The *APOA5* locus is a strong determinant of plasma triglyceride concentrations across ethnic groups in Singapore

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Abstract Singapore comprises three ethnic groups: Chinese (76.7%), Malays (14%), and Asian-Indians (7.9%). Overall, Singaporeans experience coronary heart disease rates similar to those found in the United States. However, there is a dramatic interethnic gradient, with Asian-Indians having significantly higher risk than Chinese and Malays. These differences are associated with HDL cholesterol levels and cannot be solely explained by environmental exposure, and may be driven by genetic factors. The gene encoding apolipoprotein A-V (*APOA5*) has been located on chromosome 11, and it is emerging as an important candidate gene for lipoprotein metabolism. We investigated associations between *APOA5* polymorphisms and plasma lipids in 3,971 Singaporeans to establish whether they accounted for some of the ethnic differences in plasma lipids. We found significant associations between the minor alleles at each of four common polymorphisms and higher plasma triglycerides (TGs) across ethnic groups. Haplotype analyses showed significant associations with TGs, explaining 6.9%, 5.2%, and 2.7% of the TG variance in Malays, Asian-Indians, and Chinese, respectively. Conversely, we observed significant inverse associations between the minor alleles and HDL cholesterol concentrations for Chinese and Malays. These data suggest that *APOA5* plays a role in the ethnic differences observed for plasma TG and HDL cholesterol concentrations.—Lai, C-Q., E-S. Tai, C. E. Tan, J. Cutter, S. K. Chew, Y-P. Zhu, X. Adiconis, and J. M. Ordovas. **The *APOA5* locus is a strong determinant of plasma triglyceride concentrations across ethnic groups in Singapore. *J Lipid Res.* 2003.** 44: **2365–2373.**

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Singapore is a highly developed country in Southeast Asia, populated by ~4.5 million people living in a mostly urbanized area of ~700 km^2 and representing three ethnic groups (Chinese 76.7%, Malay 14%, and Indian 7.9%, with other minor ethnicities making up the other 1.4%). Despite their ethnic and cultural differences from Western populations, Singaporeans suffer from high cardiovascular disease (CVD) rates similar to those found in the United States and Australia (1). Most interestingly, the effects of urbanization have not affected all three major ethnic groups equally. Asian-Indians have the highest rate of CVD, followed at a significant distance by Malays and Chinese (1–4). In addition, the ethnic difference is further reflected by the diverse lipid profile among these ethnic groups. In particular, HDL cholesterol levels mirror CVD rates, with Asian-Indians having the lowest, followed by the Malays, and with the Chinese displaying the highest concentrations. Conversely, plasma triglyceride (TG) concentration is the highest in Malays and Asian-Indians and the lowest in Chinese. These differences occur despite the high socioeconomic status and the consumption of diets that have similar biochemical and nutritional compositions regardless of the ethnic origin (2, 5). This provides an ideal situation in which to examine the contribution of genetic factors to disease risk heterogeneity and to study the interaction between genetic and environmental factors.

High TG and LDL cholesterol and low HDL cholesterol concentrations are independent risk factors for CVD (6, 7). Identifying genetic and environmental factors that influence plasma lipid levels represents a key step toward developing strategies for preventing and treating CVD. Previous studies have shown significant associations between genetic variability in several members of the apolipoprotein gene family and plasma lipid concentrations (8–11). The apolipoprotein A5 (*APOA5*) gene has been recently identified by comparison of human and mouse DNA sequences (12). This locus is located ~27 kb distal to

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the *APOA1/C3/A4* gene cluster and encodes apolipoprotein A-V (apoA-V). It has been reported that the human *APOA5* (*hAPOA5*) transgenic mouse has significantly decreased TG and the *APOA5* knockout mouse significantly increased plasma TG concentrations as compared with wild-type mice (12). These observations prompted the exploration of genetic variants at the *APOA5* locus for potential association with plasma TG concentrations in humans. In this regard, Pennachio et al. (12) identified several common *APOA5* single-nucleotide polymorphisms (SNPs) and demonstrated significant associations of these SNPs with plasma TG and VLDL cholesterol levels in a sample of 500 random unrelated normolipemic White subjects. Carriers of the minor alleles had 20–30% higher TG concentrations than homozygotes for the most common allele. These investigators replicated these findings on other unrelated Caucasian populations (13, 14), while other researchers supported these findings in Japanese (15, 16), Chinese (17), African-American, and Hispanic populations (14). In addition, a significant decrease in HDL cholesterol levels was observed in Japanese children (16) and Chinese (17) carriers of the less-common alleles. Furthermore, *APOA5* variants have been shown to increase the risk for familial combined hyperlipidemia in Caucasians (18) and to affect postprandial lipaemia (19). The surprising homogeneity of these results supports the notion that the *APOA5* locus may be a significant determinant of plasma TG concentrations and that this effect is consistent across ethnic boundaries and environmental factors.

In this study, we have determined the allele frequencies of several previously reported genetic variants at the *APOA5* locus and calculated haplotypes for each of the major ethnic groups of Singapore. Moreover, we have analyzed the contribution of this locus to the variability in plasma TG and HDL cholesterol concentrations. This is part of our quest to characterize the genetic and environmental bases determining the dramatic ethnic-specific differences in plasma lipid levels observed in this population (20).

MATERIALS AND METHODS

Subjects

We studied 1,833 men (1,220 Chinese, 344 Malays, and 269 Asian-Indians) and 2,138 women (1,491 Chinese, 363 Malays, and 284 Asian-Indians) from the 1998 Singapore National Health Survey. The detailed methodology of this study has been previously described (20).

SNP genotyping

DNA was isolated from blood samples using DNA blood Midi kits (Qiagen, Hilden, Germany) following the protocol recommended by the vendor.

For *APOA5* genotyping, we identified 12 SNPs on the National Center for Biotechnology Information Human SNP Database and tested their informativeness in these ethnic groups on a pilot experiment including 278 subjects (i.e., three 96-well plates of DNA samples including 147 Chinese, 93 Malays, and 38 Asian-Indians). Based on this study, five of the SNPs (ss4383597 with frequencies of 0.000, 0.048, and 0.015 in Chinese, Malay, and Asian-Indian, respectively; ss1943492 with frequencies of 0.000, 0.029, and 0.141; ss2569372 with frequencies of 0.000, 0.000, and 0.028; ss3184133 with frequencies of 0.046, 0.043, and 0.000; and ss3563852 with frequencies of 0.000, 0.000, and 0.000) were either monomorphic or had average frequencies of the minor allele of <0.05 in our population, and with the exception of the previously reported ss4383597 (S19W, 13, 14), those SNPs were not included in the genotyping of the entire population. Three other SNPs (ss4383598, ss2990302, and ss1943494) were not given further consideration, because of their low reliability for genotyping across all three populations. At the end, five previously reported SNPs (12–14) were included for the analyses presented in this research. Our nomenclature is based on that suggested by the Human Genome Variation Society (21) and it is as follows: −1131T>C in the 5′ region and [previously reported as SNP3 (12)]; −3A>G in the 5′ region as previously reported (13, 14); 56C>G in the third exon [previously reported as S19W (13, 14)]; IVS3+476G>A in intron 3 (previously reported as SNP2); and 1259T>C in exon 4 (previously reported as SNP1). Their relative positions are illustrated in **Fig. 1**.

Genotyping was carried out using the ABI Prism SNaPshot multiplex system (Applied Biosystems, Foster City, CA). The primers and probes used are listed in **Table 1**. The SNaPshot multiplex systems has been adapted and successfully used in our laboratory since its commercialization (20).

Statistical analysis

To test Hardy-Weinberg equilibrium (HWE) and investigate the strength of pair-wise linkage disequilibrium (LD) between SNPs, we used the Genetic Data Analysis software [GDA (22)]. The setup included 30,000 runs, with missing data discarded. The pair-wise LDs between each two SNPs were estimated as D′ (23) using the Helixtree software package (Golden Helix, Inc., Bozeman, MT). This package was also used to estimate haplotype frequencies within each ethnic group using the Expectation-Maximization (EM) algorithm (24).

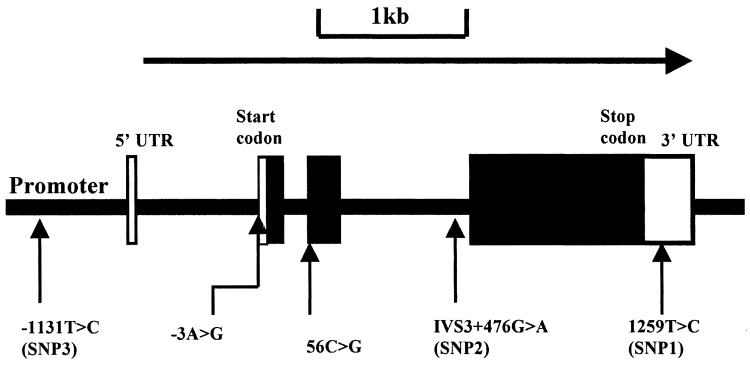

Fig. 1. Genomic structure and relative position of five single-nucleotide polymorphisms (SNPs) at the *APOA5* locus. The gene is transcribed as indicated by the large horizontal arrow. Exons are the larger rectangular boxes, with the protein coding regions shaded black. The small vertical arrows below the bar depict the relative positions of five SNPs. The SNP names in parentheses were previously used in (12).

TABLE 1. Probes and primers used for genotyping for the five polymorphisms at the *APOA5* locus

SNP Name	GenBank_ID	Probe	Left	Right
−1131T>C	ss3199915 (SNP3)	AGGAACTGGAGCGAAAGT	AGCCAGGCAGGGTGAAGATG	AGAGGCCCTGCGAGTGGAGT
−3A>G	ss1943493	GCCATGCTTGCCATTA	CCGAAAACGCTGTGGAGAGG	CCAGCCCAAGGAAGGGGTAA
56C>G	ss4383597(S19W)	CCTCTCCACAGCGTTTT	TCTGGCTGAAGTAGTCCCAGAAGC	GAGCCCAGGCCCTGATTACC
IVS3+476G>A	ss3199914 (SNP2)	GGACAAAGGAGATGATGG	TTCCCCCAGAGGATCAGTGC	TCCAGGCCGTCAGACTGCTA
1259T>C	ss3199913 (SNP1)	GCTGCTGTCTCCTGCA	CACCAAAGGGGCTGCTGTCT	CAGATTGGGGAGTCGCAGGA

SNP, single-nucleotide polymorphism.

Most other statistical analyses were carried out using SAS (Windows version 8.02). Allele and genotype frequencies were tested for homogeneity with χ^2 test with Bonferroni correction. TG concentrations were log10-transformed to achieve approximate normal distribution before analysis. To determine the association between *APOA5* polymorphisms and CVD risk factors, we used analysis-of-covariance model with the general linear model procedure. In these analyses, the dependent variables were plasma lipid measurements, and the independent variables were each of the individual *APOA5* SNPs. Analyses were adjusted for potential confounders [age, sex, body mass index (BMI), smoking, alcohol intake, and exercise] using a linear regression model. To determine the association between haplotypes and plasma lipid levels, we used haplotype trend regression analysis (25). After adjusting for the other potential confounding factors, the plasma lipid levels were regressed on composite haplotype frequencies. This method is insensitive to departure from HWE (25, 26).

Estimation of additive genetic variance

Assuming random mating within populations, the additive genetic variance associated with each SNP can be estimated as $Va = 2pq[p(X_{11}-X_{12})+q(X_{12}-X_{22})]^2$ (27). The frequency of the major allele is p ("1" allele), whereas q is the frequency of the minor allele ("2" allele). X represents estimated means for the homozygotes (X_{11} or X_{22}) and heterozygotes (X_{12}) for TG and HDL cholesterol after adjusting for confounding effects. The percentage of total phenotypic variance associated with each SNP (heritability) was calculated by dividing the additive genetic variance by the total phenotypic variance of TG and HDL cholesterol, which were calculated within each ethnic group.

Assuming additivity of allelic effect and the assumptions indicated above, one can estimate the additive genetic variance associated with haplotypes at *APOA5* locus as $Va = 2\Sigma\ p_i\ a_i^2$ (28), where p_i is the frequency of haplotype i and a_i is the estimated TG effect associated with haplotype i.

RESULTS

Diverse allele distribution and strong linkage disequilibria within *APOA5* regions in three ethnic groups of Singapore

We have examined allele frequency distributions for five SNPs within the *APOA5* locus in Chinese, Malay, and Asian-Indian subjects. The locations of these SNPs are depicted in Fig. 1, and their minor allele frequencies are presented in **Table 2**. The genotype frequencies for Malay and Asian-Indian men and women and Chinese men were as expected from HWE. However, we detected a slight departure from HWE for Chinese women, which remained after careful assessment of the data.

Allele frequencies for the −1131T>C, −3A>G, 56C>G, and 1259T>C SNPs (see Materials and Methods) were significantly different among the three ethnic groups, whereas the allelic differences for the IVS3+476G>A SNP were close to being statistically significant (P ranging from 0.09 to 0.07). Malays had significantly higher frequencies of the minor allele (designated as "2" vs. "1" for the major allele) for the −3A>G ($P < 0.0001$), and −1131T>C ($P = 0.0019$) SNPs when compared with Asian-Indians, and for 1259T>C SNP ($P = 0.0017$) when compared with Chinese. Conversely, Chinese had higher frequencies for the minor alleles at the −3A>G ($P < 0.0001$) and −1131T>C ($P = 0.0003$) SNPs when compared with those observed for Asian-Indians. The frequencies for the minor allele at the 56C>G SNP, also known as S19W (13, 14), was 0.001 for Chinese, 0.017 for Malays, and 0.031 for Asian-Indians. Because of its low frequency, our population size did not provide enough statistical power to carry out association studies with this SNP and it was not included in later single-marker association study analysis.

The pair-wise LDs between all five SNPs among three ethnic groups, expressed as D′, are given in **Table 3**. With the exception of the 56C>G SNP, all other SNPs were in significant LD. These data demonstrate that, except for the 56C>G SNP, LD within the *APOA5* region in our three ethnic groups is similar to that in Whites (13, 14). The observed deviation of LD with 56C>G from those in previous studies (13, 14) is most likely due to its lower frequencies of the minor allele in the Singaporean population.

APOA5 variants are strongly associated with lipid levels in the Singaporean population

Table 4 presents means and SEM for the most relevant anthropometrical and biochemical variables separately for each ethnic group. **Tables 5–8** show mean values and SEM for BMI, plasma lipids, and lipoproteins separated by ge-

TABLE 2. Minor allele frequencies and SEM of five SNPs at *APOA5* in three major ethnic groups in a Singaporean population

	n	−1131T>C	−3A>G	56C>G	IVS3+476G>A	1259T>C
Chinese	2,711	0.294 (0.007)	0.260 (0.007)	0.001 (0.001)	0.193 (0.006)	0.184 (0.006)
Malay	707	0.296 (0.013)	0.289 (0.014)	0.017 (0.004)	0.210 (0.012)	0.227 (0.012)
Asian-Indian	553	0.232 (0.013)	0.183 (0.013)	0.031 (0.001)	0.160 (0.012)	0.183 (0.013)

Allele frequencies were estimated by direct counting. Differences in frequencies between ethnic groups were compared using the χ^2 test. n = the sample size. SEM shown in parentheses.

TABLE 3. Pair-wise linkage disequilibrium expressed as D′ and *P* for χ^2 test in three ethnic groups

	−3A>G		56C>G		IVS3+476G>A		1259T>C	
	D′	*P*	D′	*P*	D′	*P*	D′	*P*
Chinese								
−1131T>C	0.502	<0.00001	0.137	0.287	0.467	<0.00001	0.499	<0.00001
−3A>G			0.223	0.0818	0.507	<0.00001	0.524	<0.00001
56C>G					0.556	0.6982	0.536	0.0567
IVS3+476G>A							0.458	<0.00001
Malay								
−1131T>C	0.492	<0.00001	0.271	0.636	0.452	<0.00001	0.454	<0.00001
−3A>G			0.199	0.459	0.513	<0.00001	0.508	<0.00001
56C>G					0.384	0.773	0.600	0.425
IVS3+476G>A							0.430	<0.00001
Asian-Indian								
−1131T>C	0.484	<0.00001	0.547	0.049	0.482	<0.00001	0.389	<0.00001
−3A>G			0.709	0.044	0.481	<0.00001	0.408	<0.00001
56C>G					0.446	0.076	0.583	0.064
IVS3+476G>A							0.446	<0.00001

The pair-wise linkage disequilibrium (LD) among SNPs was estimated as D′ (23); and LD was tested for significance (*P*) by χ^2 test within each ethnic group using Genetic Data Analysis (22).

notype and ethnic group. We did not detect any significant interaction between sex and genotype for any of the variables analyzed. Therefore, we performed all subsequent analyses by combining data for men and women and results are presented for both genders combined.

We found significant associations between the minor alleles at each one of the four common SNPs and increased plasma TG concentrations. These associations were consistent across the three ethnic groups. Conversely, there were inverse associations between the minor alleles and HDL cholesterol concentrations reaching statistical significance in Chinese and Malays, but not in Asian-Indians. As TG levels are highly and negatively correlated with HDL cholesterol, TG levels were then adjusted when determining the genotypic association with HDL cholesterol. SNP −1131T>C and −3A>G were still significantly associated with HDL cholesterol in Chinese, and −1131T>C and IVS3+476G>A were still significantly associated with HDL cholesterol in Malays after adjusting TG levels in the model, whereas other SNPs did not show significant association after adjusting for TG levels (data not shown). In addition, we observed a significant association between the presence of the minor alleles for the −3A>G, 1259T>C, and −1131T>C SNPs and LDL cholesterol concentrations in the Chinese subgroup.

Overall, our data suggest that the presence of the minor alleles is significantly associated with higher TG across ethnic groups, with higher TG and lower HDL cholesterol in Chinese and Malays. **Figure 2** shows the percentage increase of TG means between the homozygote of the TG-raising minor allele and the homozygote of the normal allele for all four SNPs on each ethnic group. This indicates that −1131T>C and −3A>G show the strongest association in the Chinese, −3A>G in Malays, and −3A>G and 1259T>C in the Asian-Indians. Moreover, in Chinese, we found that the minor alleles of −1131T>C, −3A>G, and 1259T>C were significantly associated with higher LDL cholesterol concentrations. Finally, the minor allele of the IVS3+476G>A SNP was significantly associated with lower systolic blood pressure (SBP), a paradoxical finding in the context of the atherogenic nature of the other associations with lipid variables.

Identifying haplotypes influencing lipid concentrations in three ethnic groups

To gain further understanding of the genetic basis for the observed associations between the *APOA5* locus and plasma lipid concentrations, we constructed haplotypes with all five SNPs using the EM algorithm (24). Subjects missing any genotype were excluded from these analyses. The sample sizes for haplotype analyses were 1,361, 388, and 349 for Chinese, Malays, and Asian-Indians, respectively. Haplotypes with frequencies larger than 0.01 are presented in **Fig. 3**; the 5 SNPs of each haplotype were arranged in the order of from 5′ to 3′ (see Fig. 1). The most common was the 11111 haplotype that was present in 71% of Chinese, 67% of Malays, and 72% of Asian-Indian haplotypes. This was followed by the 22122 haplotype, present with frequencies of 16% in Chinese, 19% in Malays, and 15% in Asian-Indians. These two haplotypes (11111 and 22122) account for 87%, 86%, and 87% of Chinese, Malay, and Asian-Indian haplotypes, respectively. In addition,

TABLE 4. Lipid profiles (mean and SEM) and clinical characteristics (mean and SEM) for both genders combined in three ethnic groups

	Chinese	Malay	Asian-Indian	*P*
Total cholesterol (mmol/l)	5.43 (0.02)	5.81 (0.04)	5.50 (0.05)	<0.0001
Triglyceride (mmol/l)	1.41 (0.02)	1.71 (0.05)	1.65 (0.05)	<0.0001
HDL cholesterol (mmol/l)	1.42 (0.01)	1.30 (0.01)	1.15 (0.02)	<0.0001
LDL cholesterol (mmol/l)	3.39 (0.02)	3.85 (0.04)	3.69 (0.04)	<0.0001
SBP (mmHg)	120.8 (0.3)	124.4 (0.6)	121.23 (0.7)	<0.0001
Body mass index	22.7 (0.1)	25.6 (0.2)	25.1 (0.2)	<0.0001

SBP, systolic blood pressure.

TABLE 5. Lipid profiles (mean and SEM) and clinical characteristics (mean and SEM) according to −1131T>C genotypes for three ethnic groups

	Chinese				Malay				Asian-Indian			
Genotype	11	12	22	P^a	11	12	22	P	11	12	22	P
n[b]	1,235	942	239		334	261	65		298	184	26	
Total cholesterol	5.39[c]	5.49	5.47	0.0331	5.57	5.66	5.80	0.2043	5.54	5.66	5.72	0.3721
(mmol/l)	0.04	0.04	0.07		0.09	0.10	0.15		0.08	0.09	0.21	
Triglyceride	1.34	1.50	1.90	<0.0001	1.56	1.78	2.64	<0.0001	1.67	1.87	2.38	<0.0001
(mmol/l)	0.05	0.05	0.08		0.11	0.12	0.17		0.07	0.08	0.18	
HDL cholesterol	1.42	1.36	1.28	<0.0001	1.28	1.25	1.07	<0.0001	1.14	1.14	1.06	0.368
(mmol/l)	0.01	0.01	0.02		0.03	0.03	0.04		0.02	0.03	0.06	
LDL cholesterol	3.37	3.47	3.50	0.0066	3.72	3.78	3.74	0.7406	3.75	3.83	3.86	0.567
(mmol/l)	0.03	0.04	0.06		0.08	0.09	0.14		0.07	0.09	0.19	
SBP	121.3	120.5	120.2	0.2629	125.9	124.1	122.7	0.1823	120.4	120.6	124.3	0.4111
(mmHg)	0.5	0.6	0.9		1.4	1.5	2.3		1.1	1.3	3.0	
BMI	22.9	22.8	22.8	0.8	25.4	25.1	24.2	0.1512	24.7	24.2	24.5	0.4518
	0.1	0.2	0.2		0.4	0.5	0.7		0.3	0.4	0.9	

BMI, body mass index.
[a] P calculated by ANCOVA, using General Linear models, and adjusted for age, sex, BMI, smoking status, alcohol use, and exercise.
[b] n = sample size.
[c] All means calculated by ANCOVA using General Linear models, and adjusted for age, sex, BMI, smoking status, alcohol use, and exercise.

a unique haplotype, 11211, representing the minor allele of SNP 56C>G and being independent of all other TG-raising minor alleles, was present at low frequencies (0.014 and 0.033) in Malays and Asian-Indians and was extremely rare in Chinese.

Using haplotype trend regression analysis (25), we analyzed the overall associations between haplotypes and plasma TG concentrations for each ethnic group after adjusting for BMI, sex, smoking status, exercise, and alcohol drinking. Only those haplotypes with frequencies greater than 1% were included in these analyses. Because of the multiple comparisons involved, we adjusted the threshold of significance to a $P \leqslant 0.01$. Our analysis shows that the haplotypes comprising the five SNPs at the *APOA5* locus were significantly associated with TG levels (adjusted $P = 2.98 \times 10^{-8}$, 1.72×10^{-6}, and 2.57×10^{-5} for Chinese, Malays, and Asian-Indians, respectively) in all three ethnic groups. Furthermore, we estimated the TG effect associated with each haplotype after log10-transformation, and normalized these effects relative to the effect of the 11111 haplotype. The TG effect associated with each haplotype and its significance levels are shown in Fig. 3. The common 22122 haplotype was significantly associated with higher TG levels among all ethnic groups. In addition, we observed similar associations with a less-frequent haplotype, 22111, which were shared among all three ethnic groups, and with the 22121 haplotype, with a significant effect on TG concentration observed only among Chinese and Malays. These results (Fig. 3) indicate that haplotypes carrying the minor allele of the −3A>G SNP have major effects on TG levels in Chinese and Malays, whereas it is less obvious which variant is the most prominent affecting TG in Asian-Indians.

Our haplotype analyses revealed a marginal association of 5-SNP haplotypes with lower HDL cholesterol levels that nearly reached statistical significance in Chinese (adjusted $P = 0.013$) and Malays (adjusted $P = 0.023$), but not in Asian-Indians (data not shown).

TABLE 6. Lipid profiles (mean and SEM) and clinical characteristics (mean and SEM) according to −3A>G genotypes for three ethnic groups

	Chinese				Malay				Asian-Indian			
Genotype	11	12	22	P^a	11	12	22	P	11	12	22	P
n[b]	1,133	715	167		275	202	52		293	131	15	
Total cholesterol	5.40[c]	5.46	5.52	0.1552	5.53	5.64	5.92	0.0347	5.56	5.56	5.57	0.9965
(mmol/l)	0.04	0.05	0.08		0.10	0.11	0.17		0.08	0.11	0.26	
Triglyceride	1.39	1.50	1.99	<0.0001	1.59	1.68	2.94	<0.0001	1.67	1.88	2.56	<0.0001
(mmol/l)	0.05	0.06	0.10		0.13	0.14	0.20		0.07	0.09	0.23	
HDL cholesterol	1.42	1.36	1.27	<0.0001	1.28	1.26	1.09	<0.0001	1.15	1.14	0.99	0.0913
(mmol/l)	0.01	0.02	0.03		0.03	0.03	0.05		0.02	0.03	0.07	
LDL cholesterol	3.37	3.46	3.50	0.0205	3.67	3.76	3.75	0.5408	3.76	3.70	3.71	0.8468
(mmol/l)	0.04	0.04	0.07		0.09	0.10	0.15		0.07	0.10	0.24	
SBP	121.7	120.8	120.6	0.2742	125.5	123.5	121.7	0.1716	121.3	119.2	124.3	0.2582
(mmHg)	0.6	0.6	1.1		1.6	1.7	2.5		1.1	1.5	3.7	
BMI	23.0	22.8	22.8	0.5673	25.1	24.7	25.0	0.5905	24.7	24.1	25.6	0.3319
	0.2	0.2	0.3		0.5	0.5	0.8		0.4	0.5	1.2	

[a] P calculated by ANCOVA, using General Linear models, and adjusted for age, sex, BMI, smoking status, alcohol use, and exercise.
[b] n = sample size.
[c] All means calculated by ANCOVA, using General Linear models, and adjusted for age, sex, BMI, smoking status, alcohol use, and exercise.

TABLE 7. Lipid profiles (mean and SEM) and clinical characteristics (mean and SEM) according to IVS3+476G>A genotypes for three ethnic groups

	Chinese				Malay				Asian-Indian			
Genotype	11	12	22	P^a	11	12	22	P	11	12	22	P
n[b]	1,381	618	94		355	186	26		325	119	14	
Total cholesterol	5.45[c]	5.51	5.42	0.4458	5.51	5.72	5.44	0.0681	5.60	5.60	5.40	0.7786
(mmol/l)	0.04	0.05	0.10		0.10	0.12	0.22		0.08	0.11	0.27	
Triglyceride	1.43	1.61	1.85	<0.0001	1.56	1.84	2.17	0.0032	1.74	2.01	2.23	0.0043
(mmol/l)	0.04	0.05	0.11		0.04	0.05	0.11		0.12	0.14	0.27	
HDL cholesterol	1.40	1.35	1.30	0.0003	1.25	1.24	1.01	0.0004	1.14	1.15	1.05	0.4345
(mmol/l)	0.01	0.02	0.03		0.03	0.03	0.06		0.02	0.03	0.07	
LDL cholesterol	3.42	3.49	3.43	0.2256	3.67	3.79	3.69	0.3901	3.77	3.72	3.56	0.6403
(mmol/l)	0.03	0.04	0.09		0.09	0.11	0.20		0.07	0.10	0.25	
SBP	122.0	120.3	117.0	0.0002	125.8	123.6	125.7	0.2924	121.4	120.3	121.5	0.7875
(mmHg)	0.5	0.7	1.5		1.4	1.7	3.3		1.1	1.6	3.8	
BMI	22.9	22.7	22.5	0.2641	25.1	24.9	25.3	0.7924	24.5	24.0	24.8	0.6506
	0.1	0.2	0.4		0.5	0.5	1.0		0.3	0.5	1.2	

[a] P calculated by ANCOVA, using General Linear models, and adjusted for age, sex, BMI, smoking status, alcohol use, and exercise.
[b] n = sample size.
[c] All means calculated by ANCOVA, using General Linear models, and adjusted for age, sex, BMI, smoking status, alcohol use, and exercise.

DISCUSSION

We have demonstrated that common allelic variants at the *APOA5* locus are significantly and consistently associated with plasma TG concentrations in a representative sample of the Singaporean population, regardless of gender and ethnic group. The presence of the minor alleles at each of the SNPs examined was associated with increased TG concentrations. Moreover, we have shown that the potential risk associated with the minor alleles is compounded in Chinese and Malays with significantly lower HDL cholesterol levels and in Chinese with the additional risk brought by increased LDL cholesterol concentrations. The potential impact of these associations over the observed ethnic-specific CVD risk should be the result of both the strength of the LD within the *APOA5* region and the allele frequency in each ethnic group. However, the variants significantly associated with TG levels in Asian-Indians were not significantly associated with lower HDL cholesterol nor increased LDL cholesterol levels. This may be due to the lower number of Asian-Indians in our population and the subsequent loss of statistical power. Alternatively, it could be due to the presence of ethnic-specific confounders. In this regard, it should be noted that in Singapore, diabetes is more common among Asian-Indians than between the other two major ethnic groups. To examine this possibility, we reanalyzed the data for the three ethnic groups after including diabetes status in our model. The statistical significances were not modified by this inclusion, suggesting that diabetic status had little influence on the relationship between *APOA5* variants and lower HDL cholesterol or increased LDL cholesterol levels in our population. Another possibility is that the smaller effect of *APOA5* on HDL cholesterol and LDL cholesterol in the Asian-Indian population is attributable to other ethnic-specific differences in genetic background.

To capitalize on the additional information provided by the analyses of multiple SNPs at a single locus, we estimated haplotype frequencies comprising all five SNPs using the EM algorithm (24). The magnitude of the effect of each of these haplotypes on plasma TG levels was calculated using haplotype trend regression analysis (25). Simi-

TABLE 8. Lipid profiles (mean and SEM) and clinical characteristics (mean and SEM) according to 1259T>C genotypes for three ethnic groups

	Chinese				Malay				Asian-Indian			
Genotype	11	12	22	P^a	11	12	22	P	11	12	22	P
n[b]	1,423	583	94		341	199	30		306	133	17	
Total cholesterol	5.42[c]	5.43	5.58	0.2705	5.49	5.75	5.69	0.0187	5.60	5.69	5.57	0.6669
(mmol/l)	0.04	0.05	0.10		0.09	0.11	0.20		0.08	0.11	0.25	
Triglyceride	1.40	1.60	1.86	<0.0001	1.59	1.90	2.33	0.0002	1.71	2.03	2.61	<0.0001
(mmol/l)	0.04	0.05	0.11		0.12	0.13	0.25		0.07	0.10	0.22	
HDL cholesterol	1.40	1.34	1.32	<0.0001	1.26	1.25	1.09	0.008	1.15	1.14	1.09	0.7133
(mmol/l)	0.01	0.02	0.03		0.03	0.03	0.06		0.02	0.03	0.07	
LDL cholesterol	3.39	3.43	3.59	0.0501	3.65	3.81	3.85	0.1232	3.78	3.85	3.66	0.654
(mmol/l)	0.03	0.04	0.09		0.09	0.10	0.18		0.08	0.10	0.23	
SBP	121.5	120.9	118.4	0.0779	125.7	123.4	122.6	0.1984	121.1	120.3	126.4	0.2522
(mmHg)	0.5	0.7	1.4		1.5	1.6	3.1		1.1	1.5	3.5	
BMI	22.8	22.9	22.8	0.905	25.4	24.9	25.5	0.508	24.3	24.3	26.5	0.1505
	0.1	0.2	0.4		0.4	0.5	1.0		0.4	0.5	1.1	

[a] P calculated by ANCOVA, using General Linear models, and adjusted for age, sex, BMI, smoking status, alcohol use, and exercise.
[b] n = sample size.
[c] All means calculated by ANCOVA, using General Linear models, and adjusted for age, sex, BMI, smoking status, alcohol use, and exercise.

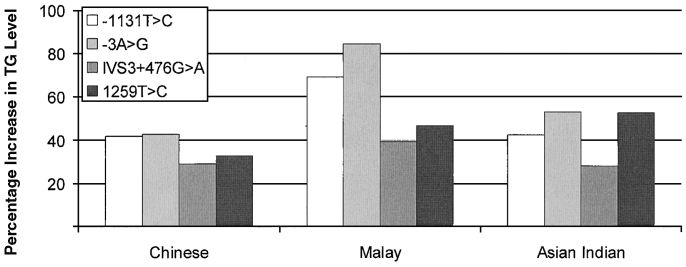

Fig. 2. Percentage increase in TG levels between the homozygote of the TG-raising allele and the homozygote of the normal allele of each SNP among three ethnic groups. The percentage was calculated as the difference between the TG means of two homozygotes (i.e., "11" and "22") divided by the TG mean of the "11" genotype and 0.01 (for the percentage).

lar to results obtained using individual SNPs, significant associations between haplotypes and TG levels were demonstrated. Our data show that the three ethnic groups share two common haplotypes (i.e., 22122 and 22111) associated with elevated TG levels. We also identified TG-raising haplotypes that were ethnic specific (see Fig. 3). Pennacchio et al. (13), using the same five SNPs, were able to identify three major haplotypes at the *APOA5* locus representing 95% of the haplotypes found in Caucasians. By comparison, the Singaporean population has a more diverse composition of haplotypes at this locus. In addition, this study showed that unlike previous reports (13, 14), the 11211 haplotype representing an independent TG-raising variant, 56C>G (S19W), was present at low frequencies in Malays and Asian-Indians and was extremely rare in Chinese.

The association between individual SNPs and HDL cholesterol concentrations was also observed for the haplotype analyses, but it was only marginally significant for the Chinese and Malay subgroup. As suggested above for the SNP analyses, this may be due to the loss of statistical power brought on by the reduction of sample size in these two populations during haplotype building.

In this study, Malays and Asian-Indians had higher TG levels (1.71 mmol/l and 1.65 mmol/l, respectively) compared with Chinese (1.41 mmol/l). After adjusting for sex, smoking status, exercise, and alcohol use, the TG levels of Malays and Asian-Indians were still significantly higher than those of Chinese, whereas those of Malays and Asian-Indians were similar (data not shown). We calculated the genetic variance for TG and HDL cholesterol attributable to individual SNPs (see **Table 9**). The values ranged from 0.97% to 3.31% for TG and 0.53% to 1.99% for HDL cholesterol. Variant −1131T>C had the largest effect on genetic variance in TG levels and HDL cholesterol in Chinese (2.43% and 1.45%) and Malays (2.88% and 1.99%), whereas 1259T>C contributed to the largest genetic variance in TG levels in Asian-Indians (3.31%). To estimate the combined effect of polymorphisms within the *APOA5* locus, we estimated additive genetic variance associated with the calculated haplotypes (see Table 9).

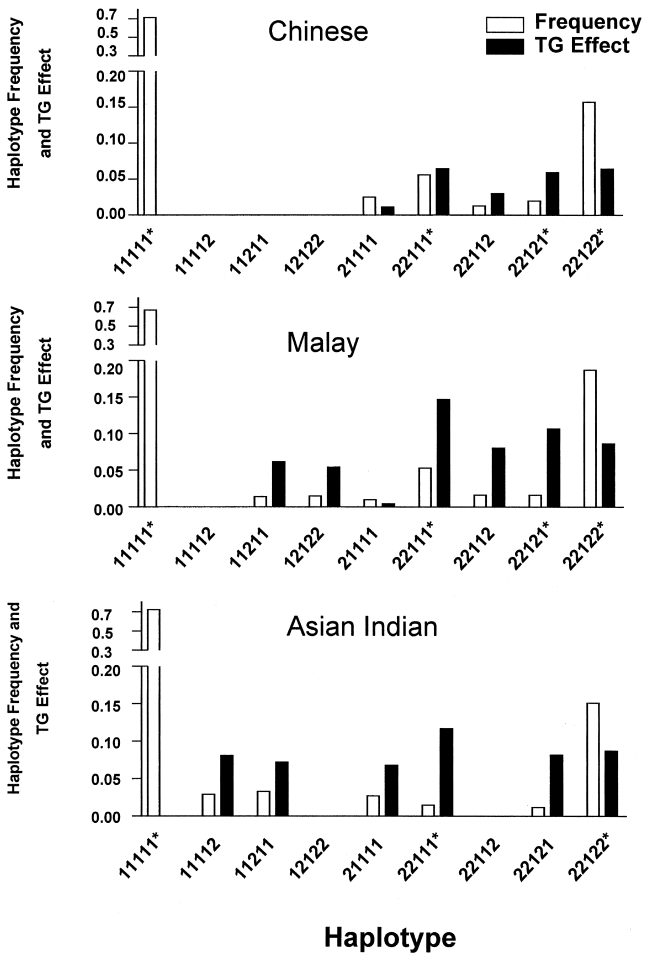

Fig. 3. Haplotype frequency and TG effect. The five SNPs in the haplotypes were arranged in the order of 5′ to 3′ (see Fig. 1), i.e., −1131T>C−3A>G 56C>G, IVS3+476G>A 1259T>C. Haplotype frequencies and associated TG effects were estimated using the Expectation-Maximization algorithm (24) and haplotype trend regression analysis (25, 26) with HelixTree software after TG concentration was log10 transformed. Thus, represented TG effects were log10 transformed and normalized to the effect of "11111" haplotype. Haplotypes marked with an asterisk indicate a significant effect ($P < 0.01$) on TG concentration.

TABLE 9. Estimation of additive genetic variance associated with APOA5 variants expressed as percentage of the total phenotypic variance (heritability)

	Chinese		Malay		Asian-Indian	
	TG	HDL	TG	HDL	TG	HDL
			%			
−1131T>C	2.43	1.45	2.88	1.99	2.51	
−3A>G	2.06	1.22	2.71	1.38	2.50	
IVS3+476G>A	0.97	0.53	1.11	0.77	1.60	
1259T>C	1.19	0.63	1.88	0.62	3.31	
Haplotype	2.73		6.86		5.16	

TG, triglyceride.

Genetic variability at the *APOA5* locus explained 6.86% and 5.16% of the TG variance in Malays and Asian-Indians, respectively, and lower, but still significant variances in Chinese (2.73%). These data suggest that the *APOA5* locus could play a significant role in the ethnic differences observed for plasma TG concentrations.

While all four common polymorphisms at the *APOA5* locus are strongly associated with TG levels in all ethnic populations, the possibility that any one of them represents a functional variant is very small; therefore, the most plausible explanation is that they are in strong LD with one or more functional variants that are directly affecting TG levels. Nevertheless, it is of great interest to determine which SNP shows the strongest association. The increase in TG means for homozygotes for the minor allele as compared with those homozygous for the normal allele for each of the four SNPs (see Fig. 2) indicates that −1131T>C and −3A>G show strongest association in the Chinese, −3A>G in Malays, and −3A>G and 1259T>C in Asian-Indians. The difference in the increased percentage of the TG levels for the same SNP between races suggests that *1*) LD between markers and unknown functional variants is not homogenous across the three ethnic groups, which is also reflected by the pair-wise LD among SNPs within this region (Table 3); and *2*) the effects of the TG-raising allele are influenced by ethnic differences in genetic background. Combined with results of haplotype analysis (Fig. 3) and the estimation of genetic variance associated with each SNP (Table 9), there is some support for the notion that −1131T>C and −3A>G show the strongest association with TG levels across the three ethnic groups, although 1259T>C seems to be equally prominent in Asian-Indians. The −1131T>C, which has been previously reported as SNP3, and its association with TG has demonstrated the same effect in Caucasian (12), African-American, Hispanic (14), and Japanese (15, 16) and Chinese (17) populations. This SNP is localized within the promoter region of *APOA5*, and it has the potential to affect gene expression. On the basis of the functional analysis of the *APOA5* promoter region in human hepatocytes, Vu-Dac et al. (29) and Prieur, Coste, and Rodriguez (30) identified a peroxisome proliferator response element (PPRE) ~328 bp from −1131T>C, and about 787 bp from −3A>G in *APOA5* promoter. In addition, the authors demonstrated that fibrates dramatically enhance *APOA5* expression of human hepatocytes through peroxisome proliferator-activated receptor α (*PPARα*) as a crucial regulator, and the PPRE is required to confer the function of *PPARα* activator. All these observations suggest that −1131T>C and −3A>G represent the variants that show strongest association with TG levels.

Another polymorphism within the *APOA5* locus, the 56C>G (or S19W), was shown by others to be associated with increased TG concentrations (13, 14), and this effect was independent of that demonstrated for the −1131T>C polymorphism. The allele frequencies ranged from 0.06 to 0.08 for Caucasian (13, 14) and Africans to 0.15 in Hispanics (14). In our population, the minor allele frequency was much lower than in other ethnic groups, ranging from 0.01 to 0.03, preventing us from establishing its association with TG concentrations. Therefore, this variant does not appear to be a major player affecting plasma TG levels in Asian populations.

A novel finding of our study relates to the statistically significant ethnic-dependent association between the −3A>G, 1259T>C, and −1131T>C SNPs and LDL cholesterol that we observed in Chinese. This was not observed in previous studies, including a wide range of other ethnic groups, suggesting some additional gene-gene interaction occurring in Chinese. Another interesting, but puzzling, observation also applying to the Chinese subgroup was the association between the minor allele of IVS3+476G>A SNP and reduced SBP. Taking into consideration the association of this SNP with high TG, high LDL cholesterol, and low HDL cholesterol, we would expect to find also a deleterious effect on SBP; however, the opposite association was observed. This single observation in Singaporean Chinese needs further confirmation in other Chinese populations.

The *APOA5* gene is distal to the *APOA1/C3/A4* cluster, the four genes being tightly linked within a 60 kb region on the long arm of chromosome 11. It is possible that genetic variants within this cluster could be in strong LD with each other. This study showed that the frequency distribution of the 56C>G SNP in Singapore was different from those observed in White populations, but little is known about the LD within this cluster in these three ethnic groups. Therefore, the influence of the *APOA5* locus on plasma lipid levels could be potentially confounded by the other neighboring loci. Further studies should focus on the construction of haplotypes with variants from other loci within this cluster and determine overall haplotype association with plasma levels.

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