The *APOA4* T347S variant is associated with reduced plasma TAOS in subjects with diabetes mellitus and cardiovascular disease

Wai-man R. Wong,[1,*] Jeffrey W. Stephens,[1,*,†] Jayshree Acharya,[*] Steven J. Hurel,[†] Steve E. Humphries,[*] and Philippa J. Talmud[2,*]

Centre for Cardiovascular Genetics, British Heart Foundation Laboratories,* Royal Free and University College London Medical School, London WC1E 6JF, United Kingdom; and Department of Diabetes and Endocrinology,† University College London Hospitals, London W1T 3AA, United Kingdom

Abstract Apolipoprotein A-IV (apoA-IV) has been postulated to be antiatherogenic. Transgenic *APOA4/Apoe*$^{-/-}$ mice are protected against atherosclerosis, with plasma apoA-IV displaying antioxidant activity in vitro. In humans, there is an inverse relationship between apoA-IV levels and risk of coronary heart disease (CHD). Furthermore, the *APOA4* T347S rare allele has been associated with increased risk of CHD and reduced apoA-IV levels. Reduced total antioxidant status (TAOS) due to increased oxidative stress is implicated in the process of atherogenesis. Thus, this study aimed to examine the association between the *APOA4* T347S variant and TAOS in diabetic patients with (n = 196) or without (n = 509) cardiovascular disease (CVD). A higher percentage of CVD patients were present in the lowest quartile of TAOS, compared with the rest ($P = 0.04$). Overall, there was no association between genotype and TAOS. However, in patients with CVD, homozygotes for the S347 allele had significantly lower TAOS compared with TT and TS subjects (31.2 ± 9.89% and 42.5 ± 13.04% TAOS, respectively; $P = 0.0024$), an effect that was not seen in the patients without CVD. This study offers direct support for an antioxidant capacity of apoA-IV, thus providing some explanation for the antiatherogenic role of apoA-IV and the higher CVD risk in S347 homozygotes.—Wong, W-m. R., J. W. Stephens, J. Acharya, S. J. Hurel, S. E. Humphries, and P. J. Talmud. **The *APOA4* T347S variant is associated with reduced plasma TAOS in subjects with diabetes mellitus and cardiovascular disease. *J. Lipid Res.* 2004.** 45: **1565–1571.**

Supplementary key words apolipoprotein A-IV • association study • oxidized low density lipoprotein • total antioxidant status

Apolipoprotein A-IV (apoA-IV) is a 46 kDa glycoprotein that circulates freely or in association with chylomicrons and HDLs (1). In humans, apoA-IV is synthesized predominantly by the enterocytes within the small intestine and is secreted into the lymph bound to chylomicrons. ApoA-IV is a polymorphic protein exhibiting several common isoforms. The apoA-IV-1A isoform is generated by the ACT>TCT substitution at codon 347, resulting in a threonine by serine (T347S) change that does not alter the electrophoretic mobility and is thus undetectable by isoelectric focusing. The CAG>CAT base change at codon 360 results in the substitution of histidine for glutamine (Q360H), and a more basic isoform, apoA-IV-2, which can be identified by isoelectric focusing (2–4).

Three case control studies have demonstrated significantly lower apoA-IV plasma levels in subjects with coronary heart disease (CHD) compared with controls (5, 6). We have previously reported, in a prospective study of middle-aged men, that homozygosity for the *APOA4* S347 variant was associated with increased risk of CHD and that this could be related to the reduced plasma apoA-IV levels in *APOA4* S347 homozygotes (7). Furthermore, haplotype analysis of nine single-nucleotide polymorphisms (SNPs) within the apolipoprotein gene cluster (*APOC3-A4-A5*) revealed that the risk-associated haplotype was defined by the presence of the *APOA4* S347 allele, and that the risk was independent of any lipid parameters, suggesting a mechanism unrelated to reverse cholesterol transport (7).

Although the precise physiological function of apoA-IV has yet to be deduced, there are several mechanisms to explain its antiatherogenic role. ApoA-IV plays a direct role in reverse cholesterol transport by activating enzymes in the pathway, namely, lecithin:cholesterol acyl transferase (8, 9) and cholesteryl ester transfer protein (10); and, in addition, by modulating lipoprotein lipase activity (11), the efflux of cholesterol from the periphery for transportation back to the liver is thus promoted (12–14). The protection of the *APOA4* transgene in apoE-deficient mice

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[1] W-m. R. Wong and J. W. Stephens contributed equally to this work.
[2] To whom correspondence should be addressed.
e-mail: p.talmud@ucl.ac.uk

(*APOA4/apoe*$^{-/-}$) supports the antiatherogenic role of apoA-IV (15). Further, these mice had reduced oxidative markers, including aldehyde-modified LDL, suggesting an antioxidant effect of apoA-IV (16), although this, per se, does not prove that the antiatherogenic effect of apoA-IV is a result of antioxidant potential. More recently, the protective effect of the human *APOA4* transgene in *APOA4/Apoe*$^{-/-}$ mice was examined in relation to lipopolysaccharide (LPS) exposure (17). LPS mimics chronic infection and increases the atherosclerotic lesion in *Apoe*-deficient mice. However, in *APOA4/Apoe*$^{-/-}$ mice, lesion size was reduced and proinflammatory cytokine production was lower, although autoantibodies to oxidized LDL (OX-LDL) were higher (17). These studies provide additional insight into the anti-inflammatory, antiatherogenic role of apoA-IV.

Thus, one possible pathological mechanism to explain the inverse association between apoA-IV levels and CHD is the ability of apoA-IV to act as an effective inhibitor of lipid peroxidation, a key process implicated in CHD progression (18, 19). A state of oxidative stress results from the excess of reactive oxygen species (ROS) in a system, overwhelming any endogenous antioxidant protection. The increased production of ROS by vascular smooth muscle and endothelial and adventitial cells, resulting from common atherogenic risk factors, such as smoking, diabetes, and hypertension, is well documented. It is postulated that ROS not only acts as a marker for vascular disease but also initiates and causes the progression of various processes involved in atherogenesis (20). ROS production promotes the formation OX-LDL, resulting in the formation of foam cells due to scavenger receptor-mediated uptake of OX-LDL by macrophages (21). Exposure of endothelial cells to ROS induces apoptosis, resulting in endothelial cell loss and a procoagulative and atherogenic state (22). The upregulation by ROS via nuclear factor-κB of many inflammatory genes, including monocyte chemotactic protein-1 and interleukin-6, has a significant role in the progression of atherosclerosis (23). On the basis of the potential antioxidant properties of apoA-IV and our previous finding of the association of *APOA4* S347 with CHD risk (7), we have here investigated the association of the *APOA4* T347S variant with plasma total antioxidant status (TAOS), which is inversely related to oxidative stress, in a sample of subjects with diabetes mellitus, a group at high risk of cardiovascular disease (CVD).

MATERIALS AND METHODS

Subjects

Patients were recruited from the University College Diabetes and Cardiovascular Study (UDACS), described elsewhere (24). This is a cross-sectional sample designed to study the association between common variants in inflammatory/metabolic genes and biochemical risk factors implicated in CVD in patients with diabetes. Briefly, this comprises 1,011 consecutive subjects recruited from the diabetes clinic at University College London Hospitals National Health Service Trust (UCLH) between the years 2001 and 2002. All patients had diabetes according to World Health Organization criteria (25). Analyses were confined to Caucasian subjects (780, of whom 731 were successfully genotyped for the gene variant) to avoid the confounding effect of ethnicity. Subjects were categorized by the presence/absence of clinically manifest CVD. CVD was recorded if a patient had one or more of CHD, peripheral vascular disease (PVD), or cerebrovascular disease (CbVD). The presence of CHD was recorded if any patient had positive coronary angiography or angioplasty, coronary artery bypass, a positive cardiac thallium scan or exercise tolerance test, or documented evidence of myocardial infarction or symptomatic/treated angina. The presence of PVD was recorded in any patient with absent peripheral pulses and abnormal lower-limb doppler pressures or an abnormal lower-limb angiogram, previous angioplasty, or limb bypass graft. CbVD was recorded if a patient had been investigated for symptoms or signs consistent with a cerebrovascular accident and had a brain computed tomography scan showing any evidence of infarction (diffuse/localized) or hemorrhage. Subjects who were asymptomatic for CHD/CbVD/PVD or who had negative investigations were categorized as having no CVD.

Ethical approval was obtained from the UCL/UCLH ethics committee.

Measurement of plasma TAOS

Plasma TAOS was measured by a photometric microassay previously described by Sampson et al. (26). The TAOS of plasma was determined by its capacity to inhibit the peroxidase-mediated formation of the 2,2-azino-bis-3-ethylbensthiazoline-6-sulfonic acid ($ABTS^+$) radical. In the assay, the relative inhibition of $ABTS^+$ formation in the presence of plasma is proportional to the antioxidant capacity of the sample. Therefore, there are two arms to the assay, a control arm and test arm. In the control arm, phosphate-buffered saline is used instead of plasma. The assay is performed in a 96 well ELISA plate using 2.5 μl of plasma. The difference in absorbance (control absorbance minus test absorbance) divided by the control absorbance (expressed as a percentage) was used to represent the percentage inhibition of the reaction. Plasma TAOS is therefore inversely related to oxidative stress (the higher the oxidative stress, the lower the TAOS). The inter-assay coefficient of variation (CV) was 14.1%, and the intra-assay CV was 4.3%.

DNA extraction and genotyping

DNA was extracted using the salting out method (27). Genotyping for the *APOA4* T347S variant was carried out by polymerase chain reaction and *Hinf*I restriction digestion using published primers and conditions (28). Fragments were resolved by microplate array diagonal gel electrophoresis (29) and confirmed by two independent technicians blind to subject outcome, with discrepancies resolved by repeat genotyping.

Statistical analysis

Analysis was performed using SPSS (version 11.5, SPSS Inc., Chicago, IL). Data are reported for those individuals for whom *APOA4* T347S genotyping and plasma TAOS measurements was successful (n = 705). Deviations from Hardy-Weinberg equilibrium were considered using χ^2 tests. Allele frequencies are shown with the 95% confidence interval. Results are presented as mean ± standard deviation or median (interquartile range). To assess the differences between CVD and CVD-free patients, two-sided *t*-tests were performed on normally distributed data or after appropriate transformation (log or square root). ANOVA was used to assess the association between genotype and plasma TAOS in subjects with and without CVD, a priori combining the 347TT and TS groups, due to the reported recessive effect on apoA-IV levels of the S347 allele (7). The relationships between baseline param-

eters and plasma TAOS were tested by Spearman rank correlation coefficient. The interaction between the T347S genotype and CVD on plasma TAOS levels was determined using linear regression by including an interaction term for genotype and CVD status. In all cases, a *P* value of less than 0.05 was considered statistically significant.

RESULTS

In total, 731 (94.0%) Caucasian patients within the UDAC study were successfully genotyped for the *APOA4* T347S variant. Genotype distributions were found to be in Hardy Weinberg equilibrium with a rare allele frequency of 0.21 (95% confidence interval, 0.19–0.23).

There were no significant differences in allele frequencies between subjects with or without CVD ($P = 0.4$). CVD was present in 202 individuals whose mean age, plasma creatine, and C-reactive protein levels were significantly higher than those without CVD. A significantly higher proportion of patients with CVD were taking angiotensin-converting enzyme inhibitors (ACEIs), aspirin, insulin, and statins (**Table 1**), explaining the lower LDL-cholesterol (LDL-C) levels and diastolic blood pressure in subjects with CVD compared with those without. Plasma TAOS was positively correlated with HDL-C plasma levels and negatively correlated with triglycerides and glucose (correlation coefficient, $r = 0.12$, -0.14, and -0.11, respectively, all $P < 0.05$). T347S genotype had no effect on any of the baseline characteristics (**Table 2**).

APOA4 T347S and plasma TAOS

There was no heterogeneity of effects due to gender with respect to TAOS; thus, analysis was performed on both men and women. In the sample, there was no significant difference in the mean plasma TAOS by CVD status (Table 1); however, a significantly higher percentage of subjects with CVD were found to be present in the lowest quartile of TAOS compared with the upper quartiles ($P = 0.04$; **Fig. 1**). In the group as a whole, there was no association of T347S genotype with TAOS levels ($P = 0.63$). However, when the patients were stratified on the basis of CVD status (**Fig. 2**), in those patients with CVD, homozygotes for the *APOA4* S347 allele had significantly lower plasma TAOS compared with carriers of the T347 allele (42.5% vs. 31.2%, respectively; $P = 0.0024$). This association remained significant after adjustment for age, triglycerides, HDL-C, glucose, and HbA_{1c} (SS vs. T allele; $P = 0.019$). No such differences were observed in those subjects without CVD ($P = 0.32$; Fig. 2). The interaction between CVD and genotype on plasma TAOS was statistically significant ($P = 0.014$).

DISCUSSION

The major novel finding in this study is that homozygosity for the *APOA4* S347 allele is associated with reduced TAOS and, therefore, increased oxidative stress in diabetic patients with CVD. This does not imply causation, but we describe an interesting observation that may shed light on the association of this gene variant and CVD risk (7). T347S genotype was not significantly associated with any CVD risk trait that may confound this effect, including levels of any lipid or lipoprotein (data not shown), and the effect of genotype on TAOS remained statistically significant after adjustment for such potential confounders. This relationship was not seen in those free of CVD. A significantly greater percentage of subjects with CVD were

TABLE 1. Baseline differences of 731 Caucasian subjects by CVD status in UDACS

Trait	No CVD (n = 529)			CVD (n = 202)			*P*
Age (years)	60.2 (14.1)			68.8 (10.1)			<0.001
Sex (F/M)	229/300 (43.2%/56.8%)			59/143 (29.4%/70.6%)			0.001
Duration (years)[b]	11 (5–11)			11 (6–18)			0.81
Systolic BP (mmHg)[a]	138 (127–150)			141 (128–153)			0.66
Diastolic BP (mmHg)[a]	81 (74–88)			76 (70–84)			<0.001
Body mass index (kg/m^2)[a]	28.1 (24.9–31.6)			29.0 (26.1–32.9)			0.28
HbA_{1c} (%)[a]	7.8 (6.8–9.0)			7.6 (6.6–8.8)			0.16
Glucose (mmol/l)[a]	10.1 (7.0–14.4)			9.6 (7.0–13.3)			0.74
Creatinine (mmol/l)[a]	87 (76–100)			98 (84–121)			<0.001
CRP (mg/l)[a]	1.61 (0.91–2.90)			1.86 (1.03–3.45)			0.05
LDL-C (mmol/l)	2.9 (0.9)			2.6 (0.9)			<0.001
TAOS (%)	44.8 (12.8)			44.2 (13.10)			0.51
ACEI (no/yes)	314/215 (59.4%/40.6%)			87/115 (43.1%/56.9%)			<0.001
Aspirin (no/yes)	348/181 (65.7%/34.3%)			53/149 (26.0%/74.0%)			<0.001
Insulin (no/yes)	278/251 (52.7%/47.3%)			127/75 (62.9%/37.1%)			0.013
Statin (no/yes)	440/89 (83.1%/16.9%)			91/111 (45.2%/54.8%)			<0.001
APOA4 T347S distribution	TT	TS	SS	TT	TS	SS	0.786
N =	321	193	15	126	69	7	
Rare allele frequency	0.21	[0.15–0.27]		0.21	[0.15–0.26]		

ACEI, angiotensin-converting enzyme inhibitor; BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; LDL-C, LDL cholesterol; TAOS, total antioxidant status; UDACS, University College Diabetes and Cardiovascular Study. Mean (SD) or median (interquartile range) shown. Analysis was performed by two-sided *t*-tests after appropriate transformation. χ^2 tests were used to compare groups.

[a] Log transformed.

[b] Square root transformed.

TABLE 2. Baseline characteristics by *APOA4* T347S in UDACS

Trait	T347S Genotype				
	TT N = 447	TS N = 262	SS N = 22	*P* (3-way)	*P* (TT/TS vs. SS)
Age (years)	62.1 (13.7)	63.2 (13.6)	64.2 (15.4)	0.50	0.57
Sex (F/M)	182/265 (40.8%/59.2%)	103/159 (39.4%/60.6%)	4/18 (18.2%/81.8%)	0.12	0.04
Duration (years)[b]	10.5 (5.0–10.0)	11.0 (5.0–20.0)	10.5 (4.0–22.0)	0.55	0.69
Systolic BP (mmHg)[a]	138 (126–150)	140 (128–151)	136 (114–143)	0.14	0.10
Diastolic BP (mmHg)[a]	80 (73–87)	80 (73–87)	80 (71–84)	0.16	0.07
Body mass index (kg/m^2)[a]	28.5 (25.2–31.7)	28.4 (25.4–31.6)	30.5 (24.3–32.7)	0.70	0.40
HbA_{1c} (%)[a]	7.7 (6.6–8.9)	7.9 (6.8–9.1)	7.5 (7.0–8.2)	0.15	0.64
Glucose (mmol/l)[a]	9.8 (7.0–14.0)	10.3 (7.5–14.7)	8.1 (5.0–13.3)	0.06	0.07
Creatinine (mmol/l)[a]	90 (77–107)	89 (78–104)	95 (79–113)	0.98	0.94
CRP (mg/l)[a]	1.66 (0.92–2.83)	1.68 (0.93–3.32)	1.64 (0.98–2.70)	0.92	0.90
LDL-C (mmol/l)	2.8 (1.0)	2.9 (0.9)	2.9 (1.3)	0.51	0.64
Plasma TAOS (%)	42.40 (13.17)	43.18 (12.67)	41.35 (10.60)	0.67	0.64
CVD (%)	321/126 (71.8%/28.2%)	193/69 (73.7%/26.3%)	15/7 (68.2%/31.8%)	0.79	0.66
ACEI (no/yes)	236/211 (52.8%/47.2%)	154/108 (58.8%/41.2%)	11/11 (50.0%/50.0%)	0.27	0.64
Aspirin (no/yes)	246/201 (55.1%/44.9%)	146/116 (55.6%/44.4%)	8/14 (38.1%/61.9%)	0.30	0.12
Insulin (no/yes)	261/186 (58.3%/41.7%)	134/128 (51.1%/48.9%)	11/11 (50.0%/50.0%)	0.16	0.60
Statin (no/yes)	324/123 (72.4%/27.6%)	192/70 (73.3%/26.7%)	16/6 (72.7%/27.3%)	0.97	1.00

TAOS, total antioxidant status. Mean (SD) or median (interquartile range) shown. Analysis was performed by two-sided *t*-tests after appropriate transformation. χ^2 tests used for categorical data.

[a] Log transformed.

[b] Square root transformed.

found to be present in the lowest quartile of TAOS ($P = 0.04$), suggesting greater oxidative stress in patients with CVD and in agreement with previous reports (18). Plasma TAOS provides a net measure of plasma oxidative stress. Previous studies have suggested that much of this effect may be related to plasma levels of proteins and uric acid (30). Unfortunately, these measures were not available in our current studies and hence could not be adjusted for in the analysis. However, after adjustment for those variables significantly correlated with plasma TAOS, the results remained unchanged. Even though plasma TAOS is not a highly specific measure of plasma oxidative stress, for a large number of samples, it is a practical and inexpensive method of assay. Other plasma markers may be difficult, expensive, and very time-consuming to measure, (e.g., plasma F_2-isoprostanes). Furthermore, there is evidence supporting the use of plasma TAOS as a marker of plasma oxidative stress (24, 31). In a previous study (31), we observed a highly significant correlation ($r = -0.65$; $P = 0.003$) between plasma TAOS and esterified F_2-isoprostanes.

Our previous findings in the prospective Northwick Park Heart Study II (NPHSII) demonstrated that individuals homozygous for the S347 variant had increased risk of CHD, independent of lipid parameters, which could be related to reduced plasma apoA-IV levels in S347 homozygotes, as seen in the healthy young men participating in the European Atherosclerosis Research Study II (7). This current report, demonstrating reduced TAOS in S347 homozygotes, provides insight into the possible mechanism for the S347 risk association. The data suggest that individuals homozygous for the S347 allele have reduced protection against free radical attack by ROS. Elevated ROS, and subsequent lipid peroxidation, would then result in the generation of a number of potentially atherogenic products. These include reactive aldehydes such as FDP (3-formyl-3,4-dehydropiperidino) lysine generated during the oxidation of LDL (32). Such aldehydes can react with OX-LDL apoB, leading to the internalization of OX-LDL particles by the scavenger receptors of macrophages, and the subsequent formation of foam cells (33, 34), hence contributing to the progression of atherosclerosis. We cannot at this point determine: *a*) whether the increased risk associated with S347 homozygosity is a result of inherent lower apoA-IV levels and therefore lower antioxidant potential; *b*) whether the apoA-IV S347 protein itself has poorer antioxidant activity compared with apoA-IV T347, and therefore S347 homozygotes have lower antioxidant potential; or *c*) whether, by accelerating postprandial lipid clearance, the S347 variant causes changes in lipid metabolism (e.g., increased hepatic oxidation of dietary polyunsaturated fats) that are themselves associated with an increased oxidation status.

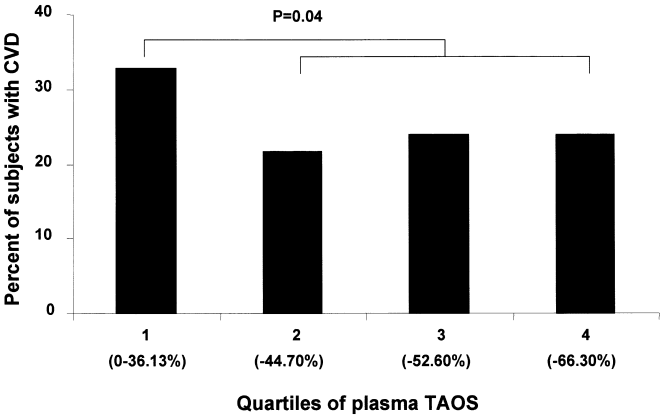

Fig. 1. Bar chart representing the percentage of patients with cardiovascular disease by quartiles of total antioxidant status (TAOS).

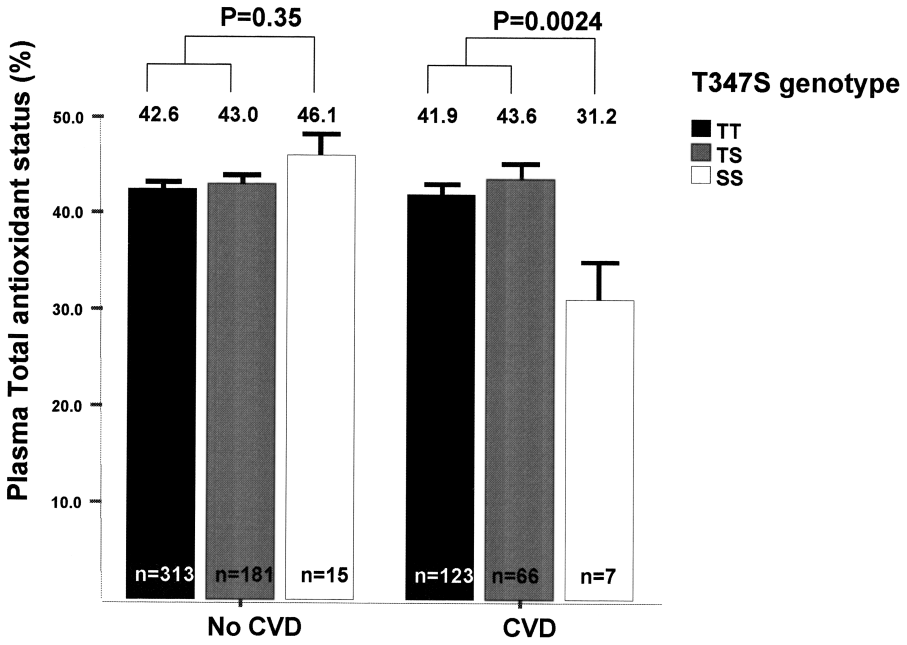

Fig. 2. Mean plasma TAOS (%, ±SE) in relation to the *APOA4* T347S genotype in the University College Diabetes and Cardiovascular Study subjects. Numbers of subjects are shown at the base of each column.

APOA4/Apoe$^{-/-}$ mice were found to be protected against atherosclerosis, without an increase in HDL concentration, and displayed reduced oxidative markers, including aldehyde-modified LDL (16). ApoA-IV was found to have accumulated in the damaged arterial wall of these animals and thus could be acting as an antioxidant in situ (16). Inhibition of Cu^{2+}-induced lipoprotein oxidation by human apoA-IV results in reduced tryptophan fluorescence, suggesting the prevention of Cu^{2+} binding to lipoprotein particles and causing lipid peroxidation, implying a chelating role of apoA-IV in preventing lipid peroxidation (35).

Analyzing the predicted amphipathic helices of apoA-IV from residues 330–351, the threonine at position 347 is found to be located within the hydrophilic face of the helix. Substitution of another neutral amino acid, serine, at this position is unlikely to alter the charge distribution of the helix. Thus, the T347S polymorphism would at first sight be predicted to have no effect on helix conformation or lipid affinity. However, computational analysis revealed that compared with wild type, this isoform has a slight increase in hydrophilicity (2). Secondary structure prediction using the method of Garnier, Osguthorpe, and Robson (36) suggests that the threonine at position 347 is at the end of 13 amino acids within a helical conformation. Serine at position 347 is predicted to extend an adjacent region containing a stretch of four residues in coil conformation to five (2). Thus, these structural predictions of the S347 isoprotein suggest that it could have reduced lipid affinity (2) and thus possibly be more prone to degradation. Rewers et al. (37) reported that patients with diabetes who carried the apoA-IV-2 isoform, encoded by the H360 allele, had significantly increased risk of myocardial infarction compared with those homozygous for the apoA-IV-1 isoform (Q360). This risk difference was even higher in H360 carriers if they were obese. However, in nondiabetic individuals, this genotype effect on risk was not seen. The H360 allele is thought to delay postprandial triglyceride clearance (4) and thus alter lipid metabolism. In diabetic patients with perturbed lipid metabolism, this may have a more pronounced effect on risk of CHD.

It is worth noting that *APOA4* S347 is in strong linkage disequilibrium (LD) with flanking SNPs, and that the effect of the *APOA4* S347 variant on apoA-IV plasma levels could be directly modulated by the *APOA4-APOC3* intergenic −2854T>G variant in strong LD with it ($D' = 0.76$; $P < 0.0005$) (38). The −2854T>G is located in close proximity to a hepatic nuclear factor 4 (HNF4) responsive element, and the two variants, S347 and −2854T, together define a haplotype associated with increased CHD risk (7). Enhancers that mapped within the *APOA4-APOC3* intergenic region are known to coregulate the expression of the entire *APOA4-C3-A1* cluster and not just *APOA4* (39). Hence, altered enhancer activity due to the –2845T>C change could directly impact on apoA-IV levels and possibly, as well, on apoC-III and apoA-I levels.

Functional studies to deduce whether the T347S amino acid change could affect the antioxidative properties of apoA-IV need to be performed, and these studies are under way. It is not possible to relate apoA-IV levels directly to the TAOS measures in the present study, because plasma apoA-IV measures were not available in UDACS subjects. We previously reported that S347 homozygotes had 8.4% lower plasma apoA-IV levels than T347 carriers, while in the present study of middle aged diabetic CVD patients, those who were S347 homozygotes had 26.5% lower TAOS than T347 carriers. This lack of correspon-

dence might merely reflect the very different health status of the two cohorts. Taken together our results suggest that S347 homozygotes have lower apoA-IV levels, which would directly or indirectly result in reduced TAOS, increased oxidative stress, and therefore increased risk of CHD.

We compared the frequency of the T347S variant in the UDAC study with that previously reported in 2,192 Caucasian men in the prospective NPHSII (excluding those with a diagnosis of diabetes, n = 71). The frequency of the S347 allele in the UDACS cohort [0.208 (0.19, 0.23)] was significantly higher than in the NPHSII study [0.184 (0.17, 0.20)] ($P = 0.04$). This supports the concept of the clustering of CVD risk genes in patients with diabetes and the increased progression of CVD in those patients (40). Diabetes is a major risk factor for CVD and is associated with enhanced oxidative stress and subsequently increased lipid peroxidation (41, 42). In such high-risk patients, apoA-IV may play a particularly important protective role, and our results suggest that the mechanism of this might be protection against lipid peroxidation and, thus, inhibition of the progression of atherosclerosis.

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