Plasma levels of remnant particles are determined in part by variation in the APOC3 gene insulin response element and the APOCI–APOE cluster

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Abstract **Remnant particles of triglyceride-rich lipoproteins (RLP) are known to be a strong predictor of atherogenicity. The serum concentrations of remnant-like particle triglyceride (RLPTG) and remnant-like particle cholesterol (RLPC) have been determined in a representative sample of the Czech MONICA study (n = 285). The relationship was investigated between remnant particle triglyceride/cholesterol concentrations and polymorphisms in the genes *APOC3* (−482C→T/3238C→G), *APOE* (ε2/ε3/ε4), *APOCI* (−317-321ins), *APOB* (signal peptide), hepatic lipase (*LIPE*, −480C→T), and lipoprotein lipase (*LPL*, S447X). Univariate analysis showed significant effects on RLPTG associated only with the *APOE* genotype ($P = 0.009$), the *APOC3* −482C→T genotype ($P = 0.018$), and the *APOCI* −317-321ins ($P = 0.014$) genotype and significant effects on RLPC with *APOE* ($P = 0.01$) and *APOCI* −317-321ins ($P = 0.021$). The raising effect of the *APOE* genotype for both remnant cholesterol and triglyceride was confined to the ε2/4 (n = 6) and ε4/4 (n = 3) groups, and thus when the ε2/4 group was omitted in order to analyze by allele (ε2+/ε3+/ε4+), significance was lost ($P = 0.6$). There was strong linkage disequilibrium between the *APOE* and *APOCI* alleles (χ^2, $P < 0.001$) and a multivariate ANOVA of RLPTG with all three significantly associated variants as factors demonstrated that while the *APOC3* −482C→T effect was independent of the others ($P = 0.003$), the *APOCI* −317-321ins and *APOE* effects were not. This was also true for the *APOCI* −317-321ins and *APOE* effects on RLPC. To assess whether *APOE-CI* effects on RLPC were independent of their effects on total cholesterol and triglyceride levels, multiple linear regression was used. Using multiple linear regression, it appeared that the *APOE-CI* effects on RLPC were independent of their effects on plasma cholesterol, but the effects of *APOC3* and *APOE-CI* on RLPTG could not be separated from their effects on plasma Tg levels. Further characterization of this remnant particle phenotype and its genetic determinants may lead to a better understanding of its metabolism and contribution to atherosclerosis.**—Waterworth, D. M., J. A. Hubacek, J. Pitha, J. Kovar, R. Poledne, S. E. Humphries, and P. J. Talmud. **Plasma levels of remnant particles are determined in part by variation in the *APOC3* gene insulin response element and the *APOCI–APOE* cluster. *J. Lipid Res.* 2000.** 41: **1103–1109.**

Supplementary key words insulin response element • remnant-like triglyceride • remnant-like cholesterol • hepatic lipase • lipoprotein lipase • apolipoprotein B

Remnant lipoproteins are partially catabolized chylomicrons and very low density lipoproteins (VLDLs) that are depleted of triglycerides (TG) and enriched with cholesterol esters. High remnant lipoprotein levels are associated with the presence, severity, and progression of atherosclerosis (reviewed in ref. 1) and have been shown to be an independent predictor of future coronary events in patients with coronary artery disease (CAD) (2). Smaller VLDL and intermediate density lipoprotein (IDL) particles have been consistently shown to have an increased capacity to enter the arterial wall as compared with larger VLDL and chylomicrons (1). The development of immunochemical assays for immunoseparation of remnant particles (using anti-apolipoprotein A-I [apoA-I] and anti-apoB-100 antibodies) has allowed the specific isolation of these particles and determination of their triglyceride and cholesterol content (3, 4). These antibodies allow the removal of most of the apoB-containing lipoproteins (low density lipoprotein [LDL]/VLDL) and apoA-I-containing lipoproteins (chylomicrons and high density lipoprotein [HDL]), leaving behind chylomicron and VLDL remnants that are enriched in apoE. This method has been found to compare favorably with ultracentrifugation and electrophoresis methods for determining triglyceride-rich

Abbreviations: apo, apolipoprotein; BMI, body mass index; CAD, coronary artery disease; IDL, intermediate density lipoprotein; IRE, insulin response element; LDL, low density lipoprotein; LRP, LDL-related protein; MADGE, microtiter array diagonal gel electrophoresis; MI, myocardial infarction; NEFA, nonesterified fatty acids; oligo, oligonucleotide; RLP, remnant-like particle; RLPC, remnant-like particle cholesterol; RLPTG, remnant-like particle triglyceride; TG, triglyceride; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

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lipoprotein remnants and remnant-like particle cholesterol (RLPC) concentrations were found to be highly correlated with total VLDL- and IDL-cholesterol and VLDL-TG but not with LDL-cholesterol or LDL-apoB levels (4–6).

The aim of this study was to identify whether any of a number of candidate gene variants involved in key pathways of remnant lipoprotein metabolism are involved in the determination of the levels of RLPC or remnant-like particle triglyceride (RLPTG) within a representative sample of healthy men and women. The candidate genes chosen for this study were *APOE*, *APOCI*, *APOC3*, *APOB*, hepatic lipase (*LIPE*), and lipoprotein lipase (*LPL*).

ApoE is the major ligand involved in the binding and uptake of remnant particles. There are three major apoE isoforms (E2, E3, and E4) that have been extensively studied and have been found to differentially affect lipid profiles and CAD risk. In particular, the ε4 allele has been associated with context-dependent (gender, diet, and ethnic origin) higher levels of triglyceride and low density lipoprotein-cholesterol (LDL-C) and risk for atherosclerosis (7–9). The ε2 allele has been shown to be associated with high levels of TG but low levels of LDL-cholesterol, and was found to be associated with low risk of myocardial infarction (MI) (8, 9). *APOE* is closely linked to *APOCI* on chromosome 19 and an *Hpa*I site (−317 bp to *APOCI*) has shown an ethnically distinct pattern of linkage disequilibrium with the *APOE* gene variants (10). ApoC-I is a constituent of triglyceride-rich lipoproteins and has been shown to displace apoE from triglyceride-rich emulsions and interfere with their hepatic clearance. ApoC-I also interferes with the binding of β-VLDL to the LDL-related protein (LRP) receptor (11) and of VLDL/IDL to LDL receptors (12). Transgenic mice overexpressing apoC-I developed hyperlipidemia but apoC-I knockout mice exhibited normal lipid levels when fed a chow diet and developed hypercholesterolemia only on a high-fat and high-cholesterol diet (reviewed in ref. 13). The *Hpa*I site is produced by a 4-bp CGTT insertion and has been shown to increase gene transcription *in vitro*, and was found to be associated with a disordered lipoprotein metabolism in a population-based study (10).

The apoC-III content of triglyceride-rich lipoproteins (TRL) is a marker of TRL metabolism and clearance of VLDL and chylomicrons, as a greater proportion of apoC-III on HDL particles than on VLDL/chylomicron particles would indicate a recent clearance of VLDL and chylomicrons (14). ApoC-III inhibits lipoprotein lipase (LPL) in vitro (15) and displaces apoE on the particles (16), which may prolong the clearance of these atherogenic particles and their circulatory residence time (14). *APOC3* 3238C→G (*Sst*I site) has been investigated in a large number of studies and possession of the rare 3238G allele has generally been found to be associated with high triglyceride levels and CAD risk (reviewed in ref. 17). The −482C→T site is situated in an insulin responsive element (IRE) and the presence of the −482T allele has been shown to reduce responsiveness to insulin in vitro, resulting in inappropriate expression of apoC-III (18). In a study of native Canadians, the −482T allele was associated with significantly increased triglycerides in both men and women (19).

Lipoprotein lipase (LPL) is abundant in muscle, adipose tissue, and macrophages, and hydrolyzes triglyceride in chylomicrons and VLDL. A common C-to-T transversion converts Ser447 to a premature stop codon, truncating LPL by two amino acids (X447 allele). Subjects in the ECTIM study who carried the X447 had significantly lower LpCIII:B (particles containing both apoC-III and apoB) and LpE:B, both of which represent remnant particles, and VLDL-cholesterol levels (8). Similarily, in German CAD patients who carried an X447 allele had a beneficial lipid profile of lower triglyceride and lower VLDL-triglyceride than S447 subjects (20). In the EARS study, the X447 allele was also found to be associated with reduced fasting and postprandial triglyceride and risk of paternal MI (21).

Hepatic lipase (LIPE) is a lipolytic enzyme that is mainly found anchored by heparan sulfate proteoglycans (HSPG) to the vascular endothelium in the liver on hepatocytes and is involved in the hydrolysis of phospholipids and triglycerides of chylomicron remnants. LIPE may influence remnant removal by the hydrolysis of chylomicron phospholipids, unmasking apoE and thereby enhancing binding to the LRP receptor (22). LIPE may also act as a ligand for chylomicron-remnant binding to the liver (23). A variant (−480C→T) in the *LIPE* promoter has been found to influence LIPE activity in CAD patients (24), fasting lipid concentrations and pre- and postprandial LpCIII:B levels in the EARSII group of young healthy men, such that carriers of the −480T had higher levels of apoC-III:B particles (25).

ApoB is the major structural apoprotein of lipoproteins synthesized in the liver (apoB-100) and in the intestine (apoB-48), and apoB-100 acts as a ligand for the LDL receptor. SP-24 is a common variant of the apoB 27-residue signal peptide (SP-27), caused by the deletion of three hydrophobic amino acids. This variant has been shown to confer a secretion-defective phenotype in yeast (26). While other *APOB* variants are markers for apoB catabolism, variation in the apoB signal peptide is thought to affect the production and secretion of apoB-containing lipoprotein. Subjects who carried one or more SP-24 alleles have significantly reduced circulating levels of both postprandial large chylomicron remnants and large very low density lipoproteins after an oral fat tolerance test (27).

These candidate gene variants were determined in this representative sample of the Czech Slav population to examine their specific association, if any, with both remnant particle triglyceride and cholesterol levels.

MATERIALS AND METHODS

Population sample

The 129 men and 153 women, aged 25–64 years, were randomly selected in 1988 from the 1% representative Czech population sample (Benesov region) according to the protocol of the MONICA study (28) and reinvestigated in 1996. The response rate of this selected group was 72% and all subjects were white. The origins of the Czech Slav population are not clear but fre-

quency differences of common genetic variants between the Czechs and the neighboring Germanic population suggest that the the two groups may be genetically distinct (R. Poledne and J. A. Hubacek, unpublished observations, 1999). The MONICA study was a World Health Organization (WHO) project for monitoring risk factors for cardiovascular diseases in 26 countries. Written informed consent was obtained from the study participants and the study design was approved by a local ethics committee.

Biochemical and anthropomorphic measures

Information was obtained in the clinic on entry into the study on age, body mass index (BMI), smoking, and menopausal status of the women. Seven men and four women were receiving lipid-lowering medication, 19 men and 29 women were receiving diuretics, and 29 men and 42 women were receiving β-blockers. Four men and 15 women were receiving a lipid-lowering diet and no women were receiving hormone replacement therapy. Lipids were obtained after an overnight fast. Remnant particles were immunoseparated from the remaining lipoproteins and their serum concentration determined in the supernatant as remnant-like particle triglyceride (RLPTG) and remnant-like particle cholesterol (RLPC) according to the method of Nakajima et al. (3) in the WHO regional reference laboratory (Institute of Clinical and Experimental Medicine, Prague, Czech Republic).

Polymorphism detection

Polymerase chain reaction (PCR) conditions and restriction enzyme digestions were as follows (oligonucleotides [oligos] were synthesized by GIBCO-BRL [Gaithersburg, MD]): *APOC3* -482C→T; forward 5′-GGT GCT GGG AGG GGC GGT GAG AGC TCA GCC-3′, reverse 5′-CCC TCC ACC AGC CCC AAG CCC GGA ACA CAG-3′. The PCR was carried out in 30 μL containing 200 ng of genomic DNA, 100 ng of each primer, 0.2 mM dNTPs (U.S. Biochemical, Cleveland, OH), 2.75 mM $MgCl_2$, NH_4 polymerase buffer (from Bioline, UK), and 0.25 units of *Taq* polymerase (GIBCO-BRL). After an initial denaturation for 5 min, denaturing was at 95°C for 1 min, with a combined annealing and extension step of 72°C for 1 min for 35 cycles. The PCR product was digested with 2 units of *Mva*I (MBI Fermentas, Amherst, NY) overnight at 37°C. The rare allele was detected by the presence of a cutting site. Polymorphisms in the *APOC3* 3238C→G (29), *APOE* (30), *APOCI* −317-321ins (31), *LIPE* −480C→T (24), *LPL* (S447X) (21), and *APOB* (signal peptide) (32) genes were determined as described previously, except that for the *APOC3*, *LIPE*, and *LPL* PCRs, the fragments were separated by 5–10% polyacrylamide microtiter array diagonal gel electrophoresis (MADGE) (33).

Statistical analysis

Data for each genotype was tested for deviation from Hardy–Weinberg equilibrium, using a χ^2 test in EXCEL. Linkage disequilibrium (Δ) was calculated with EXCEL and the method of Chakravarti et al. (34). Statistical analysis was performed with the SPSS (Chicago, IL) package. RLPTG and RLPC were both log-transformed for analysis. Differences between geometric means were tested by analysis of variance (ANOVA) (unique method) and included sex and BMI as covariates. The assumption of equal variance in the differerent genotype groups was tested and was not found to be violated in any of the comparisons. Age, smoking, and menopausal status were not found to be significant predictors of RLPTG or RLPC and were therefore not subject to adjustment. Linear regression analysis was used in an attempt to differentiate between the effect on total lipid and remnant particle lipid. Log RLPTG was regressed for log triglyceride, BMI, and sex, and the resulting residuals were tested for association with genotype by ANOVA. Likewise, log RLPC was regressed for cholesterol, BMI, and sex. No appreciable difference was observed in any of the associations when drugs (β-blockers, diuretics, and fibrates) or a lipid-lowering diet was entered into the ANOVA as covariates. A value of $P < 0.05$ was selected for a significance threshold.

RESULTS

General characteristics

The general characteristics of the sample are shown in **Table 1.** Men and women were similar with respect to age and BMI, but RLPTG was 114% higher ($P = 0.0001$) and RLPC 29.3% higher ($P = 0.09$) in the men than the women. For all variants the distribution of genotypes was as expected for Hardy–Weinberg equilibrium. The rare allele frequencies and 95% confidence interval (95%CI) in the Czech sample were not significantly different in men and women and were as follows: *APOCI* −317-321ins, 0.175 (0.14–0.21); *APOB* SP-24, 0.319 (0.28–0.36); *APOC3* −482T, 0.339 (0.30–0.38); *APOC3* 3238G, 0.123 (0.10–0.15); *LIPE* −480T, 0.209 (0.18–0.24); *LPL* X447, 0.094 (0.07–0.12); ε2, 0.081 (0.06–0.10); ε3, 0.826 (0.79–0.86); and ε4, 0.094 (0.07–0.12).

Associations with RLPTG levels

For all the analyses there was no heterogeneity of effect between men and women ($P = 0.57$) and data were analyzed for the group as a whole to increase the power of the study. For the *APOC3* gene, univariate analysis (with sex and BMI as covariates) showed (**Fig. 1** and **Table 2**) that *APOC3* (−482C→T) was significantly associated with RLPTG ($P = 0.02$), with the raising effect (largely) confined to the −482TT group (TT 25.6% higher than CC group). There was no significant relationship observed with the *APOC3* 3238C→G (*Sst*I) site ($P = 0.58$), even though the two variants are in strong allelic association ($\Delta = 0.49$, $P < 0.001$).

The only other genes found to modulate RLPTG levels significantly were *APOE* ($P = 0.009$) and *APOCI* −317-321ins ($P = 0.01$) and all three univariate associations (*APOC3*, *APOCI*, and *APOE*) are shown in Fig. 1 and Table 2. As with *APOC3* −482C→T, no significant genotype–gender interactions were observed (*APOE*, $P = 0.95$; *APOCI*, $P = 0.86$). Further scrutiny of the data showed that the significant effects of *APOE* and *APOCI* on RLPTG were derived primarily from a large raising effect in rare

TABLE 1. General characteristics of the study population

	Mean (SD)		
	Men (n = 131)	Women (n = 154)	*P* Value
Age, years	55.3 (11.6)	55.5 (11.2)	NS
BMI, wt/ht^2	28.4 (4.1)	29.1 (5.7)	NS
Triglyceride, mmol/l	2.2 (1.8)	1.9 (1.2)	0.09[a]
Total cholesterol, mmol/l	5.4 (1.1)	5.6 (1.2)	NS
HDL cholesterol, mmol/l	1.2 (0.3)	1.4 (0.4)	0.0001[a]
RLP-triglyceride, mmol/l	0.45 (1.04)	0.21 (0.40)	0.0001[a]
RLP-cholesterol, mmol/l	0.32 (0.54)	0.25 (0.31)	0.087[a]

[a] Test performed on log-transformed values.

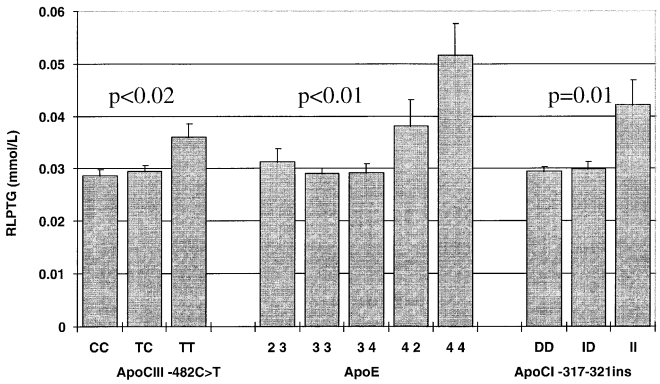

Fig. 1. Geometric means and SEM of RLPTG by genotype, with univariate ANOVA *P* values.

genotypes, with the ε2/4 group (n = 6) having 30.8% higher and the ε4/4 group (n = 3) having 77.3% higher RLPTG levels than the ε3/3 group, and the *APOCI* −317-321ins/ins group (n = 7) having 42.5% higher levels than the −317-321del/del group. Therefore, when *APOE* was analyzed by alleles (ε2, ε3, ε4) instead of genotype, using the standard procedure of omitting the ε2/4 group and grouping into ε2+, ε3+, and ε4+, significance was lost ($P = 0.34$). The possible nonindependence of the *APOE* and *APOCI* findings may result from the strong linkage disequilibrium between the two variants (Pearson χ^2 test, $P < 0.0001$), reflected in the fact that four of the seven *APOCI* −317-321ins/ins subjects also carry either ε2/4 or ε4/4. No significant gene–gene interactions were present between any of the variants investigated on RLPTG. There was no significant association between RLPTG and variation examined at the *APOB*, *LIPE*, or *LPL* loci.

These three significantly associated gene variants were subsequently entered as factors in pairwise combinations into an ANOVA to determine if any nonindependent genetic effects were present (sex and BMI were included as covariates). In a model containing both *APOC3* −482C→T and *APOCI* −317-321ins, both genetic effects remained significant ($P = 0.013$ and $P = 0.012$, respectively), as did the combination of *APOC3* −482C→T and *APOE* ($P = 0.003$ and $P = 0.006$, respectively). However, when *APOE* and *APOCI* −317-321ins were entered pairwise in both orientations, significance was lost in the variant that was entered second, regardless of the order (*APOE*, $P = 0.009$ and *APOCI*, $P = 0.18$, respectively/*APOCI*, $P = 0.01$ and *APOE*, $P = 0.29$, respectively), thus confirming that the effects of *APOCI* −317-321ins and *APOE* were not independent of each other.

Associations with RLPC levels

Univariate analysis for cholesterol content of RLP of each genetic variant showed significant results for *APOCI* −317-321ins, where the ins/ins group had 43% higher RLPC than the del/del group, and for the *APOE* gene, where the ε2/4 group (n = 6) and the ε4/4 group (n = 3) had, respectively, 38 and 75.7% higher RLPC than the ε3/3 group ($P = 0.02$ and $P = 0.01$, respectively). There was no significant association with *APOC3* −482C→T and RLPC ($P = 0.28$), although a similar trend as seen with RLPTG was noted (**Fig. 2** and Table 2). None of the other variants investigated showed a significant association with RLPC. As with RLPTG, no significant gene–gender or gene–gene interactions were observed. In a multivariate ANOVA with RLPC, effects of the *APOCI* and *APOE* variants were not found to be independent of each other, significance being retained once again only in the variant entered first (*APOE*, $P = 0.01$ and *APOCI*, $P = 0.27$, respectively/*APOCI*, $P = 0.004$ and *APOE*, $P = 0.34$, respectively). The similar findings for the genetic associations with RLPC and RLPTG were substantiated by the strong correlation observed between the two measures ($r = 0.84$, $P < 0.001$).

TABLE 2. Mean (SE) lipids and RLPTG and RLPC by genotype

	n	Cholesterol	RLPC	Triglyceride	RLPTG
			mmol/l		
APOC3 −482C→T					
CC	117	5.51 (0.10)	0.18 (0.05)	1.60 (1.05)	0.14 (0.01)
CT	124	5.52 (0.10)	0.18 (0.03)	1.58 (1.05)	0.15 (0.01)
TT	31	5.67 (0.19)	0.24 (0.03)	2.10 (1.11)	0.27 (0.01)
P value		0.72	0.28	0.05	0.02
APOE ε2/3/4					
E2/3	35	5.21 (0.23)	0.18 (0.03)	1.69 (1.12)	0.18 (0.01)
E3/3	178	5.49 (0.08)	0.18 (0.03)	1.57 (1.04)	0.15 (0.01)
E4/3	38	5.68 (0.15)	0.19 (0.07)	1.60 (1.05)	0.15 (0.01)
E4/2	6	5.56 (0.20)	0.38 (0.03)	2.66 (1.31)	0.33 (0.02)
E4/4	3	7.01 (0.02)	0.78 (0.03)	4.22 (1.40)	1.09 (0.02)
P value		0.08	0.01	0.03	0.01
APOCI −317-321ins					
D/D	190	5.51 (0.08)	0.18 (0.03)	1.59 (1.04)	0.15 (0.01)
D/I	84	5.49 (0.12)	0.19 (0.03)	1.68 (1.07)	0.16 (0.01)
I/I	7	6.07 (0.42)	0.42 (0.06)	2.93 (1.30)	0.47 (0.021)
P value		0.47	0.02	0.02	0.01

ANOVA was performed on log-transformed values for triglyceride, RLPTG and RLPC (BMI and sex were included in the ANOVA as covariates) but means and SE have been anti-logged for presentation.

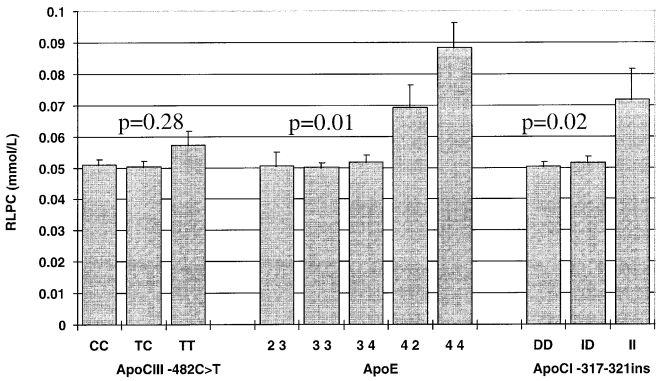

Fig. 2. Geometric means and SEM of RLPC by genotype, with univariate ANOVA *P* values.

Are these associations specific to remnant particles?

As cholesterol and RLPC, and triglyceride and RLPTG, are strongly correlated (r = 0.53, $P < 0.001$; r = 0.79, $P < 0.001$, respectively), the question arose whether the observed effects of these variants on remnant particles were a reflection of the total cholesterol and triglyceride levels, or whether they were specific to the remnant particle TRL fraction. For each genotype, means and significance values for all four measures are shown in Table 2. Similar relationships were observed in almost all cases between the remnant fraction and total triglyceride/cholesterol levels, except for *APOCI* −317-321ins, where a significant relationship with RLPC ($P = 0.02$) was not seen on total cholesterol levels ($P = 0.47$) although a similar trend was evident. In an attempt to ascertain whether the genetic effects on RLPTG are independent of their effect on triglyceride, RLPTG was adjusted for triglyceride level by multiple linear regression (including BMI and sex) and ANOVA was performed on the resulting residuals. No significant effects were observed for any of the three associated gene variants (*APOE, APOCI* −317-321ins, and A*POC3* −482C→T) on the adjusted RLPTG (data not shown). A similar analysis was performed for RLPC and total cholesterol and significant effects were still observed for *APOE* ($P = 0.017$) and *APOCI* ($P = 0.043$) (data not shown). Adjusting RLPC for TG levels resulted in the loss of these significant associations for both *APOE* ($P = 0.4$) and *APOCI* ($P = 0.4$).

DISCUSSION

Reports emphasizing the importance of remnant particles as a risk factor for atherosclerosis (1, 2) led us to investigate possible genetic influences on this important phenotype. The ability to measure remnant particles in samples from epidemiological studies has made it feasible to assess the genetic factors that determine their serum concentration. Of the seven candidate gene variants chosen to study, only *APOC3* −482C→T, *APOE* (ε2, ε3, ε4), and *APOCI* −317-321ins influenced serum RLPTG concentrations in this sample. However, as expected from their strong genetic relationship, the effect of the *APOE* and *APOCI* variants were not independent of each other. The *APOE* and *APOCI* variants also significantly influenced serum RLPC concentration in a nonindependent manner. None of the other variants showed any significant effect on remnant particle concentration in this study, although there would be limited power to detect effects of smaller magnitude.

The raising effect of the *APOC3* −482C→T variant on RLPTG was confined to homozygous carriers of the −482T allele and there was no heterogeneity of effect between men and women. There was no effect observed on this phenotype by the *APOC3* variant 3238C→G (*Sst*I)(17), which is in strong linkage disequilibrium with *APOC3* −482C→T ($\Delta = 0.49$, $P < 0.001$), but it is possible that the lower frequency of the 3238G allele (0.123) as compared with the −482T allele (0.339) could reduce the possibility of detecting significant effects. The −482C→T variant is present in an insulin-responsive element and the −482T allele has been shown in vitro to be associated with the loss of insulin-mediated downregulation of *APOC3* gene expression, as compared with the −482C allele, where a 40–50% downregulation by insulin occurs (18). It should be noted that a second variant, −455T→C, which was not evaluated in this study, is also present in the IRE, and would also be likely to show an association with RLPTG as it is in strong linkage disequilibrium with the −482C→T variant. Both IRE variants were shown to be critical in conferring the response to insulin in vitro (18). Hypothetically, in a postprandial situation, where insulin downregulates apoC-III to release its inhibitory effect on LPL, carriers of the −482T allele could maintain innappropriately high levels of apoC-III and, as a consequence, a reduced triglyceride hydrolysis by LPL and a delayed clearance of triglyceride-rich lipoproteins and RLPs. ApoC-III transgenic mouse studies have suggested that the hypertriglyceridemic effect of apoC-III may be partly mediated by the displacement of apoE from TRL, leading to reduced remnant particle uptake (16). However, hypertriglyceridemia was not corrected in *APOC3* tg/*APOE* null mice, suggesting that the predominant mechanism of apoC-III-induced hypertriglyceridemia may be due to decreased lipolysis at the cell surface (35). Thus, there are a number of possible mechanisms that could explain the effect of *APOC3* −482C→T on RLPTG, but until the exact functions of apoC-III have been elucidated, whether via LPL inhibition and/or apoE displacements, or possibly another mechanism, it is not possible to speculate further.

It was not surprising that no significant effect was observed on RPLC by *APOC3* −482C→T, as cholesterol levels are not generally affected by *APOC3* variants, although a similar trend, as with RLPTG, was observed. The two measures of RLPTG and RLPC were highly correlated, but the data suggest that this variant affects the triglyceride content of the remnant particles more than the cholesterol content.

Analysis of the associations between RLPTG/RLPC and *APOE-CI* is complicated by the high degree of linkage disequilibrium that exists between these two genes. The extent of linkage disequilibrium was similar to that previously observed in European-Americans (10) with the *APOCI* −317-

321ins allele being in linkage disequilibrium with both the ε2 and ε4 alleles; specifically four of the seven *APOCI* −317-321ins/ins subjects also carried either an ε2 or an ε4 allele. Variants in both *APOE* and *APOCI* can provide plausible mechanisms for modulating remnant particle concentrations and this data set is insufficiently powered to differentiate between them, but it could also be the case that both variants contribute to the remnant particle phenotype. It is also possible that other, as yet unknown variants in the *APOE-CI-CII* gene cluster (36), which are in allelic association with those we have investigated, could be responsible for these observed effects.

Elevated RLPTG was observed in both the ε2/4 and ε4/4 groups, reflecting previous findings of raised triglyceride in carriers of ε2 and ε4 alleles (8, 9). However, mean levels of RLPTG were similar in the group of subjects who carried at least one ε3 allele compared with the ε3/3 group, suggesting a dominant protective effect of the ε3 allele. The highest levels were observed in the ε4/4 group, suggesting that high remnant particle concentrations may be contributing to the increased risk of CAD commonly observed in this group (37). However the small sizes of the ε2/4 and ε4/4 groups in this study limit the interpretation of this result. A similar pattern was observed with RLPC, an effect that is probably due to the strong correlation between the two measures of RLPC and RLPTG.

The presence of the *APOCI* −317-321ins allele has been shown in vitro to increase expression of *APOCI* by 50%. Elevated *APOCI* levels have been shown to inhibit both apoE-mediated uptake of triglyceride-rich emulsions in perfused rat liver and remnant clearance in apoC-I transgenic mice (13). Thus, a direct inhibitory mechanism would most likely explain the high levels of RLPTG and RLPC observed in *APOCI* −317-321ins/ins subjects. This effect appeared to be recessive, with no obvious effect in heterozygous carriers.

We have attempted to ascertain the specificity of these associations by comparing the magnitude of the effects of these associated gene variants between the remnant particles and their respective total lipid levels. As these associations were generally reflected in the total lipid level, we attempted to test for any specific effect on remnant particles by adjusting the remnant particle levels for the respective total cholesterol or TG level (as well as BMI and sex) by regression. This analysis indicated that the effects associated with the *APOC3* and *APOE-CI* variants on RLPTG were not independent of their effects on triglyceride levels, suggesting a simple linear relationship between the two variables. However, there was some evidence of specific effects on RLPC by *APOE* and *APOCI* that were not reflected in effects on total cholesterol, suggesting a nonlinear relationship between the two variables. This suggests that the RLP may be specifically enriched for cholesterol in subjects who carry either ε4/4, ε2/4, or *APOCI* −317-321ins/ins. These associations with RLPC were not, however, independent of TG level, as evidenced by the loss of significant association with *APOCI* and *APOE* when RLPC was adjusted for TG.

This study has allowed us to perform a preliminary screen of putative candidate genes involved in the remnant particle phenotype and we have been able to indicate which genetic variants warrant further examination. Clearly *APOC3*, *APOCI*, and *APOE* are the strongest candidates for effects on clearance of these particles, but much larger studies will be required to delineate whether these effects are specific to the *APOE* or *APOCI* region because of the high degree of linkage disequilibrium that exists between these variants. Xu and co-workers (10) attempted to differentiate between these gene effects by examining another ethnic group (African-American) in which linkage disequilibrium between *APOE* and *APOCI* is weaker; however, their results are difficult to interpret as the different genetic background may also contribute to and interact with the various lipid phenotypes studied. However, because of the important predictive power of remnant particles with respect to atherosclerosis (1, 2), the elucidation of the mechanisms by which genetic variation modulate this phenotype will be of clinical importance.

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