An apolipoprotein A-V gene SNP is associated with marked hypertriglyceridemia among Asian-American patients[S]

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Abstract Apolipoprotein A-V (apoA-V) is an important regulator of plasma levels of triglyceride (TG) in mice. In humans, *APOA5* genetic variation is associated with TG in several populations. In this study, we determined the effects of the p.185Gly>Cys (c.553G>T; rs2075291) polymorphism on plasma TG levels in subjects of Chinese ancestry living in the United States and in a group of non-Chinese Asian ancestry. The frequency of the less common cysteine allele was 4-fold higher (15.1% vs. 3.7%) in Chinese high-TG subjects compared with a low-TG group (Chi-square = 20.2; $P < 0.0001$), corresponding with a 4.45 times higher risk of hypertriglyceridemia (95% confidence interval, 2.18–9.07; $P < 0.001$). These results were replicated in the non-Chinese Asians. Heterozygosity was associated, in the high-TG group, with a doubling of TG ($P < 0.001$), mainly VLDL TG ($P = 0.014$). All eleven TT homozygotes had severe hypertriglyceridemia, with mean TG of 2,292 ± 447 mg/dl. Compared with controls, carriers of the T allele had lower postheparin lipoprotein lipase activity but not hepatic lipase activity. In Asian populations, this common polymorphism can lead to profound adverse effects on lipoprotein profiles, with homozygosity accounting for a significant number of cases of severe hypertriglyceridemia.[jlr] This specific apoA-V variant has a pronounced effect on TG metabolism, the mechanism of which remains to be elucidated.—Pullinger, C. R., B. E. Aouizerat, I. Movsesyan, V. Durlach, E. J. Sijbrands, K. Nakajima, A. Poon, G. M. Dallinga-Thie, H. Hattori, L. L. Green, P-Y. Kwok, R. J. Havel, P. H. Frost, M. J. Malloy, and J. P. Kane. **An apolipoprotein A-V gene SNP is associated with marked hypertriglyceridemia among Asian-American patients. *J. Lipid Res.* 2008.** 49: **1846–1854.**

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Hypertriglyceridemia, a common disorder in the United States, is now recognized as an independent risk factor for coronary heart disease (CAD) (1–4). It is a significant element in the metabolic syndrome, a constellation of clinical features linking insulin resistance, hyperlipidemia, low HDL cholesterol (HDL-C), diabetes, obesity, and hypertension with CAD.

Unlike the other members of the multigene apolipoprotein family, *APOA5* was discovered only recently. The mRNA was upregulated along with that from several novel genes following partial hepatectomy (5), suggesting that it plays a role in the provision of lipid for membranes of newly formed cells. It was also reported as a result of a comparative study of the human and mouse genomes (6). The apolipoprotein A-V (apoA-V) protein is present in plasma chylomicrons, VLDL, and HDL (7). The existence of functional farnesoid X receptor and peroxisome proliferator-activated receptor α response elements in the promoter suggests an important role in hepatic triglyceride (TG) metabolism. When the gene was overexpressed in mice,

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of four tables.

either as a transgene or by adenovirus transfection, there was a noticeable decrease in the levels of TG (6). ApoA-V knockout mice, in comparison, displayed a 4-fold increase in TG (6).

The gene for apoA-V (*APOA5*) has a close homology with that for apoA-I (*APOA1*) and apoA-IV (*APOA4*). *APOA5* lies close to these two genes as well as that for apoC-III (*APOC3*) in the cluster on chromosome 11q23. Variations at this locus have been known for a long time to be important in influencing the characteristics and levels of lipoproteins. It has particularly been implicated, at least in some kindreds, in the familial combined hyperlipidemia phenotype (8–14). We have shown in three dyslipidemic populations that the rare allele of the single nucleotide polymorphism (SNP) rs662799 (−1131T>C) in the promoter of *APOA5* is associated with elevated TG and VLDL cholesterol (VLDL-C) and with lower levels of HDL (15). A number of other studies have also shown a correlation between the −1131T>C variant and plasma TGs (6, 16–23). Its importance with regard to hypertriglyceridemia was recently reinforced in a genome-wide association study (24).

The *APOA5* SNP rs2075291 (c.553G>T; p.185Gly>Cys) was associated with hypertriglyceridemia in a Taiwanese population (25). Subsequently, a population with CAD in Nanjing, China, was reported to have a higher frequency of the T allele than a control group (26). Carriers had 64 mg/dl higher TG than noncarriers. We hypothesized that the c.553G>T polymorphism in the *APOA5* gene is an important regulator for TG metabolism in individuals of Asian origin. We examined the effect of this SNP in a population of Chinese ancestry living in a Western environment to replicate association studies done in Taiwan and China. In four other Asian groups, we also examined the frequency and impact of this *APOA5* SNP. Haplotypes were constructed with 11 *APOA5* gene SNPs in the carriers of the c.553T polymorphism to determine the effect of the alleles.

Additional biochemical analyses were performed in subgroups of carriers and noncarriers of the T allele to gain a better understanding of the mechanism underlying the hypertriglyceridemia associated with this polymorphism. We measured plasma levels of apoA-I, apoA-V, apoB, apoC-II, apoC-III, apoE, angiopoietin-like protein 3 (angptl3), lipoprotein-lipid composition, and postheparin LPL and HL activities.

METHODS

Study design

This report is a genetic association study of the *APOA5* gene p.185Gly>Cys (rs2075291) SNP (exon 4) among samples from the University of California, San Francisco (UCSF), Genomic Resource in Arteriosclerosis (GRA) (27). Informed consent was obtained for all subjects, and the UCSF Committee of Human Research Internal Review Board approved the study protocol.

All subjects in the GRA of Chinese ancestry living in northern California were selected for the study. Plasma levels of total cholesterol and TG were available for all subjects, and lipoprotein lipid measurements were also available for most subjects. All values were obtained after an overnight fast. None of the subjects was taking lipid-lowering medications. Subjects were dichotomized on the basis of fasting levels of plasma TG above (high TG; n = 152) or below (low TG; n = 148) 150 mg/dl. The National Cholesterol Education Program (28) has defined TG values below 150 mg/dl as normal.

All additional subjects of Asian ancestry in the GRA were screened to establish the SNP frequency in non-Chinese Asian-American populations. In this replication study, high TG (n = 139) and low TG (n = 101) were selected as above.

Of the 541 Asian subjects selected for these two studies, most attended UCSF clinics, and 19% were volunteers who attended health fairs in San Francisco.

Lipid and lipoprotein analysis

Cholesterol and TG contents of plasma and lipoproteins were determined by automated chemical analysis (29). VLDL ($d < 1.006$ g/ml) was prepared by ultracentrifugation (30). HDL-C was measured after precipitation of apoB-containing lipoproteins with dextran sulfate and magnesium (31). LDL cholesterol (LDL-C) was calculated as TC minus HDL-C plus VLDL-C or using the Friedewald equation when the TG was <400 mg/dl (32). Standards were provided by the Centers for Disease Control.

ELISA was used to determine the plasma contents of apoA-V (23, 33), apoC-II (34), apoC-III (34), apoE (34), and angptl3 (35). ApoA-I and apoB were analyzed with a commercially available assay (Wako Chemicals USA, Inc.) on a Cobas Mira autoanalyzer (Roche Diagnostics).

Postheparin LPL and HL activities were analyzed in plasma collected from fasting subjects at 10 min after intravenous heparin administration (20 U/kg). Enzyme activities were determined by a modification of the method of Boberg and Carlson (36). LPL was determined after inhibition of HL with an antibody raised to purified HL (37). HL was determined after inhibition of LPL with 1 M NaCl. Lipase activities are expressed as micrograms of free fatty acids released per hour per milliliter of postheparin plasma.

Polymorphism detection

A method of template-directed dye terminator incorporation with fluorescence polarization (38) was established to detect the p.185Gly>Cys SNP (rs2075291). PCR primers were GAAGACACCAAGGCCCAGTT (5′) and CCTTCCTCAGTCCCAGTGCC (3′), and the extension primer was GCGTGGTGCACCACACC. The PCRs contained 2.4 ng of dried DNA, 3 μl of PCR primer mix (0.4 μM each primer), and 3 μl of PCR reagent mix with the following protocol: 95°C for 2 min; 35 cycles of 96°C for 20 s, 67°C for 20 s, and 72°C for 30 s; and 7 min at 72°C. The PCR reagent mix was as follows: Platinum Taq, 0.02 μl (5 U/μl; Invitrogen, Carlsbad, CA); 10× buffer, 0.5 μl; $MgCl_2$ (50 mM), 0.35 μl; deoxynucleoside triphosphate (2.5 mM), 0.1 μl; water, 2.03 μl. Following exo-sap cleanup of the PCR product, the extension primer was used for the separate template-directed dye terminator incorporation reaction (38).

Haplotype analysis

Haplotypes were constructed using the program PHASE version 2.1 (39) (http://www.stat.washington.edu/stephens/software.html). The 5′ flanking SNP, rs662799, was detected as described previously (15). The c.−3G>A (rs651821) and p.Ser19Trp (rs3135506) SNPs were determined as restriction fragment-length polymorphisms using *Bsp*MI and *Eae*I, respectively, with PCR primers AGGGGTAACAGGATTTCGGG (5′) and CTACGGAGTTGTCAAGGCGG (3′). The rs34282181 and rs12287066 SNPs were determined by sequencing using PCR primers AGGGGTAACAGGATTTCGGG (5′) and CTACGGAGTTGTCAAGGCGG (3′). The rs2072560 and rs3135507 (p.Val153Met) SNPs were de-

TABLE 1. Characteristics of the Chinese-American groups

Variable	High TG (n = 152) (mg/dl)	Low TG (n = 148) (mg/dl)	*P*
Age, years	54.3 ± 1.5 (152)	49.4 ± 1.6 (148)	0.026[a]
Female, %	47.4 (152)	62.8 (148)	0.007[b]
BMI, kg/m^2	24.7 ± 0.4 (139)	23.1 ± 0.4 (136)	<0.001[a]
Total cholesterol	276.8 ± 9.0 (152)	232.2 ± 5.6 (148)	<0.001[a]
TG	477.2 ± 65.3 (152)	101.7 ± 2.3 (148)	–[c]
VLDL-C	96.4 ± 16.0 (85)	14.2 ± 1.3 (75)	<0.001[a]
VLDL-TG	466.2 ± 102.1 (85)	52.5 ± 2.8 (75)	<0.001[a]
LDL-C	151.2 ± 5.3 (133)	154.6 ± 5.5 (146)	0.662[a]
LDL-TG	55.4 ± 3.4 (85)	29.7 ± 1.1 (75)	<0.001[a]
HDL-C	45.4 ± 1.2 (144)	60.7 ± 1.6 (146)	<0.001[a]
Myocardial infarction, %	8.7 (150)	3.4 (146)	0.059[b]
Angina, %	12.2 (147)	4.9 (142)	0.027[b]
Family history of coronary heart disease, %	28.5 (130)	30.3 (132)	0.744[b]
Stroke, %	4.0 (149)	4.7 (148)	0.767[b]
Diabetes, %	18.2 (148)	6.8 (147)	0.003[b]
Hypertension, %	41.6 (149)	22.4 (147)	<0.001[b]

BMI, body mass index; -C, -cholesterol; TG, triglyceride. Values shown are ± SEM, with the numbers of subjects in each instance in parentheses.

[a] Calculated by unpaired Student's *t*-test (TG values were log-transformed prior to testing).

[b] Calculated by Chi-square test.

[c] Use of this variable as a case selection criterion precludes the reporting of a statistical significance.

termined by sequencing using PCR primers GCTAGGACAAGAGCCCTCGAC (5′) and CCTTCCTCAGTCCCAGTGCC (3′). The p.Q341H (rs7120555), c.*31C>T (rs619054), and c.*76C>T (rs34089864) SNPs were also determined by sequencing using primers GAGCTCTTCCACCCATACGCCG (5′) and CAGGAGACAGCAGCCCCTTTGG (3′). Sequencing was performed using BigDye Terminator version 3.1 and a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA).

Throughout this article, we have followed the rules for nomenclature for the description of sequence variations as compiled by the Human Genome Variation Society (40) (http://www.hgvs.org/mutnomen/).

Statistical analysis

Allele and genotype frequencies were determined by the gene-counting method. Hardy-Weinberg equilibrium was assessed for biallelic markers using a Chi-square test. Comparisons between high-TG and low-TG groups were performed using Fisher's exact test for independent qualitative data. Means of log-transformed or normally distributed variables were compared using the independent samples *t*-test. Odds ratios (ORs), adjusted for other covariables, were determined using logistic regression models. All statistical tests were performed using the program SPSS (SPSS, Inc., Chicago, IL).

In order to evaluate the role of *APOA5* genotype, body mass index (BMI), sex, age, diabetes, hypertension, alcohol consumption, and smoking on the binary outcome of hypertriglyceridemia, a logistic model was fit. The following interactions were assessed: *APOA5* genotype and BMI, sex, age, diabetes, hypertension, alcohol consumption, and smoking. Backward step-wise regression was conducted manually with predictor variables with $P < 0.2$ retained for the final fitted model. Model assumptions and fit were assessed by examination of standardized Pearson residuals to identify outlying observations, while model fit was assessed by goodness-of-fit test.

RESULTS

Characteristics of the Chinese-American case and control groups

In this association study of the *APOA5* gene c.553G>T (p.185Gly>Cys) SNP, the groups were chosen on the basis of fasting levels of TG in plasma above 150 mg/dl (high TG) or below 150 mg/dl (low TG). The characteristics of the two groups are presented in **Table 1**. Because there were significant differences in age, sex ratio, BMI, and frequency of diabetes between the groups, we subsequently adjusted the ORs for these parameters. There was an increased incidence of angina and hypertension in the high-TG group, but no differences were seen in the frequency of stroke or family history of CAD. Total cholesterol and VLDL-C were significantly higher in the high-TG group, but there was no difference in LDL-C. As expected, the higher levels of TG in the high-TG group are reflected in higher levels of VLDL-TG. Levels of HDL-C in this group were significantly lower, consistent with the well-established inverse hyperbolic relationship between levels of HDL-C

TABLE 2. APOA5 c.553G>T allele and genotype frequencies in Chinese-American subjects

Subjects	High TG	n	Low TG	n	*P*[a]
Female and male		152		148	
GG	0.737	112	0.926	137	
GT	0.224	34	0.074	11	<0.001
TT	0.039	6	0	0	
GT + TT	0.263	40	0.074	11	<0.001
T allele	0.151	46	0.037	11	<0.001
Female		72		93	
GG	0.819	59	0.925	86	
GT	0.167	12	0.075	7	0.093
TT	0.014	1	0	0	
GT + TT	0.181	13	0.075	7	0.054
T allele	0.097	14	0.038	7	0.039
Male		80		55	
GG	0.663	53	0.927	51	
GT	0.275	22	0.073	4	0.001
TT	0.063	5	0	0	
GT + TT	0.338	27	0.073	4	<0.001
T allele	0.2	32	0.036	4	<0.001

[a] *P* values were calculated by Fisher's exact test.

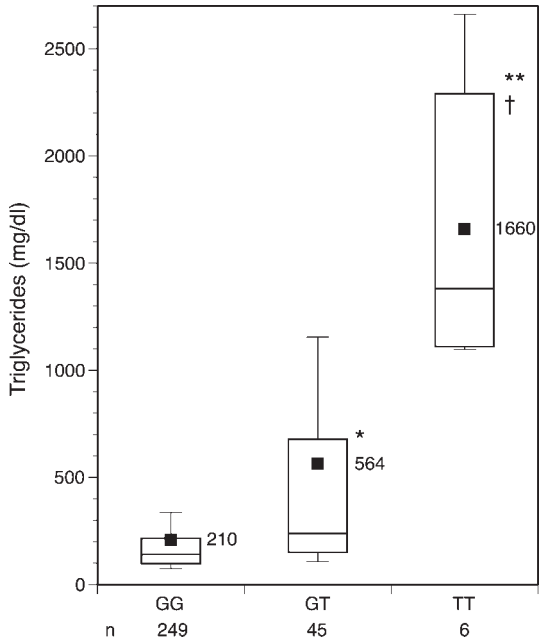

Fig. 1. Association of the *APOA5* c.553G>T single nucleotide polymorphism (SNP; p.185Gly>Cys) with plasma levels of triglycerides in the Chinese-American population. The mean values are shown next to the boxes, which show the 25th, 50th, and 75th percentiles (the error bars show outlier caps). * GG versus GT ($P < 0.0001$); ** GG versus TT ($P < 0.0001$); † GT versus TT ($P < 0.001$) calculated from log-transformed data. The T allele frequency is 9.5%.

and TG due to the transfer of cholesteryl esters from HDL to TG-rich lipoproteins (41).

Rare allele frequencies

Table 2 reveals the striking, and highly significant, 4-fold increased frequency of the c.553T allele in the Chinese-American high-TG group compared with the low-TG group (15.1% vs. 3.7%). There was an even larger, 5.5-fold, higher frequency in males. The 2.6-fold increase in the T allele frequency with females was less significant. Of note, the frequency of the T allele was found to be 4.5% in a population of 101 unselected free-living Chinese Americans recruited at a San Francisco Chinatown health fair (data not shown). The unadjusted OR was 4.45 (95% confidence interval, 2.18–9.07; $P < 0.001$) (see supplementary Table I). When adjusted for age, sex, and BMI, the OR was 4.40 (95% confidence interval, 1.99–9.74; $P < 0.001$) (data not shown). When diabetes was added as an additional covariable, the OR was 4.27 (95% confidence interval, 1.91–9.56; $P < 0.001$).

TABLE 4. Chinese-American low-TG group plasma lipids, age, and BMI, by *APOA5* c.553G>T genotype

Variable	GG	GT	*P*
Age, years	47.0 ± 1.7 (137)	55.9 ± 6.5 (11)	0.149
BMI, kg/m^2	23.1 ± 0.4 (127)	22.2 ± 0.8 (9)	0.545
Total cholesterol	232 ± 6 (137)	234 ± 15 (11)	0.939
Total TGs	101 ± 2.4 (137)	115 ± 7.2 (11)	0.101
VLDL-TC	13.6 ± 1.1 (72)	29.0 ± 15.5 (3)	0.064[a]
VLDL-TG	52.0 ± 2.8 (72)	63.0 ± 18.0 (3)	0.444
LDL-C	155 ± 6 (136)	145 ± 15 (10)	0.636
LDL-TG	29.6 ± 1.1 (72)	30.7 ± 6.5 (3)	0.856
HDL-C	60.4 ± 1.6 (136)	65.1 ± 4.5 (10)	0.446

All lipid values are mg/dl and are expressed as means ± SEM. Numbers of subjects are in parentheses.
[a] Calculated from log-transformed data.

Non-Chinese Asian-American subjects

The characteristics of the replication cohort of non-Chinese American-Asian groups are given in supplementary Table II. Our results with Chinese-American subjects were confirmed in this cohort. The T allele frequency was 2.5-fold higher in the high-TG group (13.7% vs. 5.4%; Chi-square = 20.2; $P < 0.0001$). The OR was 2.75 (95% confidence interval, 1.32–5.74; $P = 0.007$) (see supplementary Table I).

The genotypes of all groups (females, males, high TG, and low TG) were found to be in Hardy-Weinberg equilibrium.

Genetic association of lipids and lipoproteins

The association of the c.553T genotype with levels of TG in the complete Chinese-American group is presented in **Fig. 1**, which shows a pronounced gene-dosage effect. In the high-TG and low-TG groups, separately, we evaluated the association of the SNP on plasma lipid and lipoprotein concentrations. In the high-TG group, the plasma levels of total cholesterol and TG, as well as VLDL-C and VLDL-TG, were notably and significantly elevated in those subjects carrying the T allele, again with a clear gene-dosage effect (**Table 3**). The ratio of cholesterol to TG in VLDL, however, was unchanged. The presence of the T allele was associated with a decrease in LDL-C and an increase in

TABLE 3. Chinese-American high-TG group plasma lipids, age, and BMI, by *APOA5* c.553G>T genotype

Variable	GG	GT	TT	*P*
Age, years	51.0 ± 1.8 (112)	52.2 ± 2.5 (34)	49.0 ± 5.8 (6)	0.895
BMI, kg/m^2	24.9 ± 0.4 (105)	24.4 ± 0.5 (29)	23.8 ± 1.0 (6)	0.715
Total cholesterol	270 ± 9 (112)	273 ± 23 (34)	422 ± 30 (6)	0.001[a]
Total TGs	343 ± 66 (112)	709 ± 160 (34)	1660 ± 274 (6)	<0.001[a]
VLDL-C	66 ± 16 (61)	130 ± 41 (19)	333 ± 25 (5)	<0.001[a]
VLDL-TG	326 ± 106 (61)	632 ± 262 (19)	1546 ± 331 (5)	<0.001[a]
LDL-C	163 ± 6 (105)	113 ± 11 (23)	80 ± 11 (5)	<0.001[a]
LDL-TG	47.9 ± 2.5 (61)	61.2 ± 8.3 (19)	125 ± 21 (5)	<0.001[a]
HDL-C	47.8 ± 1.4 (108)	39.1 ± 2.2 (31)	32.2 ± 7.1 (5)	0.001

All lipid values are mg/dl and are expressed as means ± SEM. *P* values were calculated by ANOVA. Numbers of subjects are in parentheses.
[a] Calculated from log-transformed data.

TABLE 5. Effect of *APOA5* c.553G>T heterozygosity on lipid profiles in Pacific Islander, Southeast Asian, Japanese-American, and Korean-American populations

Variable	T Allele Frequency (%)	GG	GT	*P*
Pacific Islander	7.5			
Total TGs		221 ± 24 (83)	614 ± 137 (14)	<0.001[a]
HDL-C		51.1 ± 1.9	40.9 ± 4.2 (14)	0.044
Southeast Asian	5.1			
Total TGs		217 ± 26 (53)	657 ± 304 (6)	0.007[a]
HDL-C		49.9 ± 2.8 (51)	47.8 ± 6.2 (4)	0.837
Japanese	13.5			
Total TGs		238 ± 50 (42)	380 ± 159 (19)	0.423[a]
HDL-C		63.8 ± 4.6 (41)	52.8 ± 5.1 (18)	0.161
Korean	9.6			
Total TGs		413 ± 160 (16)	1043 ± 566 (4)	0.056[a]
HDL-C		51.3 ± 3.8 (16)	32.2 ± 6.8 (4)	0.036

All lipid values are mg/dl and are expressed as means ± SEM. Numbers of subjects are in parentheses.
[a] Calculated from log-transformed data.

LDL-TG. The decrease in HDL-C in carriers, again with a gene-dosage effect, reflects the transfer of cholesteryl esters from HDL to TG-rich lipoproteins, as mentioned above. In the low-TG group, the T allele was associated with doubled levels of VLDL-C and a 14% increase in TG (**Table 4**), but this was not significant, possibly reflecting low power.

The effects of the polymorphism on plasma TG and HDL-C in the four non-Chinese Asian population subgroups in this study are shown in **Table 5**. The frequency of the T allele was highest in the Japanese group and lowest in the Southeast Asians. In all four groups, the levels of TG were higher in the heterozygotes than in the more common GG homozygotes, and these differences were significant in the Pacific Islander and Southeast Asian subjects. Levels of HDL-C were lower for heterozygotes in all four populations, and this was significant in the Pacific Islander and Korean groups. It should be noted that the numbers of people in each of these subgroups was considerably lower than for the Chinese-American population. The overall difference in TG levels between the GG and GT groups is similar to that seen in the Chinese-American population (Fig. 1). One Pacific Islander, one Japanese, and one Korean individual were homozygous for the T allele. All three were severely hypertriglyceridemic, as noted below. The polymorphism was not seen in South Asians (India or Pakistan) and was only present at an extremely low frequency in Caucasians: among 779 non-Asian individuals, only three carriers were found, two Caucasian and one Hispanic.

The characteristics of all 11 subjects identified as homozygotes are given in **Table 6**; all had pronounced hypertriglyceridemia. Subject 2 developed hypertension at age 62 and had a myocardial infarction at age 77. Subjects 6 and 8 developed hypertension at ages 32 and 58, respectively. In addition, subject 8 developed diabetes at age 45. Little clinical information was available for subjects 5, 7, and 9. One homozygote was Caucasian. This was somewhat surprising, given the overall very low frequency of the T allele in this ethnic group.

We analyzed the combined data from the two studies in an attempt to explain the large range of TG values observed in heterozygous individuals (see supplementary Table IV). In a regression model, we included, in addition to *APOA5* genotype, several potential confounding variables: BMI, sex, age, diabetes, hypertension, alcohol consumption, and smoking. The analysis revealed that only sex (i.e., male sex) was a modest positive confounder. Although BMI, diabetes, and hypertension were all independent predictors of TG, they did not affect the rela-

TABLE 6. Characteristics of *APOA5* c.553G>T homozygous patients

Subject	Sex	Ethnic Origin	BMI	Age	Total Cholesterol	TG	VLDL-C	VLDL-TG	LDL-C	LDL-TG	HDL-C
1	Male	Chinese	25.8	48	405	1,110	288	990	81	85	36
2	Male	Chinese	22.9	75	306	1,370					
3	Male	Chinese	21.5	49	519	1,393	371	1,225	120	138	28
4	Male	Chinese	27.4	51	485	2,702	405	2,483	61	172	19
5	Male	Chinese	24.1	33	405	2,290	328	2,190	57	68	20
6	Female	Chinese	21.2	39	414	1,095	273	845	83	164	58
7	Male	Chinese	25.0	49	133	687					
8	Male	Japanese	29.2	34	610	4,968					
9	Male	Korean	—	25	676	2,220	538	1,980	52	125	25
10	Female	Pacific Islander	19.9	51	543	5,040					
11	Female	White European	—	31	649	2,339	543	1,953	47	83	33
		Mean	24.1	44.1	468	2,292	392	1,667	71.6	119	31.3
		SEM	1.0	4.2	48	447	42	242	9.6	16	5.0
		n	9	11	11	11	7	7	7	7	7

Lipid measurements are expressed as mg/dl, and BMI is expressed as kg/m^2.

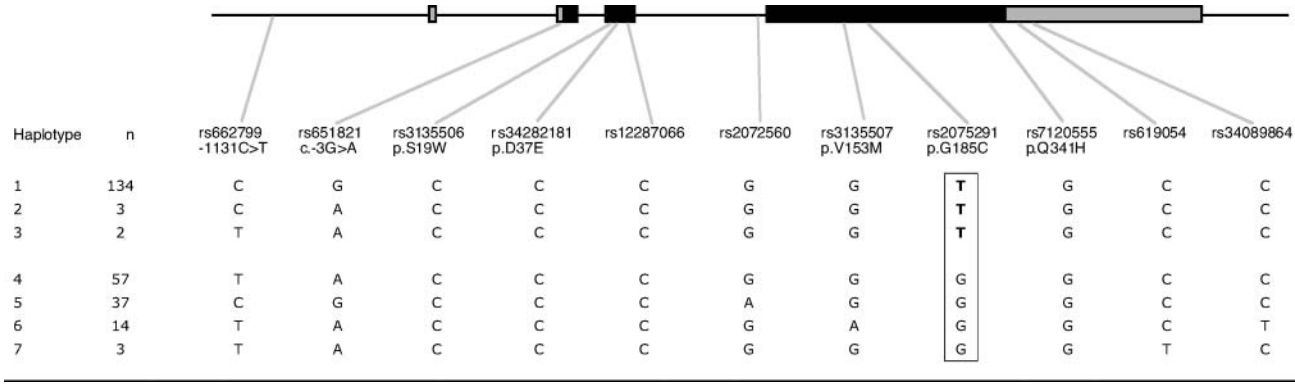

Fig. 2. Schematic diagram of the *APOA5* gene showing the positions of 11 SNPs genotyped. Haplotypes were constructed using the program PHASE version 2.1. Three haplotypes, 1, 2, and 3, carried the c.553T variant.

tionship of *APOA5* genotype with TG. Neither alcohol consumption nor smoking was a predictor of TG, and neither affected the association between *APOA5* genotype and TG. Although the estimate of the coefficient for age was not significant, this variable was retained in the model to maintain validity.

Haplotype analysis

Haplotypes were inferred for all of the 125 c.553T carriers that we identified; in 90% of cases, this was with >92% certainty, and in 10% of cases, it was with 71–91% confidence. One haplotype (haplotype 1) accounted for almost all T alleles (**Fig. 2**). All Chinese-American carriers displayed this haplotype except one, who had haplotype 2. Of the other two subjects with haplotype 2, one was a Pacific Islander and the other was of mixed ancestry: Pacific Islander and Japanese. The two subjects with haplotype 3 were both Pacific Islanders. All 11 T homozygotes were homozygous for haplotype 1.

One-third of c.553T heterozygotes had, as the other allele, haplotype 5 (Fig. 2), which has the less common C in SNP rs662799 (−1131C>T). Further analysis of heterozygous carriers revealed that those with haplotype 5 had the highest TGs compared with those with haplotypes 4, 6, or 7 (see supplementary Table III). Those with haplotype 5 had a mean TG of 832 ± 216 mg/dl, compared with 444 ± 70 mg/dl for subjects with the other haplotypes ($P = 0.018$). When haplotype 5 was the second allele, levels of VLDL-C and VLDL-TG were also significantly elevated and HDL-C was significantly lower (see supplementary Table III). Figure 2 shows that the −1131 and c.−3G>A SNPs were in very strong, but not complete, linkage disequilibrium, as reported previously in a group of Japanese Americans (22).

Additional biochemical studies

Further, in-depth biochemical studies were performed on plasma samples from a subset of c.553T carriers. For comparison, a random group of GG homozygotes was selected from the total Asian population set. Individuals for this study were included irrespective of their levels of TG. These results are presented in **Table 7**. As before, we found striking, and highly significant, increases in levels of TG. As before, HDL-C was decreased, here by 23%, but the amount of apoA-I was decreased by only 10%, and not significantly. The levels of apoC-II, apoC-III, and apoE were moderately and significantly increased (by 61, 42, and 52%, respectively) in the carrier group, reflecting the higher levels of TG-rich lipoproteins. In contrast, apoA-V was increased by only 13%.

Postheparin LPL activity measured in 7 c.553T carriers was 34.5% lower than in 31 low-TG controls (3.8 ± 1.1 and 5.8 ± 0.3 U/ml, respectively; $P = 0.027$). In contrast, there was no difference in hepatic lipase activity between carriers and controls (4.8 ± 0.3 and 4.8 ± 0.8 U/ml, respectively; $P = 0.987$).

DISCUSSION

We successfully replicated the previously reported association of an apoA-V polymorphism (c.553G>T;

TABLE 7. Multiple biochemical parameters and characteristics of a subgroup of subjects

Variable	GG (n = 28)	GT/TT (n = 42)	*P*
Age, years	56.0 ± 3.5 (28)	53.2 ± 2.9 (42)	0.536
Female, %	60.7 (28)	40.5 (42)	0.097
Total cholesterol	230.8 ± 8.3 (28)	249.6 ± 11.4 (42)	0.233
TGs[a]	214.0 ± 46.9 (28)	452.3 ± 78.6 (42)	<0.001
LDL-C	143.5 ± 7.2 (23)	122.7 ± 7.6 (31)	0.060
HDL-C	58.1 ± 4.4 (25)	44.6 ± 2.6 (36)	0.007
ApoA-I	141.7 ± 5.6 (24)	127.9 ± 4.6 (36)	0.064
ApoA-V,[a] ng/ml	206.2 ± 28.5 (24)	232.9 ± 40.2 (36)	0.972
ApoB	93.8 ± 3.5 (24)	90.2 ± 2.8 (36)	0.427
ApoC-II[a]	4.6 ± 0.5 (28)	7.4 ± 0.6 (42)	<0.001
ApoC-III[a]	10.2 ± 0.7 (28)	14.5 ± 0.9 (42)	<0.001
ApoE[a]	4.4 ± 0.2 (28)	6.7 ± 0.5 (42)	<0.001
Angiopoietin-like protein 3,[a] ng/ml	158.6 ± 14.9 (28)	202.2 ± 31.6 (42)	0.287

ApoA-I, apolipoprotein A-I. Values are ±SEM and are mg/dl unless indicated otherwise; numbers of observations are in parentheses. *P* values were calculated by unpaired Student's *t*-test, except for the female value, which was by Chi-square test.

[a] These values were log-transformed prior to testing.

p.185Gly>Cys) with plasma levels of TG in China and Taiwan (25, 26). Our population was of Chinese descent living in the United States. The presence of the less common T (cysteine) allele conferred a substantial 4.45 times greater risk of hypertriglyceridemia. We found that this association persisted in a non-Chinese cohort consisting of four other Asian-American ethnic groups: those of Japanese, Korean, Southeast Asian, and Pacific Islander ancestry. Overall, in both populations, there was a marked association with the presence of the T allele with a pronounced gene-dosage effect. These differences were more pronounced in men than in women. One in sixty (1.7%) of the Asian subjects we studied were homozygous carriers of the c.553T allele; all had severe hypertriglyceridemia. The 4.5% T allele frequency that we found in 101 unselected Chinese Americans is somewhat higher than the 2.2% reported in the HapMap project for 45 Han Chinese in Beijing (http://www.hapmap.org). Based on our data, homozygosity for the T allele would be expected at 1 in 500 free-living Chinese Americans.

What needs to be explained, given the markedly increased frequency of the c.553T allele and the prominent effect on levels of TG in the high-TG group, is the lack of effect on lipids and lipoprotein parameters in the low-TG groups. In the Chinese-American low-TG group, the effect was not significant, although it was similar in magnitude to the report of Kao et al. (25) in a Taiwanese control population, in which heterozygotes had 15% higher TG. This incomplete dominance could be due to an interaction between the polymorphism and other genetic factors, which could be determined in family studies. Environmental effects and diet may also play a role. Our analysis showed that while BMI, diabetes, and hypertension were all predictors of TG, they did not have an impact on the correlation of *APOA5* genotype with TG. It is worth noting that the impact of the *APOA5* genotype is two to three times greater than any of the other predictors. In Caucasian or predominantly Caucasian populations (6, 15–23), and in Chinese populations (42, 43), it has been shown that the −1131C variant is associated with a significant increase in levels of TG, although the effects were considerably lower in magnitude than seen here with the c.553G>T SNP. Our present study shows that, in combination with c.553T, the −1131C variant has a significant compounding effect.

The Chinese-American high-TG group showed a marked decrease in HDL-C associated with the presence of the T allele. We estimated, based on the curvilinear relationship between HDL-C and TG as described by Meyers, Phillips, and Havel (41), that one would expect a 16% lower HDL-C in the GT group compared with the GG group, solely due to the effect of mass transfer associated with the difference in TG (709 vs. 343 mg/dl) (Table 3). We actually observed 18% lower HDL-C; hence, the major part of the associated lower HDL-C can be explained by this effect alone.

Some previous evidence points to apoA-V being an activator of LPL (44, 45). We observed significantly lower postheparin LPL activity in c.553T carriers. Thus, given the essentially unchanged level of apoA-V in the carrier group, it is likely that the cysteine-185 apoA-V variant may be less efficient at activating the enzyme. This activation, it should be pointed out, is not direct but by some as yet unclear indirect mechanism (46). We postulated that apoA-V could affect LPL activity indirectly by affecting the level of angptl3. Angptl3 has been implicated as a regulator of TG metabolism (47), and recently, genetic variants near to the *ANGPTL3* gene were associated with levels of plasma TG (48, 49). Angptl3 decreases VLDL-TG clearance via the inhibition of lipolysis by LPL (50). Our results (Table 7) show that levels of angptl3 were indeed higher in c.553T carriers, but not significantly so.

There was a marginal, but not significant, decrease in the level of apoA-I and modest, significant increases in apoE, apoC-II, and apoC-III that probably reflect an increase in the surface area of TG-rich lipoproteins.

Our data show clearly that the presence of the *APOA5* p.185Gly>Cys variant results in considerable effects on TG levels in the Asian populations that we have studied. Among the 65 subjects with TG above 500 mg/dl, 60% were carriers: 45% heterozygous and 15% homozygous. Of the 29 with TG above 1,000 mg/dl, 79% were carriers: 45% heterozygous and 34% homozygous. All but one of the homozygotes we identified had levels of TG exceeding 1,000 mg/dl. Clearly, at the frequency we have observed, tens of millions of people worldwide are carriers with increased risk of developing hypertriglyceridemia. It was suggested recently that individuals with TG levels above 1,000–1,500 mg/dl should be treated with fibrates to lower the risk of pancreatitis (51). In the case of T allele carriers with such TG levels, we believe that it would be appropriate to screen close relatives to alert the carriers of their increased risk and to offer them intervention if necessary. Future family- and population-based studies are needed to determine, more precisely, the discriminative ability of the polymorphism to identify family members with an increased risk of severe hypertriglyceridemia.

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