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APOA5 genetic variants are markers for classic hyperlipoproteinemia phenotypes and hypertriglyceridemia

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SUMMARY

Background Several known candidate gene variants are useful markers for diagnosing hyperlipoproteinemia. In an attempt to identify other useful variants, we evaluated the association of two common *APOA5* single-nucleotide polymorphisms across the range of classic hyperlipoproteinemia phenotypes.

Methods We assessed plasma lipoprotein profiles and *APOA5* S19W and –1131T>C genotypes in 678 adults from a single tertiary referral lipid clinic and in 373 normolipidemic controls matched for age and sex, all of European ancestry.

Results We observed significant stepwise relationships between *APOA5* minor allele carrier frequencies and plasma triglyceride quartiles. The odds ratios for hyperlipoproteinemia types 2B, 3, 4 and 5 in *APOA5* S19W carriers were 3.11 (95% CI 1.63–5.95), 4.76 (2.25–10.1), 2.89 (1.17–7.18) and 6.16 (3.66–10.3), respectively. For *APOA5* –1131T>C carriers, the odds ratios for these hyperlipoproteinemia subtypes were 2.23 (95% CI 1.21–4.08), 3.18 (1.55–6.52), 3.95 (1.85–8.45) and 4.24 (2.64–6.81), respectively. The overall odds ratio for the presence of either allele in lipid clinic patients was 2.58 (95% CI 1.89–3.52).

Conclusions A high proportion of patients with four classic hyperlipoproteinemia phenotypes are carriers of either the *APOA5* S19W or –1131T>C variant or both. These two variants are robust genetic biomarkers of a range of clinical hyperlipoproteinemia phenotypes linked by hypertriglyceridemia.

KEYWORDS ***APOA5*, complex trait, DNA variant, hyperlipoproteinemia, triglyceride**

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INTRODUCTION

Hypertriglyceridemia is a common biochemical phenotype that is observed in up to 5% of adults. A plasma triglyceride concentration above 1.7 mmol/l is a defining component of the metabolic syndrome[1] and is associated with several comorbidities, including increased risk of cardiovascular disease[2] and pancreatitis.[3,4] Factors, such as an imbalance between caloric intake and expenditure, excessive alcohol intake, diabetes, and use of certain medications, are associated with hypertriglyceridemia; however, genetic factors are also important.[5,6]

Complex traits, such as plasma triglyceride levels, usually do not follow Mendelian patterns of inheritance because multiple genes contribute to the phenotypes.[7] In very rare instances, however, relevant mutations in single genes have been found. Children with hyperchylomicronemia (WHO *International Statistical Classification of Diseases*, 10th edition, classification E78.3; Fredrickson hyperlipoproteinemia type 1; *Mendelian Inheritance in Man*, 12th edition, classification 238600) often have homozygous mutations in *LPL* encoding lipoprotein lipase (LPL) or *APOC2* encoding apolipoprotein (apo) C-II, an LPL cofactor. Indeed, five of the six classic WHO or Fredrickson hyperlipoproteinemia phenotypes include varying degrees of elevated fasting plasma triglyceride in their definitions (Table 1). The exception is familial hypercholesterolemia (FH) (*International Statistical Classification of Diseases*, 10th edition, classification 78.0; hyperlipoproteinemia type 2A; *Mendelian Inheritance in Man*, 12th edition, classification 143890), which is most frequently caused by mutations in *LDLR* encoding the LDL receptor.[8] The genetic basis of hyperlipoproteinemia types 1 and 2A have, therefore, been identified. Most cases of hyperlipoproteinemia types 2B, 3, 4 and 5 are only partially characterized at the molecular genetic level, although it is known that type 3 requires homozygosity for the *APOE* E2 isoform as necessary for expression of the phenotype, but this factor is not sufficient alone.[2,9] Given

Table 1 Summary of classic hyperlipoproteinemia phenotypes.

WHO *ICD-10* code	Frederickson HLP phenotype	MIM number	Lipid levels	Lipoprotein levels	Genetic characteristics
E78.3	HLP type 1 Familial chylomicronemia	238600	↑TG	↑CM	Monogenic, autosomal recessive due to mutant *LPL* or *APOC2*. Primarily pediatric and young adults
E78.0	HLP type 2A Familial hypercholesterolemia	143890	↑TC	↑LDL	Monogenic, heterozygous form due to mutant *LDLR*, *APOB* or *PCSK9;* Homozygous form due to mutant *LDLR* or *ARH*
E78.4	HLP type 2B Combined hyperlipoproteinemia	144250	↑TC, ↑TG	↑VLDL, ↑LDL	Polygenic, multiple etiologies, some cases due to *USF1*, *APOB* or *LPL*
E78.2	HLP type 3 Dysbetalipoproteinemia	107741	↑TC, ↑TG	↑IDL	Polygenic, *APOE* E2/E2 homozygosity or mutant *APOE* necessary but not sufficient
E78.1	HLP type 4 Primary hypertriglyceridemia	144600 and 145750	↑TG	↑VLDL	Polygenic, no specific genes yet identified or replicated
E78.3	HLP type 5 Mixed hyperlipidemia	144650	↑TC, ↑TG	↑VLDL, ↑CM	Polygenic, mutant *LPL*, *APOC2* and *APOA5* in ~10% of cases

Abbreviations: *APOA5*, gene encoding apolipoprotein A-V; *APOB*, gene encoding apolipoprotein B; *APOC2*, gene encoding apolipoprotein C-II; *APOE*, gene encoding apolipoprotein E; *ARH*, gene encoding autosomal recessive hypercholesterolemia protein; CM, chylomicrons; HLP, hyperlipoproteinemia; ICD, *International Statistical Classification of Diseases, 10th Edition*; IDL, intermediate-density lipoprotein; *LDLR*, gene encoding LDL receptor; LPL, lipoprotein lipase; *LPL*, gene encoding LPL; MIM, *Mendelian Inheritance in Man*; *PCSK9*, gene encoding proprotein convertase subtilisin/kexin type 9; TC, total cholesterol; TG, triglyceride; *USF1*, gene encoding upstream stimulatory factor 1.

that hypertriglyceridemia is common, and that most classic hyperlipoproteinemia phenotypes defined in part by elevated triglyceride levels have no identified genetic basis, a DNA marker that is consistently associated with hypertriglyceridemia might help both in understanding pathogenesis and in diagnosis.

The apolipoprotein apo A-V is encoded by *APOA5* and is a key apolipoprotein whose physiological role has been demonstrated in studies showing elevated triglyceride levels in knockout mice,[10] elevated triglyceride levels in probands with rare loss-of-function mutations in *APOA5*[11,12] and associations of single-nucleotide polymorphism (SNP) genotypes or haplotypes with raised plasma triglyceride concentrations.[13,14] This apolipoprotein appears to play a focal part in the hydrolysis of triglyceride-rich lipoproteins by increasing the activity of LPL; rare mutations of apo A-V can cause familial chylomicronemia.[11,12,15] The SNPs S19W and –1131T>C in *APOA5* are relatively common (i.e. 5–10% allele frequency) in most populations; they are defining variants of unique *APOA5* haplotypes, are associated with *in vitro* dysfunction and are consistently associated with elevated plasma triglyceride levels.[12] We therefore evaluated *APOA5* S19W and –1131T>C genotypes for association with elevated plasma triglyceride levels and hyperlipoproteinemia phenotypes in patients from a lipid clinic.

METHODS

Participants

We studied 678 consecutive, unrelated white patients from a tertiary referral lipid clinic (age range 18–84 years). Patients underwent a complete medical examination and provided a history, and basic clinical, biochemical and demographic variables were recorded. Normolipidemic adult controls were selected from the European subgroup of the Study of Health Assessment and Risk in Ethnic groups (SHARE), a survey of cardiovascular risk factors in Canadian subpopulations,[16] and from population-based controls from Ontario who self-reported good health. By use of a validated sampling strategy,[16] households of white ethnicity within the same geographic area from which the patients were referred were randomly selected and mailed an introductory letter. Written contact was followed by up to 12 telephone calls inviting the individual with the earliest date of birth from the household to participate. All study participants provided informed consent for DNA analysis (University of Western Ontario Institutional Review Board protocol number #07920E).

Biochemical determinations and hyperlipoproteinemia phenotype

Plasma lipoprotein profiles were determined as previously described for lipid clinic patients[17] and for normal controls.[16] Lipid clinic patients were classified as having FH based on the presence

of characteristics that definitely met clinical and biochemical diagnostic criteria,[8] including demonstration of heterozygosity for a disease-causing mutation.[18,19] Participants were classified as having combined hyperlipidemia (hyperlipoproteinemia type 2B) on the basis of both cholesterol and triglyceride levels being higher than age-specific and sex-specific 95th and 90th percentile values, respectively, as well as cholesterol or triglyceride levels being higher than age-specific and sex-specific 90th percentile values in a blood relative.[20–23] Dysbetalipoproteinemia (hyperlipoproteinemia type 3) was diagnosed based on the presence of an *APOE* E2/E2 homozygous genotype, triglyceride levels exceeding age-specific and sex-specific 90th percentile values and/or a ratio of VLDL cholesterol to triglyceride of at least 0.30.[24,25] Participants were classified as having hypertriglyceridemia (hyperlipoproteinemia type 4) based on triglyceride concentrations exceeding age-specific and sex-specific 90th percentile values but not exceeding 10 mmol/l, with no documented chylomicronemia or other lipoprotein phenotypes. Participants were classified as having mixed hyperlipidemia (hyperlipoproteinemia type 5) based on fasting plasma triglyceride levels above 10 mmol/l documented on at least two occasions and documented chylomicronemia. We excluded children with fasting plasma triglyceride levels above 10 mmol/l with documented chylomicronemia and homozygous or compound heterozygous mutations in *LPL*.

DNA analysis

DNA samples were extracted as previously described.[17] *APOA5* S19W (SNP database number rs3135506) was genotyped with a validated TaqMan® genotyping assay (Assay ID C_25638153_10, TaqMan® SNP Genotyping Assays; Applied Biosystems, Foster City, CA). *APOA*5 –1131T>C (SNP database number rs662799) was genotyped with a custom-designed TaqMan® genotyping assay (TaqMan® SNP Custom Genotyping Assays; Applied Biosystems). A 600 nucleotide sequence (300 upstream and 300 downstream) from NT_033899.7 was submitted to RepeatMasker (www.repeatmasker.org) to detect repetitive sequences. The sequence was then submitted to BLASTN2.2.17[26] to confirm unique alignment to the National Center for Biotechnology Information human build 36 genome database.[27] After passing these criteria, the 600 nucleotide sequence was edited to place an "N" where any other SNPs or indels were present to allow the custom probe to be designed (Applied Biosystems). The custom probe uses the primers 5'-CCC TGC GAG TGG AGT TCA-3' and 5'-CTC TGA GCC CCA GGA ACT G-3'.

SNP genotyping was performed with an allelic discrimination assay that used the 7900HT Fast Real-Time PCR System (Applied Biosystems), and genotypes were read using automated software (SDS 2.3; Applied Biosystems).[28] Reactions were run in 5 μl volumes with an amplification protocol of 95 °C for 10 min, 50 cycles of 95 °C for 15 s and 60 °C for 1.5 min.

Statistical analysis

Analyses were performed using SAS version 9.1 (SAS® Institute, Cary, NC).[29] Differences between groups in discrete and quantitative traits were determined using χ^2 analysis and unpaired Student's *t*-tests, respectively. Odds ratios (ORs) were calculated with the case–control method in the FREQ procedure in SAS. Logarithmically transformed triglyceride levels were used for parametric analyses, but untransformed values are reported for the reader's benefit. Maximum likelihood linkage disequilibrium was estimated with PHASE (v2.0).[30] To assess the relationship of both SNPs with hyperlipoproteinemia and high triglyceride levels concurrently, participants who had more than one copy or no copies of either allele were further classified as having either or neither, respectively. To further explore the association of the *APOA5* variants with dyslipidemia, we stratified the 678 lipid clinic patients according to quartiles of fasting plasma triglyceride concentration and tested for between-quartile differences in the *APOA5* allele and carrier frequencies. Due to the relatively large number of comparisons, and in order to be conservative and minimize type I errors, the nominal level for significance was adjusted to $P<0.01$.

RESULTS

Clinical and biochemical characteristics

Baseline characteristics of the 678 lipid clinic patients and normolipidemic controls are shown in Table 2. Lipid clinic patients were older, had higher BMI values and plasma total cholesterol and triglyceride concentrations and had lower HDL cholesterol concentrations.

Pairwise linkage disequilibrium between *APOA5* variants

Pairwise linkage values for disequilibrium between *APOA5* S19W and –1131T>C, as estimated by the

Table 2 Clinical, biochemical and genetic characteristics of study participants according to lipoprotein phenotype.

Patients' characteristics	Hyperlipoproteinemia subtypes					Total patients (*n* = 678)	Total controls (*n* = 373)
	FH (HLP2A) (*n* = 88)	CHL (HLP2B) (*n* = 92)	DBL (HLP3) (*n* = 48)	HTG (HLP4) (*n* = 38)	MHL (HLP5) (*n* = 151)		
Demographic characteristics							
Sex (female) (%)	45.4	46.7	39.6	10.5	31.8	43.1	40.1
Mean (SD) age (years)	47.8 ± 13.0	56.6 ± 11.7	51.6 ± 12.0	59.5 ± 13.4	50.8 ± 12.7	54.7 ± 14.7	47.2 ± 15.2
Mean (SD) BMI (kg/m^2)	27.1 ± 3.2	29.1 ± 4.3	28.9 ± 3.1	31.2 ± 8.1	30.5 ± 4.8	28.7 ± 4.7	± 4.2
Mean (SD) plasma cholesterol (mmol/l)							
Total	8.9 ± 2.3	8.2 ± 1.4	9.3 ± 1.8	4.9 ± 0.8	12.0 ± 6.0	6.3 ± 2.1	± 0.84
HDL	1.0 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	0.8 ± 0.2	0.8 ± 0.4	1.2 ± 0.4	1.3 ± 0.3
Mean (SD) plasma triglyceride (mmol/l)	1.6 ± 0.5	4.7 ± 1.3	6.7 ± 2.8	4.9 ± 1.5	30.9 ± 25.2	3.8 ± 9.7	1.18 ± 0.41
Allelic variant *APOA5* S19W							
Genotype frequency (% in parentheses)							
S/S	77 (87.5)	74 (80.4)	35 (72.9)	31 (81.6)	102 (67.6)	550 (82.0)	346 (92.8)
S/W	10 (11.4)	17 (18.5)	12 (25.0)	6 (15.8)	42 (27.8)	122 (17.2)	23 (6.2)
W/W	1 (1.1)	1 (1.1%)	1 (2.1)	1 (2.6)	7 (4.6%)	6 (0.8)	4 (1.0)
Allele frequency (%)	7.3	10.3[b]	14.6[b]	9.2[b]	18.5[b]	9.4[b]	9.4[b]
Carrier frequency (%)	12.5	19.6[a]	27.1[b]	18.4[a]	32.5[b]	18.1[b]	7.2
Allelic variant *APOA5* –1131T>C							
Genotype frequency (% in parentheses)							
T/T	73 (83.0)	73 (79.3)	35 (72.9)	26 (68.4)	101 (66.9)	549 (80.2)	334 (89.5)
T/C	14 (16.0)	18 (19.6)	12 (25.0)	11 (29.0)	41 (27.1)	120 (18.6)	38 (10.2)
C/C	1 (1.0)	1 (1.1)	1 (2.1)	1 (2.6)	9 (6.0)	9 (1.2)	1 (0.3)
Allele frequency (%)	9.1	10.9[a]	14.6[a]	17.1[a]	19.5[b]	10.5[b]	5.4
Carrier frequency (%)	17.1	20.7[a]	27.1[b]	31.6[b]	33.1[b]	19.8[a]	10.5

[a]$P<0.01$. [b]$P<0.001$ compared with normolipidemic controls. Abbreviations: C/C, homozygous –1131C/C genotype; CHL, combined hyperlipoproteinemia; DBL, dysbetalipoproteinemia; FH, familial hypercholesterolemia; HLP, hyperlipoproteinemia; HTG, primary hypertriglyceridemia; MHL, mixed hyperlipidemia; S/S, homozygous S19/S19 genotype; S/W, heterozygous S19/W19 genotype; T/C, heterozygous –1131T/C genotype, T/T, homozygous –1131T/T genotype; W/W, homozygous W19/W19 genotype.

correlation coefficient *r* from PHASE, were 0.037 ($P=0.33$) and 0.017 ($P=0.83$) in lipid clinic patients and in normolipidemic controls, respectively. Estimates of pairwise linkage disequilibrium between *APOA5* S19W and –1131T>C in hyperlipoproteinemia subgroups were also nonsignificant, with *r* values ranging from 0.004 to 0.165 and *P* values ranging from 0.23 to 0.98. Thus, in these samples, the alleles of the two SNPs were not associated and SNP genotypes could be considered independent of each other.

Prevalence of *APOA5* variants in lipid clinic patients

APOA5 S19W and –1131T>C genotype frequencies did not deviate significantly from expectations of Hardy–Weinberg equilibrium. *APOA5* S19W in lipid clinic patients was found at a higher allele frequency compared with controls (9.4% versus 4.2%, $P<0.0001$; Table 2) and at a higher carrier frequency than in controls (18.1% versus 7.2%, $P<0.0001$; Table 2). *APOA5* –1131T>C was also found at higher allele frequency in lipid clinic patients than in controls (10.5% versus 5.4%, respectively, $P<0.0001$; Table 2) and at higher carrier frequency compared to controls (19.8% versus 10.4%, $P<0.0001$; Table 2). More carriers of either *APOA5* S19W or –1131T>C were in the lipid clinic group than in the control group (carrier frequency 35.2% versus 17.4%, $P<0.0001$; Table 2). The overall OR for carriers of *APOA5* S19W, –1131T>C or both among lipid

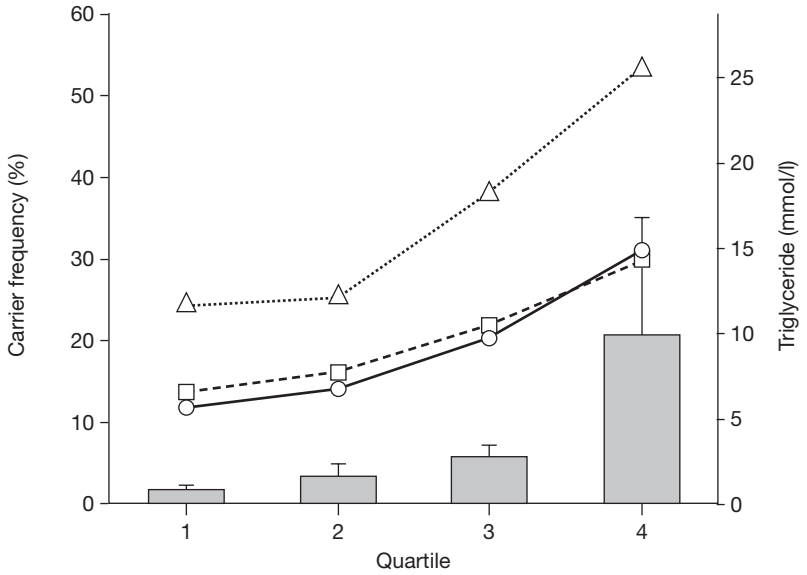

Figure 1 Frequency of carriers of *APOA5* variants according to quartile of plasma triglycerides. Histogram bars and error bars represent quartile mean (SD) of triglyceride concentration (right scale). Percentage of subjects in each quartile who were carriers of *APOA5* S19W (circles), –1131T>C (squares) or either (triangles), respectively, are shown (left scale).

clinic patients was 2.98 (95% CI 1.93–4.60), 2.01 (1.38–2.95) and 2.58 (1.89–3.52), respectively.

Association of *APOA5* variants with plasma triglycerides

We observed an increasing trend of *APOA5* S19W allele frequency across triglyceride quartiles 1, 2, 3 and 4 of 6.2%, 7.0%, 10.3% and 16.2%, respectively ($P<0.0001$ for trend). We also observed a stepwise relationship between *APOA5* S19W carrier frequency and triglyceride quartiles 1, 2, 3 and 4 of 11.8%, 13.4%, 20.0% and 30.5%, respectively ($P<0.0001$ for trend; Figure 1 and Table 3). We observed an increasing trend of *APOA5* –1131T>C allele frequency across triglyceride quartiles 1, 2, 3 and 4 of 6.5%, 7.0%, 11.5% and 15.9%, respectively ($P<0.0001$ for trend; Figure 1 and Table 3). We also observed a stepwise relationship between *APOA5* –1131T>C carrier frequency and triglyceride quartiles 1, 2, 3 and 4 of 12.4%, 14.0%, 21.2% and 28.8%, respectively ($P<0.0001$ for trend; Figure 1 and table 3). When the presence of at least one copy of S19W or –1131T>C was considered, we observed a stepwise relationship between carrier frequency and triglyceride quartile 1, 2, 3 and 4 of 23.7%, 26.7%, 38.8% and 52.1%, respectively ($P<0.0001$ for trend; Figure 1 and Table 3).

Association of *APOA5* S19W with classic hyperlipoproteinemia phenotypes

We classified 88, 92, 48, 38 and 151 patients with FH, combined hyperlipidemia, dysbetalipoproteinemia, hypertriglyceridemia and mixed hyperlipidemia (hyperlipoproteinemia types 2A, 2B, 3, 4 and 5), respectively. Patients with hyperlipoproteinemia type 1, defined as children or adolescents with LPL deficiency due to absent post-heparin LPL activity and/or mutated *LPL* or *APOC2* alleles, were excluded. Clinical, biochemical and genetic features of study participants are shown in Table 2. All phenotype classes, except for FH, had markedly elevated plasma triglyceride concentrations. Furthermore, *APOA5* allele and carrier frequencies were significantly higher than in controls for all hyperlipoproteinemia phenotypes, except for FH. Specifically, *APOA5* S19W carrier ORs for hyperlipoproteinemia types 2B, 3, 4 and 5 were 3.11 (95% CI 1.63–5.95), 4.76 (2.25–10.1), 2.89 (1.17–7.18) and 6.16 (3.66–10.3), respectively (Figure 2). *APOA5* –1131T>C carrier ORs for hyperlipoproteinemia types 2B, 3, 4 and 5 were 2.23 (1.21–4.08), 3.18 (1.55–6.52), 3.95 (1.85–8.45) and 4.24 (2.64–6.81), respectively (Figure 2). The presence of either allele was similarly associated with hyperlipoproteinemia types; the overall OR for the presence of either allele in lipid clinic patients was 2.58 (95% CI 1.89–3.52; Figure 2).

DISCUSSION

In this study, we found a higher frequency of carriers of *APOA5* variants in lipid clinic patients than in controls, a significant stepwise relationship between *APOA5* minor allele carrier frequencies and plasma triglyceride quartiles, and higher *APOA5* S19W and *APOA5* –1131T>C allele and carrier frequencies in lipid clinic patients than in controls for hyperlipoproteinemia types 2B, 3, 4 and 5. These findings indicate that *APOA5* variants S19W and –1131T>C are strongly and specifically associated with hypertriglyceridemia in lipid clinic patients and with several hyperlipoproteinemia phenotypes defined by elevated plasma triglyceride concentration. Of the phenotypes tested, only hyperlipoproteinemia type 2A (FH), which is not characterized by elevated triglyceride levels, was not associated with *APOA5* variant alleles. The findings confirm the importance of these *APOA5* variants and indicate that genotyping might be useful for studies of pathogenesis, response to intervention and diagnosis of hypertriglyceridemia and triglyceride-dependent hyperlipoproteinemia phenotypes.

Table 3 Clinical and biochemical attributes of Lipid Clinic patients divided into quartiles of plasma triglycerides.

Characteristic	Quartile of plasma triglycerides			
	1 (*n* = 169)	2 (*n* = 172)	3 (*n* = 170)	4 (*n* = 167)
Demographic characteristic				
Sex (female)	82	80	69	61
Plasma triglyceride (mmol/l)				
Range	0.1–1.2	1.2–2.1	2.1–3.8	3.8–222.0
Mean (SD)	0.8 ± 0.2	1.6 ± 0.3	2.8 ± 0.5	10.0 ± 18.1
Allelic variable *APOA5* S19W				
Genotype frequency (%)				
S/S	149 (88.2)	149 (86.6)	136 (80.0)	116 (69.5)
S/W	19 (11.2)	22 (12.8)	33 (19.4)	48 (28.7)
W/W	1 (0.6)	1 (0.6)	1 (0.6)	3 (1.8)
Allele frequency (%)	6.2	7.0	10.3	16.2[a]
Carrier frequency (%)	11.8	13.4	20.0	30.5[a]
Allelic variable *APOA5* –1131T>C				
Genotype frequency (%)				
T/T	148 (87.6)	148 (86.1)	134 (78.8)	119 (71.3)
T/C	20 (11.8)	24 (14.0)	33 (19.4)	43 (25.8)
C/C	1 (0.6)	0 (0)	3 (1.8)	5 (3.0)
Allele frequency (%)	6.5	7.0	11.5	15.9[a]
Carrier frequency (%)	12.4	14.0	21.2	28.8[a]
Either S19W or –1131T>C				
Allele frequency (%)	12.7	14.4	22.1	31.7[a]
Carrier frequency (%)	23.7	26.7	38.8	52.1[a]

[a]*P* <0.0001 for trend across quartiles. Abbreviations: Abbreviations: C/C, homozygous –1131C/C genotype; S/S, homozygous S19/S19 genotype; S/W, heterozygous S19/W19 genotype; T/C, heterozygous –1131T/C genotype, T/T, homozygous –1131T/T genotype; W/W, homozygous W19/W19 genotype.

Our data also confirm a key physiological role for apo A-V. Numerous genetic studies[12] have suggested associations between *APOA5* S19W and –1131T>C, and elevated plasma triglyceride levels, and both variants have been shown to be dysfunctional *in vitro* and *in vivo*;[12,15,31] however, the association between these variants in dyslipidemic patients across classic hyperlipoproteinemia phenotypes has not been quantified in terms of ORs. We found the associations with *APOA5* S19W and –1131T>C were consistent across the polygenic hyperlipoproteinemia type 2B, 3, 4 and 5 phenotypes. Between 30% and 60% of patients with these phenotypes were carriers of either the *APOA5* S19W or –1131T>C alleles (OR 2–7%). This finding is consistent with a complex pathogenesis as well as with a role for the dysfunctional *APOA5* S19W and –1131T>C alleles in this complexity.

APOA5 S19W and –1131T>C variants are the main tagging variant of haplotypes of APOA5*2 and APOA5*3, respectively; both the variants and the haplotypes have been shown to be associated with elevated plasma triglyceride levels.[12] *In vivo* functional studies have shown the dysfunction of the complete APOA5*2 haplotype; the evidence for dysfunction of the APOA5*3 haplotype is less compelling.[31] Although there are other SNPs defining additional rare haplotypes at *APOA5* (haplotype frequency <2%),[13] their functional relevance remains unclear. Our results indicate that S19W and –1131T>C have comparably strong associations with hypertriglyceridemia and hyperlipoproteinemia types 2B, 3, 4 and 5. Furthermore, given the absence of substantial linkage disequilibrium between *APOA5* S19W and –1131T>C, the genotypes function effectively as independent

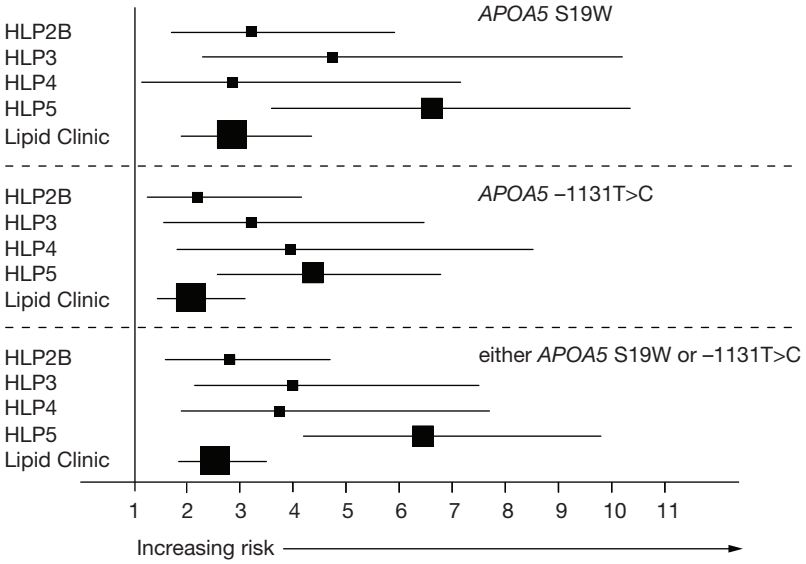

Figure 2 Forest plot of odds ratios for patients with *APOA5* S19W, –1131T>C or either for classic primary triglyceride-containing hyperlipoproteinemia phenotypes. Data are shown as odds ratios (*squares*) and 95% CI (*solid horizontal lines*). Abbreviation: HLP, hyperlipoproteinemia.

determinants of hypertriglyceridemia; therefore, the information that each provides is complementary and additive. Perhaps the most impressive example of this additivity is that more than half (57.6%) of hyperlipoproteinemia type 5 patients in this study carried one of the two *APOA5* variants, compared with only one in six normolipidemic controls (17.4%). Such strong and consistent associations, involving prevalent polymorphic alleles, are unusual in most complex metabolic traits such as lipoprotein metabolism. The associations with several seemingly unrelated hyperlipoproteinemia phenotypes suggest that the *APOA5* variants are common determinants linking them. Both the chromosome 11 apolipoprotein gene cluster, which harbors *APOA5*,[32] and the *APOA5* gene specifically, have been implicated in combined hyperlipidemia.[20] Our findings suggest that these linkages reflect a role for *APOA5* in a portion of combined hyperlipidemia probands; studies of cosegregation of *APOA5* variants and dyslipidemia phenotypes in extended combined hyperlipidemia kindreds would help to further clarify this relationship. In dysbetalipoproteinemia, *APOE* E2/E2 homozygosity provides the first genetic "hit" and several genes have been implicated in providing the second hit; this evidence for a second genetic hit is based on very few families in which mutations in *LDLR*,[33] *LIPC*,[34] *LPL*[35] or *APOB*[36] appear to cosegregate with the dysbetalipoproteinemia phenotype. *APOA5* S19W has also been associated with dysbetalipoproteinemia in *APOE* E2/E2 individuals.[24,37–39] Our findings suggest that *APOA5* S19W and –1131T>C could also provide a second hit in a substantial fraction of *APOE* E2/E2 individuals, resulting in expression of dysbetalipoproteinemia.

Hypertriglyceridemia is poorly understood at the molecular genetic level. Our findings suggest a possible role for *APOA5* S19W and –1131T>C in combination with other contributory factors in a substantial proportion of these patients. Patients with hypertriglyceridemia were older and had a somewhat higher BMI than controls, potentially representing nongenetic factors that could have played a part in the genetic associations we observed. Others have shown associations of *APOA5* variants with individual hyperlipoproteinemia types;[40,41] our study shows that increased *APOA5* variant frequency is a common feature across four of six hyperlipoproteinemia types. Finally, we have shown in another study that approximately 10% of patients with mixed hyperlipidemia (or severe hypertriglyceridemia) have heterozygous mutations in *LPL*, *APOC2* or *APOA5* (OR 52) and that another 35% of patients have the *APOA5* S19W variant (OR 5.5).[42] The present results, while limited by the fact that patients from a single site and single ethnicity were evaluated, nevertheless implicate both the *APOA5* S19W and –1131T>C variants in mixed hyperlipidemia patients. In summary, our findings indicate that *APOA5* S19W and –1131T>C are biomarkers for hypertriglyceridemia, regardless of associated biochemical abnormalities.

KEY POINTS

- Hyperlipoproteinemia types 2B, 3, 4 and 5 feature elevated plasma triglyceride concentration as part of their definition
- We found that *APOA5* variants S19W and –1131T>C are frequently present in and are strongly associated with hyperlipoproteinemia 2B, 3, 4 and 5 and also with hypertriglyceridemia in lipid clinic patients
- These two *APOA5* variants are robust genetic biomarkers of a range of complex hyperlipoproteinemia phenotypes, which had been considered distinct and disparate but which share hypertriglyceridemia as a defining feature
- These strong genetic associations might help predict susceptibility to hypertriglyceridemia or identify interindividual differences in response to interventions to lower plasma triglyceride

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Competing interests

The authors declared no competing interests.