ORIGINAL ARTICLE

Different effects of apolipoprotein A5 SNPs and haplotypes on triglyceride concentration in three ethnic origins

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Several polymorphisms in the *ApoA5* gene emerged as important candidate genes in triglyceride metabolism. The aim of this study was to determine the associations between ApoA5 polymorphisms, plasma triglyceride concentrations and the presence of cardiovascular disease (CVD) in three ethnic origins. Genotypes for 15 single nucleotide polymorphisms (SNPs) were determined in 659 older adults (mean age 71 ± 7 years) who immigrated to Israel or whose ancestors originated from East Europe (Ashkenazi), North Africa, Asia (Sephardic) or Yemen (Yemenite). The minor alleles of the four common SNPs (rs662799, rs651821, rs2072560 and rs2266788) are associated with an increase of 27–38% in triglyceride concentration among Ashkenazi and Yemenite Jews compared with the major alleles, but not among those of Sephardic origin. Conversely, among the Sephardic group, the presence of the minor allele in SNP rs3135506 compared with the major allele was associated with an increase of 34% in triglyceride concentration. The four SNPs were in significant linkage disequilibrium (D′=0.96–0.99), resulting in three haplotypes H1, H2 and H3, representing 98–99% of the population. Haplotype H2 was significantly associated with triglyceride concentration among Ashkenazi and Yemenite but not among Sephardic Jews. Conversely, haplotype H3 was associated with triglyceride concentration in Sephardic but not in Ashkenazi and Yemenite Jews. Ashkenazi carriers of H2 haplotype had a CVD odds ratio of 2.19 (95% CI: 1.05–4.58) compared with H1 (the most frequent), after adjustment for all other risk factors. These results suggest that different SNPs in ApoA5 polymorphisms may be associated with triglyceride concentration and CVD in each of these ethnic origins.
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INTRODUCTION

The apolipoprotein A5 (*ApoA5*) gene is located at 27 kb distally to ApoA4 in the *ApoA1/C3/A4* gene cluster on human chromosome 11q23. The gene consists of four exons and codes for 369 amino-acid proteins, which are expressed in the liver only.[1–3] Genetic polymorphisms in the *ApoA5* gene have been associated with differences in human plasma triglyceride (TG) concentration, as well as with very low density lipoprotein cholesterol (VLDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations, in white populations.[1,4–7] They were found to affect postprandial lipemia,[8] and to increase the risk of cardiovascular disease (CVD).[5]

Five common single nucleotide polymorphisms (SNPs) within this locus (rs662799, rs651821, rs3135506, rs2072560 and rs2266788) have been identified in studies of different designs and in some race-specific populations associated with TG, VLDL-C and HDL-C.[5,9,10]

The frequencies of the minor alleles and haplotypes vary significantly among ethnic origins. Most studies show a 15–30% increase in

TG concentrations with the presence of the minor allele at these polymorphisms and there is no clear evidence that ethnic origin determines the magnitude of the above increase.[11] There are inconsistencies in reports regarding specific ethnic origins and their association with ApoA5 polymorphisms and serum TG concentration. Sample size or sampling errors may account for these inconsistencies, as well as unidentified genetic and/or environmental factors.

Israel is a country of immigrants, or their offspring, who came from all over the world during specific periods.[12–14] Ethnically, this population can be divided into three main ethnic origins: Yemenite (those coming from Yemen or Aden), Sephardic (those coming from North Africa, parts of Asia, Middle East and some Mediterranean countries) and Ashkenazi (those coming from Europe, Russia and America).[14,15] Yemenite are regarded as a separate ethnicity, and as highly homogeneous genetically, as they lived in Yemen for hundreds of years, were culturally isolated from their surroundings, and preserved their own traditions and lifestyle throughout the centuries.[16] Sephardic Jews

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were also isolated among themselves for hundreds of years and are a separate homogeneous ethnic group,[14] and so are Ashkenazi. These three ethnic origins had socioeconomic, sociocultural (tradition intrareligion and intracommunity marriage) and behavioral differences, and they also differ in various specific genetic disorders.[12–14]

Previous studies showed that mortality and morbidity rates from coronary heart disease (CHD) were markedly dissimilar among these three ethnic origins.[17–19] CHD mortality was highest in Ashkenazi and lowest in those of Sephardic ethnic background.[20,21] Mortality from cerebrovascular disease was also different, with Sephardic having higher rates than Ashkenazi. Yemenite had the lowest rates of CHD and cerebrovascular mortality.[20] These differences in mortality and morbidity reflect differences in constitutional or environmental risk factors for CVD. Furthermore, data on ethnic differences in CVD genetic risk factors are sparse. Therefore, it would be important to reveal the possible genetic differences between these ethnic origins, accounting for the different plasma TG concentrations as an independent risk factor for CVD.[22]

The primary purpose of this study was to evaluate the genotype and haplotype frequencies of the five common SNPs at the ApoA5 locus for association with the variability in plasma TG concentrations in each of the three major ethnic origins of the Israeli Jewish population. A secondary aim was to determine whether SNPs and haplotypes associated with differences in TG concentrations showed an effect on the risk of CVD.

SUBJECTS AND METHODS
Subjects and laboratory measurements
Study population. This cross-sectional study included 659 subjects of a 30-year cohort of the Jewish Israeli population, stratified according to sex, age and ethnic origin (Yemen, Sephardic and Ashkenazi).[17] Participants completed an interview and had their lipid levels measured and blood collected for DNA analysis, in addition to all other laboratory examinations during 1999–2004.

Out of 953 survivors of the original cohort, 82 individuals completed a telephone interview but not a laboratory examination, and of the 212 non-respondents (22%), 11.5% were not located, 7.4% refused to participate, 1.7% had communication problems and 1.4% were ill. Demographic characteristics were compared between respondents, those lost to follow-up, nonrespondents and those with no laboratory examination, to reveal that respondents were characterized by a somewhat better risk factor profile in 1980 when the follow-up began.

Interview and laboratory examinations. The surveyed individuals were asked about demographic variables (age, ethnic origin), lifestyle characteristics (smoking and physical activity habits), medical history and use of medications, during a personal interview performed by trained nurses in regional clinics. Three blood pressure measurements were taken during the interviews; resting electrocardiography (ECG) was performed and blood was drawn after 12 h of overnight fasting for lipids. Weight and height were measured with subjects wearing light clothing, without shoes, on a calibrated weight scale, and body mass index (BMI, in $kg\,m^{-2}$) was calculated. Prevalence of CVD was determined by a detailed standardized medical history questionnaire regarding previous admissions to hospital due to either acute myocardial infarction (MI), other heart disease, cerebrovascular accident (CVA) or a combination of the above, as well as ECG criteria of myocardial ischemia or of an old MI based on the revised Minnesota Code for resting ECG,[23] and the use of different kinds of medications.

All laboratory examinations were performed at the Institute of Biochemical Pathology laboratory in our medical center. Levels of total cholesterol (TC), HDL-C and TG were determined using an automated enzymatic technique (Boehringer, Mannheim, Germany), and standardized against reference materials supplied by the standardization program of the Center for Disease Control and Prevention. Creatinine concentration was measured in venous plasma using an Olympus (Dallas, TX, USA) AU2700 Analyzer, by a spectroscopic method. ApoA-I, ApoB-100, ApoC-II and LP(a) serum concentrations were measured by

immunoturbidimetric technique according to the US LRC protocol[24] in a Batch analyzer (Vitalab, Dieren, the Netherlands). Serum TC, HDL-C and TG were also examined in 1980 while the mean age was 51 ± 7 years.

The study received the approval of the local institutional review board at the Chaim Sheba Medical Center and of the central local institutional review board for genetic-related studies of the Ministry of Health, and each participant was asked to sign an informed consent form.

SNPs Genotyping
DNA was isolated from blood samples using a DNA blood PureGene kit (Gentra, Minneapolis, MN, USA) according to the manufacturer's protocol.

SNP genotyping was performed using PCR amplification of genomic DNA, a short extension reaction across the polymorphic site and mass spectrometry to detect allele-specific mass difference of the extension. Allele detection and genotyping were performed using the MassARRAY system from Sequenom (San Diego, CA, USA).[25]

Fifteen SNPs were genotyped, of which five SNPs that were either monomorphic or had minor allele frequencies of <0.05 (rs2542058, rs4938312, rs7120555, rs2075291 and rs2266789) were not considered further. Five other SNPs that exhibited low reliability for genotyping (rs619054, rs7103224, rs10750097, rs12287066 and rs3135507) across all three populations were not included in the genotyping of the entire sample. The analysis includes five SNPs that were reported previously on Caucasian populations and other ethnic groups.[1,4,5,7,10] Their description is as follows: rs662799 (−1131T>C) in the 5′ region and previously reported as SNP3; rs651821 (−3A>G) in the 5′ region and previously reported as a start codon or kozak; rs3135506 (c. 56C>G) in the third exon and previously reported as S19W or SNP5; rs2072560 (IVS3+476G>A) in intron 3 and previously reported as SNP 2 and rs2266788 (1259T>C) in exon 4 previously reported as SNP 1.

Statistical analysis
Allele frequencies were obtained by direct counting. Hardy–Weinberg equilibrium was assessed by the χ^2-test. Pairwise linkage disequilibrium (LD) between SNPs was calculated from the genotype data and is expressed as D′ and r^2. Haplotype frequencies within each ethnic origin were calculated using the Bayesian method of haplotype reconstruction.[26–28] Differences in allele and genotype frequencies were tested by the χ^2-test. An omnibus likelihood ratio test, which examines the differences in haplotype frequencies between three ethnic origins, and permutation-based hypothesis tests were performed. TG concentrations were logarithmically transformed before analysis to reduce skewness distributions. Analysis of variance and the χ^2-test were used to compare a number of phenotypes between the three ethnic origins. General linear model analysis was performed to identify association between SNPs, haplotypes and plasma TG concentrations adjusted for potential confounders. Variables considered to have an impact on plasma TG concentration were selected with respect to literature and biological plausibility (age, sex, BMI, smoking, physical activity and lipid-lowering medications). The common homozygote genotype of each SNP and the most frequent haplotype were used as reference. A mixed model was used to analyze the effects of each of the ApoA5 polymorphisms on the 1980 and 2000 measures of TG. For individual tests of association, rather than applying a correction for multiple testing at a global significance level of 5% ($P<0.05$), statistical significance was defined as less than 1% ($P<0.01$). However, because there were high correlations between the phenotypes and marker alleles were interdependent, there was no necessity to adjust for multiple comparisons.[5,29,30] In addition, type I errors are random and patterns in results that confirm previous reports should be given more weight than isolated results with a single low P-value.[5,29] Moreover, correction for multiple comparisons largely increases the likelihood of type II errors and important differences are considered nonsignificant.[5] Logistic regression analysis was used to estimate odds ratio (OR) and 95% confidence intervals (95% CIs) describing the association between SNPs, haplotypes and risk of CVD.

RESULTS
Clinical characteristics, serum lipoprotein and apolipoprotein concentrations and minor allele frequencies in the three ethnic origins are presented in Table 1. A significant difference between ethnic origins

Table 1 Demographic and clinical characteristics, serum lipoprotein and apolipoprotein concentration in three ethnic origins

	Yemenite (n=125)	Sephardic (n=298)	Ashkenazi (n=236)	P-value
Age (years)	69.5 (0.60)	70.2 (0.40)	71.8 (0.45)	<0.01
Males/Female (%)	54/46	52/48	52/48	0.94
Level by education (years)	7.4 (0.43)	9.7 (0.25)	12.6 (0.26)	<0.01
BMI (kg m^{-2})	26.9 (0.38)	29.0 (0.28)	28.7 (0.31)	<0.01
Current smoking (%)	17	12	7	<0.05
Physical activity (%)	40	52	61	<0.01
CVD (%)	22	28	26	0.41
Diabetes (%)	33	30	27	0.51
Serum lipoprotein and apolipoprotein concentration (mg per 100 ml)				
TC	209 (3.43)	202 (2.24)	199 (2.25)	0.07
TG	136 (7.05)	136 (3.73)	145 (5.66)	0.30
HDL-C	48.6 (1.29)	48.8 (0.80)	48.4 (0.91)	0.94
LDL-C	133 (2.82)	126 (1.87)	123 (1.85)	<0.01
ApoA-$_I$	138 (2.34)	135 (1.46)	137 (1.71)	0.58
ApoB-$_{100}$	104 (2.12)	102 (1.46)	98 (1.52)	0.09
ApoC-$_{II}$	3.97 (0.19)	3.89 (0.12)	3.97 (0.12)	0.87
LP$_{(a)}$	24.4 (2.15)	29.4 (1.68)	22.8 (1.21)	<0.01
ApoC-$_{II}$ to TG ratio	0.032 (0.001)	0.031 (0.001)	0.030 (0.001)	0.54
Minor allele frequencies (%)				
rs662799	11.4	9.4	10.3	0.66
rs651821	11.9	9.3	10.6	0.51
rs3135506	6.5	7.3	7.9	0.80
rs2072560	10.6	9.2	9.8	0.82
rs2266788	10.3	9.5	9.8	0.94

Abbreviations: *n*, sample size; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; LP$_{(a)}$, lipoprotein$_{(a)}$.
Value represent mean and s.e.m. in the parentheses in continuous variable, and percentage in categorical variables.

was observed for mean age, BMI, level of education, LDL-C and LP$_{(a)}$. In addition, intergroup differences were observed in current smoking and physical activity. No significant differences were observed in the prevalence of CVD, diabetes and mean of TC, TG, VLDL-C, HDL-C, ApoA-$_I$, ApoB-$_{100}$, ApoC-$_{II}$ and ApoC-$_{II}$ to TG ratio.

The genotype frequencies for Yemenite, Sephardic and Ashkenazi were as expected and did not significantly deviate from the Hardy–Weinberg equilibrium ($P > 0.05$). There were no significant differences in the allele frequencies for rs662799, rs651821, rs3135506, rs2072560 and rs2266788 SNPs among the three ethnic origins. The minor allele frequencies were in the range of 6.5% (rs3135506) to 11.9% (rs651821).

The pairwise LD expressed as D$'$ and r^2 between all five SNPs among the three ethnic origins is presented in Figure 1. Four SNPs (rs662799, rs651821, rs2072560 and rs2266788) throughout the ApoA5 locus show almost complete LD, with D$'$ ranging from 0.960 to 0.999 and r^2 from 0.846 to 0.961 in the three ethnic origins. However, the SNP rs3135506 is independent of the other four SNPs in the three ethnic origins, with r^2 ranging from 0.001 to 0.008 in the three ethnic origins.

Association between *ApoA5* gene SNPs and plasma TG concentration

Plasma TG concentrations and the independent contribution of ApoA5 polymorphisms among the three ethnic origins are presented in Table 2. SNP analysis of ApoA5 polymorphisms according to TG concentration and ethnic origins revealed significant associations between the minor alleles at four common SNPs and increased plasma TG concentration in Ashkenazi. In this ethnic origin, the difference

between homozygotes and heterozygotes of the TG-raising minor allele and homozygotes of the normal allele for all four SNPs ranged from 32 to 38% (44–52 mg per 100 ml). These SNPs explained 3.04–4.05% of the residual variance in TG concentration.

The association between TG concentration and ApoA5 polymorphisms in Yemenite was statistically significant in two of the four common SNPs (rs662799 and rs651821) and close to statistical significance in the other two SNPs (rs2072560 and rs2266788, *P*-value ranging from 0.083 to 0.075). The difference between homozygotes and heterozygotes of the TG-raising minor allele and homozygotes of the normal allele for all four SNPs ranged from 27 to 31% (34–38 mg per 100 ml). These SNPs explained 3.57–5.87% of the residual variance in TG concentration.

No statistically significant association was observed between the rs3135506 SNP in Yemenite and Ashkenazi Jews.

Opposite results were observed in the Sephardic subgroup, in which none of the four common SNPs was associated with TG concentration. The rs3135506 SNP was associated with TG concentration. The difference between homozygotes and heterozygotes of the TG-raising minor allele and homozygotes of the normal allele for this SNP was 34% (46 mg per 100 ml). This SNP explained 2.09% of the residual variance in TG concentration. There were no statistically significant associations between the minor allele and TC, LDL-C, HDL-C, ApoA-$_I$, ApoB-$_{100}$, ApoC-$_{II}$ and LP$_{(a)}$ concentrations across the three ethnic origins.

Although differential use of lipid-lowering medications among the three ethnic origins may be a source of bias, no significant differences were observed in TG plasma concentration between subjects with respect to the use of lipid-lowering medication. However, lower LDL-C concentrations were observed in Sephardic and Ashkenazi
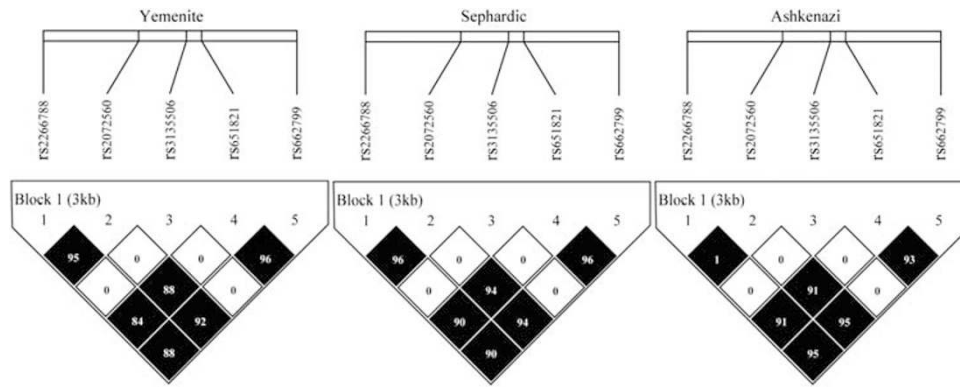
Figure 1 Pairwise linkage disequilibrium expressed as D′ (different color intensities) and r^2 (numbers) in five single nucleotide polymorphisms (SNPs) among Yemenite, Sephardic and Ashkenazi ethnic groups. Above is the relative physical distance between SNPs. The rs number of each SNP is shown at the bottom of the physical distance. The color gradient indicates the relative level of linkage disequilibrium (LD) from black (complete) to white (no LD).

Table 2 Plasma TG concentrations and the independent contribution of genotypes at ApoA5 polymorphisms among Yemenite, Sephardic and Ashkenazi ethnic groups

SNPs	Genotype	n	Yemenite	n	Sephardic	n	Ashkenazi
rs662799	TT	96	126 (12.68)	241	138 (5.42)	189	137 (7.24)
	TC+CC	27	163 (20.02)*	53	135 (13.45)	45	185 (17.21)**
	($R^2 \times 100$)		4.82**		0.00		3.43**
rs651821	AA	94	126 (12.77)	242	138 (5.41)	188	138 (7.51)
	AG+GG	28	164 (20.09)*	53	134 (13.42)	47	182 (16.35)**
	($R^2 \times 100$)		5.87**		0.01		3.04**
rs3135506	CC	108	135 (13.39)	253	135 (5.68)	200	139 (7.13)
	CG+GG	15	132 (25.25)	41	181 (11.84)**	35	165 (16.29)
	($R^2 \times 100$)		0.00		2.09**		0.76
rs2072560	GG	98	128 (12.45)	243	138 (5.41)	192	136 (7.15)
	GA+AA	25	162 (20.95)*	51	136 (13.49)	43	188 (17.25)**
	($R^2 \times 100$)		3.57**		0.02		4.05**
rs2266788	TT	97	129 (12.39)	241	138 (5.41)	192	136 (7.15)
	TC+CC	24	163 (20.83)	53	137 (13.48)	43	188 (17.25)**
	($R^2 \times 100$)		4.43**		0.14		4.05**

Abbreviations: *n*, sample size; SNP, single nucleotide polymorphism.
Value represent mean and s.e.m. in the parenthesis; all means adjusted for age, sex, BMI, smoking, physical activity and lipid-lowering medication; $R^2 \times 100$ is the proportion of the phenotypic variance explained by individual SNPs.
*$P < 0.05$; **$P < 0.01$.

who use lipid-lowering medication. To estimate the potential confounding effect caused by lipid-lowering medication treatments, we performed a sensitivity analysis. No significant differences were observed in the proportion of lipid-lowering medication users between homozygotes of the normal allele and in homozygotes and heterozygotes of the minor allele in five SNPs in the Yemenite and Sephardic. In contrast, in Ashkenazi, a higher proportion of lipid-lowering medication use was found in homozygotes and heterozygotes of the minor allele, compared with homozygotes of the normal allele in rs662799, rs651821, rs2072560 and rs2266788 SNPs (49 vs 33%, respectively, $P < 0.05$); homozygotes and heterozygotes of the minor allele had higher TG concentration compared with homozygotes of the normal allele. Therefore, among Ashkenazi, the difference in TG concentration between homozygotes and heterozygotes of the minor allele and homozygotes of the normal allele might be even larger before using lipid-lowering medication. In addition, these results repeat themselves when analyzed with respect to lipid concentrations collected in 1980. The percentage increase in TG concentration

according to ethnicity in 1980 and 2000 between the heterozygotes and homozygotes of the TG-raising allele and homozygotes of the normal allele in SNPs rs662799 and rs3135506 is shown in Figure 2. In 1980, the use of lipid-lowering medications was insignificant. The effects of the polymorphisms seem to be independent of the year of analysis and similar results are seen when analyzing these associations for TG concentrations in samples collected in 1980, when the patients were 20 years younger.

Association between haplotype *ApoA5* gene and plasma TG concentration

Haplotype frequency, plasma concentration TG effect and the independent contribution of each haplotype among the three ethnic origins are presented in Table 3. Haplotypes were inferred using all five SNPs. Three major haplotypes were identified: H1, H2 and H3. The most common was H1, present in 82% of Yemenite, 81% of Ashkenazi and 83% of the Sephardic Jews. The second most frequent haplotype was H2, present in 10% of Yemenite and Ashkenazi, and 9%
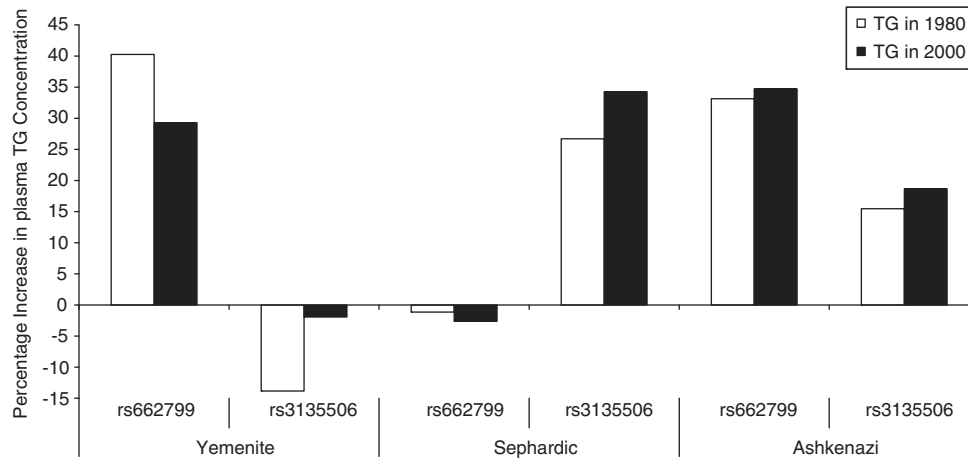
Figure 2 Percentage increase from baseline (1980) to follow-up (2000) in plasma triglyceride (TG) concentrations between the heterozygotes+homozygotes of the TG-raising allele and homozygotes of the major allele in single nucleotide polymorphisms (SNPs) rs662799 and rs3135506 among the three ethnic origins: Yemenite, Sephardic and Ashkenazi.

Table 3 Haplotype frequency estimates and significant levels of Yemenite, Sephardic and Ashkenazi ethnic groups comparison from permutation test, plasma TG concentrations and the independent contribution of each haplotypes

	Haplotype frequencies					Plasma TG concentrations ($R^2 \times 100$)		
Hap.	Yemenite	Sephardic	Ashkenazi	χ^2	P-value[a]	Yemenite	Sephardic	Ashkenazi
H1 (TACGT)	82.00	82.89	81.36	0.427	0.81	131 (10)	136 (4)	138 (5)
						1.97*	0.67*	2.66**
H2 (CGCAC)	10.00	8.89	9.75	0.352	0.84	166 (21)*	137 (13)	192 (17)**
						2.53**	0.05	2.40**
H3 (TAGGT)	6.40	7.21	7.84	0.509	0.78	132 (23)	181 (12)**	168 (16)
						0.01	1.03**	0.64
Log (ln) Likelihood				1.410[b]	0.97			

Abbreviation: Hap., Haplotype
$R^2 \times 100$ is the proportion of the phenotypic variance explained by individual Haplotypes.
*$P < 0.05$; **$P < 0.01$.
[a]P-values based on 1000 permutations.
[b]Likelihood ratio test statistical values for omnibus test.

of Sephardic Jews. The next most frequent haplotype H3, representing the minor allele of SNP rs3135506 and being independent of all other TG-raising minor alleles, was present in as low as 6% of Yemenite, 7% of Sephardic and 8% of Ashkenazi Jews. These three haplotypes (H1, H2 and H3) accounted for 98% of the haplotypes of Yemenite and Ashkenazi, and 99% of Sephardic. There were no significant differences in haplotype frequencies among the three ethnic origins.

Haplotype analysis showed that H2 was significantly associated with higher TG concentration in both Yemenite and Ashkenazi but not in Sephardic. Carriers of H2 showed TG levels higher by 21% (34 mg per 100 ml) and 28% (54 mg per 100 ml) compared with the most common haplotype (H1) in Yemenite and Ashkenazi, respectively. Conversely, H3 was associated with significantly higher TG concentration in Sephardic but not in Yemenite and Ashkenazi. Carriers of the H3 had TG concentration higher by 25% (44 mg per 100 ml) compared with the most common haplotype (H1) in Sephardic.

Association between *ApoA5* gene SNPs/haplotypes and CVD
The association between ApoA5 SNPs or haplotypes and risk of CVD is shown in Table 4. The distribution of SNPs or haplotypes was not

significantly different between the CVD and non-CVD groups in Yemenite and Sephardic. Neither SNPs nor haplotypes showed any statistically significant associations with risk of CVD before or after adjustment for other CVD risk factors in Yemenite and Sephardic Jews. However, Ashkenazi Jews had significantly different distributions in the CVD and non-CVD groups. Comparing Ashkenazi heterozygotes and homozygotes of the minor alleles of the rs662799 SNP to major alleles and carriers of haplotype H2 with the most common haplotype (H1) yielded odds ratio of 3.03 (95% CI: 1.48–6.23) and 2.67 (95% CI: 1.39–5.13), respectively, for CVD, after adjustment for age and sex. To further explore whether this risk effect could be explained by the higher TG concentration associated with this SNP or haplotype, we estimated the risk after further adjustment for TG, and similar results were obtained (OR=2.77, 95% CI: 1.33–5.78 and OR=2.37, 95% CI: 1.22–4.61, for rs662799 SNP and haplotype H2, respectively). To further minimize the effect of potentially confounding factors, the calculation was repeated after adjustment for age, sex, TG, BMI, smoking, diabetes, SBP and LDL-C (OR=2.19, 95% CI: 1.05–4.58), but the risk estimate did not materially change.

Table 4 Logestic regression models for the association between ApoA5 haplotypes and risk of CVD

		CVD (%)	Non-CVD (%)	Model 1 OR (95% CI)[a]	Model 2 OR (95% CI)[b]	Model 3 OR (95% CI)[c]
SNPs						
Yemenite	rs662799	19.2	22.7	0.66 (0.21–2.05)	0.52 (0.16–1.71)	0.51 (0.20–1.26)
	rs3135506	11.5	12.4	1.02 (0.25–4.16)	1.07 (0.39–2.90)	1.60 (0.54–4.73)
Sephardic	rs662799	21.0	16.9	1.36 (0.70–2.64)	1.37 (0.71–2.67)	1.29 (0.78–2.14)
	rs3135506	13.6	14.1	0.94 (0.44–2.02)	0.86 (0.50–1.49)	0.96 (0.53–1.72)
Ashkenazi	rs662799	31.7	14.9	3.03 (1.48–6.23)**	2.77 (1.33–5.78)**	2.60 (1.47–4.60)*
	rs3135506	14.8	14.9	0.89 (0.38–2.09)	0.83 (0.45–1.52)	0.73 (0.37–1.42)
Haplotype						
Yemenite	H1	85.2	82.8	Ref.	Ref.	Ref.
	H2	9.3	10.4	0.78 (0.27–2.49)	0.64 (0.21–1.92)	0.69 (0.22–2.22)
	H3	5.6	6.8	0.90 (0.23–3.43)	0.97 (0.25–3.78)	1.39 (0.33–5.86)
Sephardic	H1	82.3	84.3	Ref.	Ref.	Ref.
	H2	10.4	8.5	1.29 (0.69–2.41)	1.28 (0.68–2.41)	1.24 (0.63–2.45)
	H3	7.3	7.3	0.99 (0.49-2.03)	0.93 (0.45–1.91)	1.02 (0.48-2.19)
Ashkenazi	H1	76.0	84.4	Ref.	Ref.	Ref.
	H2	16.5	7.5	2.67 (1.39–5.13)**	2.37 (1.22–4.61)**	2.19 (1.05–4.58)*
	H3	7.4	8.1	0.90 (0.40–2.04)	0.83 (0.36–1.88)	0.89 (0.38–2.08)

Abbreviations: OR, odds ratio; CI, confidence interval 95%; Ref., reference group; H1, TACGT; H2, CGCAC; H3, TAGGT.
*$P<0.05$; **$P<0.01$.
[a]Adjusted for age and sex.
[b]Adjusted for age, sex and TG.
[c]Adjusted for age, sex, TG, BMI, smoking, diabetes, SBP and LDL-C.

DISCUSSION

These results show that common allelic variants at the ApoA5 locus are significantly associated with plasma TG concentration in a sample of older adults. The presence of the minor alleles of the four common SNPs (rs662799, rs651821, rs2072560 and rs2266788) was significantly associated with higher TG plasma concentrations in two ethnic origins, namely, Yemenite and Ashkenazi, whereas in the Sephardic participants, only the presence of the rs3135506 SNP showed this association. The strengths of the study are that all phenotypes, especially lipid levels examined in 1980, were available and had less confounding factors, all assayed in the same laboratory under standardized conditions. This considerably enhances the ability of the study to detect modest effects associated with genotypes and haplotypes.

A novel finding of this study relates to the inverse association between the four SNPs and TG that was observed in the Yemenite and Ashkenazi but not in the Sephardic Jews subgroup. Conversely, the Sephardic group showed an association between the rs3135506 SNP and TG but the Yemenite and Ashkenazi patients did not. This was not observed in previous studies that included a wide range of other ethnic origins. The variable results among samples of Yemenite, Ashkenazi and Sephardic indicate that there may be additional unidentified genetic and/or environmental factors that are important modifiers of gene effect.

SNPs/haplotypes and plasma TG concentration

The presence of the minor alleles at each of the four common SNPs examined was associated with an increase of 27–38% in TG concentration among the Yemenite and Ashkenazi ethnic origins but not in the Sephardic one. The minor allele of the rs3135506 SNP was associated with an increase of 34% in TG concentration among the Sephardic. However, SNPs rs2072560 and rs2266788 were close to being significantly associated with TG concentration in Yemenite.

Those results from a relatively small number of subjects in this ethnic origin may underline the subsequent loss of statistical power. Nevertheless, a trend is clear, with the minor alleles showing higher values.

In contrast, in those of Sephardic origin, the lack of association between the allelic variants at the ApoA5 locus and TG concentration is unlikely to arise from the small sample size of this ethnic origin. It could be due to the presence of ethnicity-specific confounders, namely, genetic and environmental factors that may have a role in modifying the gene effect. A significant difference between ethnic origins was observed in habitual lifestyle (smoking, physical activity) and socioeconomic status (level of education). Another possibility is that the effect of ApoA5 on TG plasma levels is smaller in Sephardic individuals and may be attributed to differences of other ethnicity-specific genetic backgrounds. Support for this can be found in the haplotype analysis and in the independent contribution of SNPs and haplotypes to TG concentration.

The four common SNPs and their association with TG have shown the same effect in Caucasian,[4,7,31] African-American,[5,7] Hispanic,[5] Japanese[31,32] and Chinese[31,32] populations.

There is an LD within the ApoA5 region in the three ethnic origins, except for rs3135506 SNP, similar to that found in other Caucasian populations.[4,5,7,9] The minor allele of the rs3135506 SNP was found in a lower frequency of 6.5–7.9% in all three ethnic origins and those of the other four SNPs were found in higher frequencies of 9.2–11.9%. Reports of the relationship between the rs3135506 polymorphism and TG plasma concentrations have not been consistent, as some studies have observed an effect[4] whereas others have not.[33,34] Pervious studies reported that the allele frequency ranged from 6 to 8% for Caucasian and Africans,[1,4,7] from 4 to 8% in African Americans,[4,35] was 15% in Hispanics[4,7] and was very rare, that is, 1–3%, in Chinese and Japanese.[10,31,32] The frequencies of the other four SNPs ranged from 9 to 11% for Caucasians,[4,7] 13 to 16% for Hispanics,[4,36] 6 to 9% for

Percentage Increase in plasma TG Concentration was normalized to the effect of haplotype H1, TACGT (reference haplotype); H2, CGCAC; H3, TAGGT.
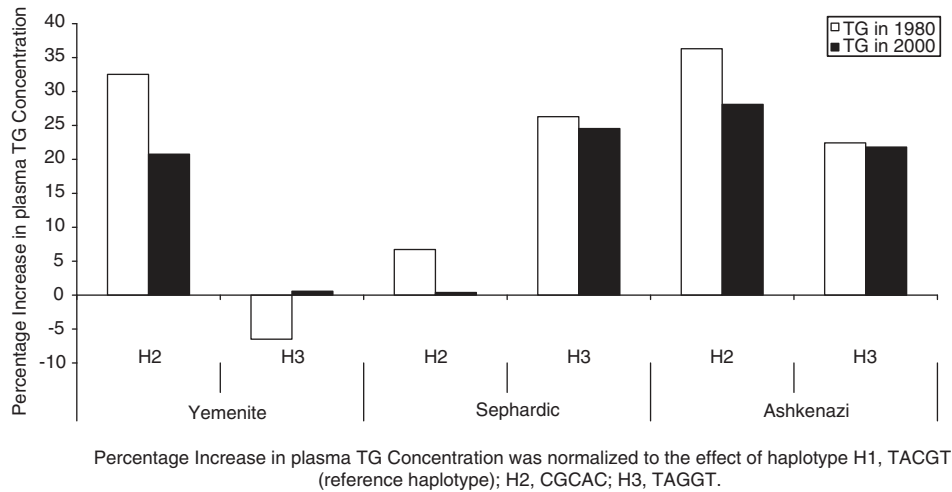
Figure 3 Percentage increase for haplotype and plasma concentration triglyceride (TG) effect in Yemenite, Sephardic and Ashkenazi ethnic groups. The five single nucleotide polymorphisms (SNPs) in the haplotype were arranged in the order of 5′–3′ as follows: rs662799, rs651821, rs3135506, rs2072560 and rs2266788.

African Americans[1,4] and are in common frequencies of 27 to 37% among East Asian populations.[10,31,36–38]

Although all four common polymorphisms at the ApoA5 locus were associated with plasma TG concentrations in Yemenite and Ashkenazi, the possibility that any one of them represents a functional variant is very small because; they are in a strong LD with one or more functional variants that directly affect the TG plasma concentration. Several other polymorphisms in the ApoC-III gene have been shown to be associated with plasma TG concentration in different studies,[39] the most prominent being SNP 1100C>T in axon 3. To assess the effect of this and other polymorphisms on plasma TG concentrations genotyped in this study population, we studied the primary polymorphisms in ApoC-III, SNPs −455T>C and −482C>T, which are found in the promoter. Neither of these polymorphisms showed any significant association with plasma TG concentrations. This finding supports the notion that the effect detected in the ApoA5 genotype and haplotypes is due to sequence variants in ApoA5 and not due to polymorphisms in the ApoC-III gene neighboring this gene in its location.

To explore fully the information provided by the SNP analysis, we carried out a haplotype analysis. The three ethnic origins share three common haplotypes (H1, H2 and H3) and identified TG-raising haplotypes with specific ethnicity (Figure 3). The H2 and H3 haplotypes are two independent haplotypes, with the former representing the four SNPs that are in strong LD and the latter representing the rs3135506 SNP. The frequencies of these two haplotypes are similar to the frequency found in other Caucasian populations.[4,7]

A number of mechanisms were proposed to explain the association between ApoA5 and TG metabolism. One mechanism suggested an inhibitory effect on VLDL production and secretion; another suggested the stimulation of lipoprotein lipase-mediated TG hydrolysis and thread, as well as acceleration of hepatic uptake of TG-rich lipoproteins and their remnants.[40,41]

SNPs/haplotypes and CVD risk
None of the single SNPs or haplotypes had a significant effect on CVD risk in Yemenite and Sephardic. The study had limited power to detect modest effect in Yemenite. However, in Ashkenazi, carriers of the minor allele of SNP rs662799 had higher risk (OR=3.03, 95% CI:

1.48–6.23) compared with carriers of major alleles, and carriers of H2 had higher risk (OR=2.67, 95% CI: 1.39–5.13) compared with carriers of the most common haplotype (H1). Although this could be due to the TG-raising effect associated with this SNP or haplotype, on the basis of statistical arguments, this seems unlikely.

The effect of ApoA5 SNPs and haplotypes on CVD risk has previously been reported.[5,42] A previous study in Caucasians reports that the minor allele frequency of SNP rs662799 was significantly higher among coronary artery disease patients than among controls (OR=1.98, 95% CI: 1.14–3.48).[43] Another study[44] found that homozygotes of the minor allele frequency of at least one of the SNPs rs662799 and/or rs3135506 were more prone to MI ($P<0.001$), but they failed to detect significantly different distributions of the ApoA5 genotype between MI patients and controls. In contrast, two prospective studies, which were branch investigations of the NPHS II study[40] and LOCAT study,[34] did not observe any positive association between SNP rs662799 and coronary artery disease. The mechanism between ApoA5 polymorphism and CVD risk effect is unclear. As the effect on risk seems unlikely to be through differences in plasma TG concentration, it raises the possibility that the SNPs might be influencing the quality of the protein ApoA5.

Limitations of the study
Ethnic origin arose primarily through geographical, social and cultural factors that are not stagnant and potentially fluid.[45] Only the country of origin of the father was used to determine ethnicity, with no information on maternal ethnic background. An interethnic marriage was characteristic of the three ethnic groups of the Jewish population along the centuries.[13] Nevertheless, this could obscure the differences between the three ethnic origins and may result in an underestimation of the effect of ApoA5 on TG concentration. To account for the heterogeneity of the Sephardic group, which included subjects from both Asia and North Africa, we performed a separate analysis for each of these two groups. The results repeated themselves with no association found between the four common SNPs and TG plasma concentrations among subjects from Asia or North Africa. Survival bias cannot be avoided in an association study of older adults, and prospective studies will be necessary to confirm the role of this allelic variation.

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