APOA5 gene variants, lipoprotein particle distribution, and progression of coronary heart disease: results from the LOCAT study

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Abstract Animal and human studies support a role for apolipoprotein A-V (apoA-V) in triglyceride (TG) metabolism. We examined the relationship of APOA5 −1131T>C and S19W with lipid subfractions and progression of atherosclerosis in the Lopid Coronary Angiography Trial. Compared with −1131TT men (n = 242), carriers of the −1131C allele (n = 54) had significantly higher total TG (P = 0.03), reflected in significantly increased VLDL mass [higher VLDL-TG, VLDL-cholesterol, VLDL-protein, and surface lipids (all P < 0.05)]. Because apoB levels were unaffected by genotype, this suggests an increase in VLDL size and not number. Compared with 19SS men (n = 268), 19W carriers (n = 44) had higher intermediate density lipoprotein (IDL)-TG, IDL-cholesterol (P = 0.04), and IDL-surface components [free cholesterol (P = 0.005) and phospholipids (P = 0.017)] but not protein content, suggesting an increase in IDL lipid enrichment resulting in an increase in IDL size. 19W carriers also showed a trend toward increased progression of atherosclerosis, as measured by change in average diameter of segments (−0.46 ± 0.011 mm compared with −0.016 ± 0.006 mm) in 19SS men (P = 0.08). There was no effect of genotype on the response of these parameters to gemfibrozil treatment. These results shed new light on the role of APOA5 variants in TG metabolism and coronary heart disease risk.—Talmud, P. J., S. Martin, M-R. Taskinen, M. H. Frick, M. S. Nieminen, Y. A. Kesäniemi, A. Pasternack, S. E. Humphries, and M. Syvänne. APOA5 gene variants, lipoprotein particle distribution, and progression of coronary heart disease: results from the LOCAT study. J. Lipid Res. 2004. 45: 750–756.

Supplementary key words apolipoprotein A-V • lipid subfractions • very low density lipoprotein • intermediate density lipoprotein • progression of atherosclerosis • Lopid Coronary Angiography Trial • gemfibrozil • pharmacogenetics

Animal and human studies of the newly identified APOA5 gene are consistent in identifying APOA5 as a major determinant of plasma triglyceride (TG) levels (1). Thus, APOA5, forming a cluster with APOA4-C3-AI on chromosome 11q23, constitutes a locus involved in TG and HDL determination (2, 3). In both transgenic and knockout mouse models, it is clear that apolipoprotein A-V (apoA-V) is inversely associated with plasma TG levels. Transgenic mice overexpressing human APOA5 (1) or adenoviral vector-mediated transfer of APOA5 into mice (4) produce a 60–70% decrease in TG, whereas apoA5 knockout mice have 4-fold higher plasma TG than controls (1). The human APOA5 gene is fairly polymorphic, and in Caucasians, three common haplotypes have been identified: wild-type haplotype APOA5-1; APOA5-2, defined by rare alleles of −1131T>C, c.3A>G, IVS3+476G>T, and c.1259T>C; and APOA5-3, defined by a rare allele of S19W (5) and thus genotyping for −1131T>C or S19W, essentially acting as tagging single nucleotide polymorphisms (SNPs) (6). The −1131C variants and to a lesser extent 19W have been associated with increased TG in healthy Caucasians (1, 5, 7–9) and in African Americans (5) and with higher relative risk of developing dyslipidemias (10, 11). The rare allele of −1131T>C is more common in Japanese compared with Caucasians (0.34 versus 0.08, respectively) (12) and shows a strong association with TG levels, even in young school-age children. However, despite these consistent associations with TG levels, the function and role of apoA-V in TG metabolism remains unclear.

To examine the relationship between APOA5 gene variants and the metabolism of TG-rich particles in more...
detail, we determined the association between APOA5 \(-1131T>C\) and S19W with lipids, lipoproteins, and apolipoproteins as well as ultracentrifuged lipoprotein subfractions in the Finnish Lopid Coronary Angiography Trial (LOCAT) study of postcoronary bypass men (13). To date, variations in the peroxisome proliferator-activated receptor \(\alpha\) gene, PPARA L162V and intron 7G>C (14), the stromelysin gene, MMP3 5A/6A (15), the platelet endothelial cell adhesion molecule-1 gene, PECAM1 53G>A (16), and the \(\alpha\)-1-antitrypsin gene, AAT V213A and 11478G>A (17), have all shown significant associations with the progression of atherosclerosis in LOCAT, with AAT V213A showing a pharmacogenetic interaction with the response to gemfibrozil treatment. In contrast, variation in the hepatic lipase (HL) gene LIPC \(-514C>T\), although showing strong association with HL activity and lipid parameters, showed no association with atherosclerosis progression (18). Thus, the strong association between APOA5 and TG, and the establishment by meta-analysis of TG as an independent coronary heart disease (CHD) risk factor (19), suggest that APOA5 gene variants might be associated with atherogenesis. LOCAT, with angiographic measures of disease progression over 3.5 years of the study, afforded us the opportunity to examine this.

METHODS

Study

Patients. The entry criteria and baseline characteristics of the study population have been described (13). In essence, the LOCAT study entry criteria included men who had undergone coronary artery bypass grafting with HDL cholesterol \(\leq 1.1\) mmol/l. Ethical approval was granted for the study, and all patients provided written consent.

Quantitative coronary angiography was performed before randomization and after 2 years of double-blind, randomized, placebo-controlled gemfibrozil treatment (1,200 mg/day). A total of 372 (of 395) patients completed the study, with the number of dropouts being equally distributed between the gemfibrozil and placebo groups (20).

Quantitative coronary angiography. Two main angiographic outcome variables were used in the present analysis. Detailed description of the angiographic variables and the main angiographic results have been provided elsewhere (13, 20, 21). First, the average diameter of coronary segments (ADS) was used as a parameter to describe the extent of diffuse coronary artery disease (CAD). The difference in ADS between baseline and the last angiogram (DADS) provided an estimate of the on-trial effect (ADS). Second, the minimum lumen diameter (MLD) was used as a parameter to characterize the extent of focal CAD. Accordingly, the on-trial increase of focal coronary artery stenosis, or reduction in the diameter of stenotic segments, was best described by the MLD.

Lipoprotein and lipid determinations. Blood samples were obtained after an overnight fast at the randomization visit, 1 year after randomization, and 2 years after randomization. Lipoproteins [VLDL, \(d < 1.006\) g/ml; intermediate density lipoprotein (IDL), \(d = 1.006-1.019\) g/ml; LDL, \(d = 1.019-1.063\) g/ml; HDL, \(d = 1.063-1.210\) g/ml] were separated by preparative ultracentrifugation as described elsewhere (13). Triglyceride, cholesterol, free (nonesterified) cholesterol, and phospholipid were measured in unfractionated serum and in the lipoprotein fractions, and protein was measured in the fractions (13). Cholesterol ester concentrations were calculated as 1.67 \(\times\) (total minus free cholesterol [in milligrams per deciliter]) (22). Lipoprotein compositions were calculated as the percentages of triglyceride, esterified cholesterol, free cholesterol, phospholipid, and protein (all in milligrams per deciliter) of the sum of these constituents. Serum apoB concentrations were determined as described (13). Height was measured at baseline; weight, waist and hip circumferences, blood pressure, and heart rate were determined at each visit.

DNA genotyping

Genotyping for the APOA5 \(-1131T>C\) and S19W was carried out using the protocol reported previously (7).

Statistical analysis

Deviations from Hardy-Weinberg (H-W) equilibrium were assessed using a Chi-square test. The linkage disequilibrium (LD) coefficient between \(-1131T>C\) and S19W was estimated using \(\Delta\) (23).

Lipid, lipoprotein, and apolipoprotein values are expressed as means \(\pm\) SD. Differences in these variables according to genotype were analyzed by ANOVA. Those lipid or lipoprotein variables that showed a skewed distribution were log-transformed before the analysis. The influence of genotype on the progression of coronary atherosclerosis was analyzed by analysis of covariance. The per-patient changes in ADS and MLD from baseline to follow-up (DADS and DMLD, respectively) were entered into the models as dependent variables (14). The genotype \(-1131T>C\) or S19W was the independent variable. All analyses were adjusted for the randomized treatment group allocation, the baseline value of the dependent variable (ADS or MLD), and the time between baseline and follow-up angiograms by entering these variables as covariates. Values of DADS and DMLD in genotypes are expressed as adjusted least-squares means \(\pm\) SE.

RESULTS

The baseline characteristics for the sample are presented in Table 1. Genotype distributions for both variants were in H-W equilibrium, with rare allele frequencies for the \(-1131T>C\) and S19W being 0.095 (95% confidence interval, 0.07, 0.12) and 0.072 (0.05, 0.09), respectively, which were similar to those reported for other European countries (8). There was no statistically significant allelic association between these two sites (\(\Delta = -0.08, P = 0.77\)).
Association of −1131T>C with baseline lipid and lipoprotein subfractions

The association of the −1131T>C with baseline lipids in total serum and lipoprotein fractions separated by ultracentrifugation are presented in Tables 2 and 3, respectively. In agreement with previous studies, APOA5 −1131T>C displayed a significant effect on plasma TG levels, and carriers of the rare −1131C allele had statistically higher TG levels than common allele homozygotes (1.85 ± 1.00 mmol/l compared with 1.56 ± 0.67 mmol/l, respectively; \( P = 0.03 \)). The availability of lipoprotein subfractions meant that the distribution of lipid components within these fractions could be determined. Compared with −1131TT men, carriers of the rare −1131C allele consistently had higher lipid, surface components (with the exception of phospholipids), and protein constituents of VLDL. Thus, in −1131C carriers, VLDL-cholesterol was higher (\( P = 0.03 \)), as were cholesteryl esters in VLDL (\( P = 0.04 \)), VLDL protein content (\( P = 0.02 \)), and surface free cholesterol (\( P = 0.05 \)), compared with −1131TT men (Table 4), which was reflected in a significantly higher VLDL mass in −1131C carriers (\( P = 0.03 \)) (Table 3). ApoB levels, however, were no different in TT versus C carriers (102.74 ± 18.90 mg/dl compared with 102.67 ± 16.31 mg/dl, respectively; \( P = 0.98 \)) (Table 2).

There was no statistically significant difference in the change of any of these parameters from baseline to on-trial in the group that was randomized to gemfibrozil (data not shown); thus, APOA5 −1131T>C did not influence the response to gemfibrozil treatment.

Effect of APOA5 −1131T>C and progression of atherosclerosis

The effect of the −1131T>C genotype on the progression of atherosclerosis over the period of study was examined using two different parameters: DADS, a measure of diffuse atherosclerosis, and DMLD, a measure of focal progression of disease. The −1131T>C had no significant effect on the change of either parameter; TT, −0.025 ± 0.007 mm, versus CT+TT, −0.004 ± 0.014 mm (\( P = 0.16 \)) for DADS; and TT, −0.075 ± 0.01 mm, versus CT+TT, −0.044 ± 0.022 mm (\( P = 0.20 \)) for DMLD.

Association of S19W with baseline lipid and lipoprotein fractions

The S19W was not associated with differences in total TG or cholesterol levels (Table 2). However, there was an association of this variant with IDL parameters (Table 5). Compared with men homozygous for the 19S allele, carriers of the 19W allele had borderline higher TG content of IDL (\( P = 0.087 \)) but statistically significantly higher IDL-cholesterol (\( P = 0.04 \)), higher surface free cholesterol (\( P = 0.005 \)), and higher phospholipids (\( P = 0.017 \)). However, the protein content of IDL was not affected by genotype, suggesting that the lipid composition of the IDL particle was altered. Again, there was no effect of genotype on the response of these parameters to gemfibrozil treatment (data not shown).

Effect of APOA5 S19W on the progression of atherosclerosis

The S19W variant was associated with a borderline significant DADS over the course of the study. Men who carried the 19W allele had a trend toward greater progression of disease (−0.046 ± 0.016 mm compared with decrease in DADS of −0.016 ± 0.006 mm in 19S homozygotes; \( P = 0.082 \)). These results, together with results showing a similar trend in DMLD, are presented in Fig. 1.
TABLE 4. Ultracentrifuged lipoprotein subfractions according to APOA5 −1131T>C

<table>
<thead>
<tr>
<th>Variable</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1131TT</td>
<td>−1131C+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.02 ± 0.58</td>
<td>1.29 ± 0.83</td>
<td>0.12 ± 0.05</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>0.04</td>
<td>0.42</td>
<td>0.23 ± 0.13</td>
<td>0.24 ± 0.12</td>
</tr>
<tr>
<td>P</td>
<td>0.09</td>
<td>0.04</td>
<td>0.08 ± 0.04</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>0.61</td>
<td>0.82</td>
<td>1.14 ± 3.31</td>
<td>11.32 ± 3.48</td>
</tr>
<tr>
<td>Protein</td>
<td>0.02</td>
<td>0.86</td>
<td>0.95 ± 6.15</td>
<td>10.01 ± 5.31</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>0.04</td>
<td>0.28</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>0.27</td>
<td>0.13</td>
<td>0.73 ± 3.98</td>
<td>7.93 ± 3.32</td>
</tr>
</tbody>
</table>

DISCUSSION

The differential results of the two common APOA5 variants, −1131T>C and S19W, along with lipoprotein compositional data available in LOCAT, provide novel insights into the role of apoA-V in TG metabolism. As in previous studies, −1131T>C and S19W showed no allelic association (5, 7); therefore, they were acting independently. These two variants are known to define the two common APOA5 variant haplotypes, APOA5-2 and APOA5-3 (5).

−1131T>C and plasma TG levels

In LOCAT, the rare allele of the −1131T>C variant, in agreement with all previous studies, was associated with significantly higher plasma TG levels compared with common allele homozygotes. The lipid compositional data suggest that this reflects an increase in VLDL-TG, and the higher levels of VLDL components, with the exception of phospholipids and apoB, are corroborated by the significant increase in total VLDL mass. Although the protein content of VLDL was associated with genotype, the levels of apoB did not differ among genotypes, which might reflect a change in apoB and/or apoC-III content.

The potential effect of lower apoA-V resulting from −1131T>C, which acts as a marker for haplotype APOA5-2, would be an increase in plasma TG synthesis leading to an increase in VLDL mass, which could reflect either an increase in VLDL secretion or a decrease in catabolism or both. We can only speculate on these options. These results are in agreement with two previous studies that showed that the −1131C carriers displayed significantly higher VLDL mass measures (1, 9).

−1131T>C and LD with other variants in cluster

The −1131T>C is in complete positive LD with a −3G>A, and together these variants help define the APOA5-2 haplotype (5). As detailed elsewhere, both of these variants could potentially be functional (24). We have examined the association of these two variants with lipid parameters in a study of Japanese Americans, and this haplotype appears to be a major genetic determinant of LDL particle size and triglyceride levels.

In addition to the potential direct functional effects of these variants, we previously showed that there is overall strong LD across the APOA5-A4-C3 gene cluster, and to investigate whether the association of APOA5 variants with TG levels acted independently of APOC3 variants (also associated with differences in TG levels), we examined the association of nine SNP haplotypes across the APOA5-A4-C3 genes with lipid levels in a large prospective study of middle-aged men (7). Results from that study showed that the five haplotypes that were associated with the highest TG levels carried either APOA5 19W or APOC3 −482T,

TABLE 5. Ultracentrifuged lipoprotein subfractions according to APOA5 S19W

<table>
<thead>
<tr>
<th>Variable</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>19W+</td>
<td>S19W</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>1.06 ± 0.65</td>
<td>1.12 ± 0.57</td>
<td>0.12 ± 0.05</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>P</td>
<td>0.41</td>
<td>0.49 ± 0.32</td>
<td>0.54 ± 0.29</td>
<td>0.22 ± 0.12</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.29</td>
<td>0.30</td>
<td>0.26 ± 0.13</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>0.31</td>
<td>0.31</td>
<td>0.10 ± 0.06</td>
<td>0.97</td>
</tr>
<tr>
<td>Protein</td>
<td>16.37 ± 11.79</td>
<td>17.87 ± 10.88</td>
<td>9.40 ± 5.93</td>
<td>10.75 ± 6.33</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>26.45 ± 15.42</td>
<td>28.17 ± 14.42</td>
<td>7.28 ± 3.71</td>
<td>8.79 ± 4.62</td>
</tr>
</tbody>
</table>
demonstrating that these two variants independently determined TG levels. The APOA5 −1131T>C was in strong positive LD with APOC3 −482T>C, a functional change disrupting an insulin-responsive element (25), and although the haplotype associated with the second highest TG levels carried both the APOA5 −1131C and APOC3 −482C, it was not possible to identify the independence of these effects.

We examined the recently published in vitro structural analysis of apoA-V to gain some insight into the potential basis of these results (26, 27). ApoA-V was identified as a molecule with high lipid affinity (26), low elasticity, and slow binding kinetics, with a suggestion that it may retard the second step in VLDL assembly. We previously speculated that if apoA-V were to act by limiting the TG content of growing lipoprotein particles [e.g., if it were to influence microsomal triglyceride transfer protein (MTP) function], the resulting VLDL would be TG-enriched (7). Weinberg et al. (27) report that a Basic Local Alignment Search Tool search revealed 55% identity between the C-terminal domain of apoA-V and residues 239–260 of mouse MTP, suggesting that apoA-V might have MTP-like activity. They also showed that apoA-V overexpression in COS-1 cells led to poor apoA-V secretion, supporting the idea that the primary role of apoA-V is in intercellular hepatic metabolism (27). This would explain why plasma apoA-V levels are low (4). Thus, the predicted reduced APOA5 transcription or translation, attributable to the functional changes resulting from either −1131T>C or 3G>A or both, could explain the resulting increase in VLDL mass in −1131C/−3A men compared with −1131T/−3G men. Taking into account these structure/function studies of apoA-V (26, 27), it seems that the likely cause of VLDL mass increase is a decrease in apoA-V synthesis and an increase in TG incorporation into VLDL.

−1131T>C has little effect on disease progression

In Northwick Park Heart Study II (NPHSII), a prospective study of UK men, there was very little similarity in the ranking of the haplotypes by TG or CHD prevalence (28). Men who carried the haplotype defined by APOA5 −1131C/APOC3 −482T showed CHD prevalence well below that of men who carried the common haplotype defined by all nine common alleles (28), supporting the idea that the −1131C is not associated with increased CHD risk. This emphasizes that high TG levels alone are not a good discriminator of CHD risk (29).

S19W and increase in IDL number

Although we and others (1, 5, 7) have previously reported the association of the S19W with differences in plasma TG levels, this association has not been as consistent as the findings with −1131T>C. We reported that in the European Atherosclerosis Study II, a study of university students comparing those whose fathers had suffered premature myocardial infarction before the age of 55, called “cases,” with age-matched “controls” drawn from the same university environment, 19W was not associated with significantly higher plasma TG levels in either group, although there was significant heterogeneity of effect between groups (8). In the controls, 19W carriers had 2% lower TG levels than 19SS men. In LOCAT, the difference in total TG levels according to S19W was not statistically significant (P = 0.34) and represented only a 5% increase in TG. In NPHSII, TG levels in 19W carriers were 10% higher compared with SS men (P < 0.006) (P. J. Talmud and E. Hawe, unpublished observations), although when comparing rare homozygotes with common homozygotes, WW men had 52% higher TG than SS men (P < 0.003) (7). The lack of a significant association of 19W with TGs in LOCAT may reflect the considerably smaller sample size compared with NPHSII (297 versus 2,497, respectively). In LOCAT, men who carried the 19W allele had borderline statistically significantly higher TG in IDL (P = 0.087); however, other IDL constituents, such as cholesterol (P = 0.04) and particularly the surface lipids, namely free cholesterol (P = 0.005) and phospholipids (P = 0.017), were higher in 19W carriers than in 19S homozygotes. The protein content of IDL remained unaffected by the variant. Taken together, these results suggest that S19W is associated with an increase in IDL particle size, with primarily cholesterol enrichment. These cholesterol-enriched remnant particles could provide a good substrate for cholesteryl ester transfer protein, resulting in a depletion of cholesteryl ester in exchange for TG enrichment. It has been suggested that TG-rich IDL particles, after hydrolysis by HL, could lead to an increase in small dense proatherogenic LDL particles (30), which might explain the increased risk associated with APOA5 S19W.

As with the other APOA5 variants, the direct functional role of S19W has not been confirmed, although this amino acid change within the apoA-V signal sequence has the potential to be functional.

S19W and atherosclerosis progression

In contrast to the data from −1131T>C, the S19W variant was associated with differences in atherogenesis over time in LOCAT. Although only showing borderline effects, 19W carriers had increased progression of diffuse atherosclerosis, as suggested by the change in DADS (P =
0.082) but not focal disease (DMLD). Again, comparing these data with results from our previous study, NPHSII, the haplotype defined by the 19W on a background of common alleles was associated with the highest plasma TG levels, and men carrying this haplotype had the fourth highest prevalence of CHD, suggesting that this variant, unlike −1131T>C, promotes atherosclerosis by generating a proatherogenic particle.

No effect of APOA5 variants on the response to gemfibrozil treatment

LOCAT is a gemfibrozil trial, and although gemfibrozil, compared with placebo, was effective at reducing lipid levels and disease progression (13, 20), there was no association between APOA5 variants and this response. This is not surprising, because although APOA5 has a PPAR responsive element (PPRE) and gemfibrozil is a PPARα ligand, the PPRE is a considerable distance from the −1131 site (860 bp). Furthermore, we previously examined the effect of functional PPARα variants on disease progression in LOCAT (14); that study also found no association of PPARα variants with lipid levels.

In agreement with other studies (1, 9, 24), our data suggest a role for apoAV in the determination of lipid subtraction composition. By considering this lipoprotein subtraction data in the LOCAT study, we can speculate on potential different mechanisms for the effect of APOA5 variants on the secretion of TG-rich particles, which goes some way to explain their individual roles in atherosclerosis progression. Further confirmation, in additional studies and in-depth molecular experiments, are needed to determine the functional basis for these effects.

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