7.5 ± 0.3	5.6 ± 0.2
LDL-chol (mM)	3.66 ± 0.09	4.07 ± 0.20	3.84 ± 0.13	5.47 ± 0.19	3.62 ± 0.17
TG (mM)	1.51 ± 0.07	1.64 ± 0.18	1.52 ± 0.10	6.73 ± 4.46	1.56 ± 0.13
ApoB (mg/dl)	100 ± 3	109 ± 6	102 ± 3	147 ± 21	101 ± 5
ApoA-I (mg/dl)	139 ± 2	139 ± 6	146 ± 3	139 ± 8	142 ± 5
ApoC-III (mg/dl)	8.3 ± 0.3	10.2 ± 0.7	8.2 ± 0.5	13.2 ± 1.4	8.7 ± 0.6

Values are expressed as means ± SEM. *P* values were tested using analysis of variance. "1-2-1-2/1-2-2-2," all combinations with M1, T_{317}, S1, T_{1100} alleles; Chol, cholesterol; TG, triglycerides.

TABLE 8. Association of specific combinations of haplotypes and plasma traits in probands

	1-1-1-1/1-1-1-1	1-1-1-1/1-2-2-2	1-1-1-1/2-2-1-2	2-2-1-2/1-2-2-2	"1-2-1-2/1-2-1-2"
N	8	4	7	3	1
Chol (mM)	7.5 ± 0.6	8.0 ± 0.7	9.9 ± 2.7	12.0 ± 2.6	8.3
TG (mM)	3.4 ± 0.5	3.5 ± 0.3	14.4 ± 10.9	14.5 ± 9.0	2.3
ApoA-I (mg/dl)	135 ± 22	127 ± 24	120 ± 7	130 ± 11	145
ApoC-III (mg/dl)	14.5 ± 2.1	11.4 ± 2.1	21.3 ± 12.0	14.6 ± 3.9	9.2

Values are expressed as means ± SEM. *P* values were tested using analysis of variance. "1-2-1-2/1-2-1-2," all combinations with M1, T_{317}, S1, T_{1100} alleles; Chol, cholesterol; TG, triglycerides.

ested to know whether the minor alleles preferentially reside in the *cis* configuration with these high risk minor alleles. As shown in Table 5, the 1-2-2-2 and 2-2-1-2 haplotypes were quite frequent, indicating no specific preference for either the *Msp*I or *Sst*I locus. Both the C_{317}-T and the C_{1100}-T variants were in strong, although not complete, linkage disequilibrium with each other and with the *Msp*I and *Sst*I variants, because the frequencies of the C_{317}-T and the C_{1100}-T variants were much higher than the frequencies of the *Msp*I and *Sst*I variants. The 1-2-2-2 and 2-2-1-2 haplotypes together with the wild-type haplotype accounted for about 90% of those occurring in these FCHL families. Therefore the two distinct haplotypes found in this study, predisposing to FCHL, can be attributed to the presence of the M2 and S2 minor alleles, which are more specific. The haplotype combinations carrying one of these high risk alleles are associated with elevated lipid levels in probands as well as in spouses, and the combination of these two, 2-2-1-2/1-2-2-2, has a more distinct hyperlipidemic pattern, as described previously (18, 19). This is consistent with the model described by Chakravarti (40), in which allelic variants result in greater or lesser susceptibility to a complex disease, rather than with the model in which a complex disease occurs because of mutations at multiple genes.

In conclusion, this study provides evidence that the newly identified genetic variants in the apoA-I gene, C_{317}-T, and the apoC-III gene, C_{1100}-T, do not affect apoA-I concentrations, but show association with plasma triglyceride and apoC-III levels. However, the haplotype combination with the M2 and S2 alleles show a severe hyperlipidemic phenotype, suggesting the importance of specific haplotype combinations, relative to single genotype effects.

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