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SHORT COMMUNICATION

The apolipoprotein A-V genotype and plasma apolipoprotein A-V and triglyceride levels: prospective risk of type 2 diabetes. Results from the Northwick Park Heart Study II

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Abstract
Aims/hypothesis We sought to establish the relationship between plasma apolipoprotein A-V (APOA5, previously known as apoA-V) and triglyceride levels and to determine the impact of the *APOA5* genotype on APOA5 levels and development of type 2 diabetes in a 15-year follow-up study of healthy UK men.
Materials and methods *APOA5* −1131T>C and S19W genotypes were determined in 2,490 men, of whom 145 subsequently developed type 2 diabetes. In a subset of 299 men, we also determined APOA5 levels.
Results Plasma APOA5 levels positively correlated with triglycerides (r=0.18, p<0.002) and were not different in men who subsequently developed type 2 diabetes compared with healthy men (p=0.7). Carriers of either *APOA5* W19 or −1131C had, as expected, higher plasma triglycerides. However, while W19 carriers had significantly higher APOA5 levels (p=0.0003), APOA5 levels were not associated with −1131T>C (p=0.63), reinforcing the idea that the reported −1131C association with triglycerides levels is due to linkage disequilibrium with variants in the *APOC3* gene, and not due to the direct effect on APOA5 levels. Overall no effect of *APOA5* −1131T>C or S19W was found on type 2 diabetes risk.
Conclusions/interpretation In contrast to animal studies, in man, plasma APOA5 positively correlates with plasma triglyceride levels. In prospective analysis, with the caveat that numbers were small, *APOA5* genotypes do not appear to have an impact on risk of development of type 2 diabetes.

Keywords APOA5 · ApoAV levels · Gene · Type 2 diabetes risk

Abbreviations

APOA5	Apolipoprotein A-V
APOC3	Apolipoprotein C III
HR	Hazard ratio
NPHSII	Northwick Park Heart Study II
SNP	Single-nucleotide polymorphism
TG	Triglyceride

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Introduction

Overexpression and knock-out mouse models of apolipoprotein A-V (APOA5, previously known as apoAV) demonstrate an inverse relationship between APOA5 and triglyceride (TG) levels [1] and show that APOA5 plays a key role in TG metabolism. This is supported by epidemiological studies reporting that variation in the *APOA5* gene

is consistently associated with differences in plasma TG levels in healthy individuals [2, 3] of different ethnicity [2]. Since hypertriglyceridaemia reflects the dyslipidaemia associated with type 2 diabetes, variation in *APOA5* could contribute to the development of type 2 diabetes. Two haplotypes, *APOA5*2* and *APOA5*3,* show association with raised plasma TG levels [2] and can be 'tagged' by the rare genotype for −1131T>C and coding single nucleotide polymorphism *(*SNP) 56C>G (S19W), respectively [4].

In this study we examined the correlation of plasma APOA5 with TG levels and the association with *APOA5* genotypes in a subset of the men participating in the prospective Northwick Park Heart Study II (NPHSII). We also examined the association of two *APOA5* SNPs with the 15-year prospective risk of developing type 2 diabetes in these middle-aged UK men, who entered the study in good health.

Subjects, materials and methods

Northwick Park Heart Study II

Between 1989 and 1994, a total of 3,012 healthy middle-aged Caucasian men were recruited from nine primary care practices in the UK for prospective surveillance. The study was approved by local ethics committees and all subjects gave written informed consent. Full details of the study are presented elsewhere [5]. In 2004, information on the development of type 2 diabetes since baseline was obtained from the medical practices by searching individual records for a diagnosis and treatment of type 2 diabetes [6]. Type 2 diabetes was diagnosed on the basis of the WHO guidelines in place at the time of diagnosis.

Determination of plasma APOA5

Plasma APOA5 levels were determined by ELISA [7] in 299 randomly selected samples.

DNA extraction and APOA5 genotyping

DNA extraction and *APOA5* −1131T>C (rs662799) and S19W (rs3135506) genotyping methods have already been fully detailed [3]. Genotype was confirmed by two independent researchers blind to subject outcome, with discrepancies resolved by repeat genotyping.

Statistical analysis

Baseline characteristics for plasma TG and APOA5 levels were log transformed to a normal distribution. Results are presented as hazard ratios (HRs) obtained from Cox regression models with their corresponding 95% CIs, adjusted for age and practice (recruitment site) with further adjustment as described. Frequencies were compared by Fisher's exact test. Statistical significance was taken as $p<0.05$.

Results

Subject characteristics

Of the 3,052 men recruited into the study, 76 with type 2 diabetes on entry were excluded from further analysis. Full phenotypic and *APOA5* genotypic data were available on 2,490 men for −1131T>C and 2,431 men for the S19W, and in this group 145 men developed type 2 diabetes during follow-up. The baseline characteristics of these men have been reported elsewhere [6]. Baseline BMI, TG, cholesterol, HDL and blood pressure were all associated with increased risk of developing type 2 diabetes, with BMI conferring the highest risk (HR=1.86 [1.65–2.10], $p<0.0001$, per increase of 1 SD) [6].

Plasma APOA5 levels

Plasma APOA5 levels were measured in a subset of 299 men chosen at random. Baseline characteristics of these men were not significantly different from those in the entire cohort (data not shown). The mean plasma APOA5 levels were 113.6±50.3 ng/ml (SD) and, contrary to expectation, APOA5 was positively correlated to TG ($r=0.18$, $p<0.002$), total cholesterol ($r=0.15$, $p=0.008$), C-reactive protein ($r=0.15$, $p=0.01$) and BMI ($r=0.18$, $p=0.002$). There was no difference in APOA5 levels when stratified by future development of type 2 diabetes (non-type 2 diabetes, 112.8± 50.3 ng/ml ($n=273$), vs type 2 diabetes, 117.8±48.1 ng/ml, $p=0.71$, $n=16$), but numbers of men with type 2 diabetes in this group are small. Carriers of W19 had significantly higher plasma APOA5 levels than S19 homozygotes ($p=0.0003$) independently of BMI, TG and cholesterol ($p=0.0001$), and in the sample as a whole had significantly higher TG levels ($p=0.007$) (Fig. 1). Carriers of the −1131C allele had higher TG levels than −1131TT men ($p=0.003$); however, there was no genotype association with plasma APOA5 levels.

Effect of APOA5 genotype on plasma TG and future risk of diabetes

The distributions of the *APOA5* S19W and −1131T>C genotypes were in Hardy–Weinberg equilibrium. There was no significant genotype or allele frequency difference between those who subsequently developed type 2 diabetes and those who did not (Table 1).

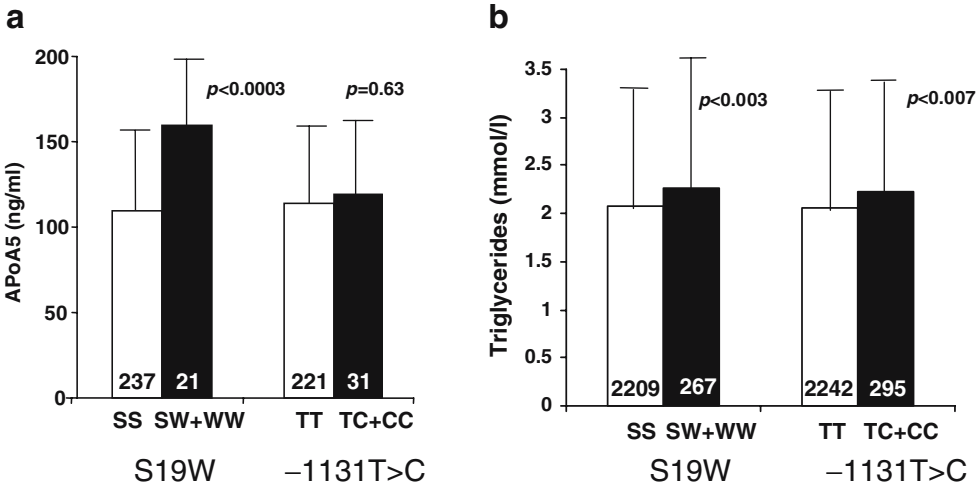

Fig. 1 Mean unadjusted plasma APOA5 (±SEM) (**a**) and triglycerides (±SEM) (**b**) in men by *APOA5* S19W and −1131T>C genotype. The number of each genotype class is shown at the *foot of the bar*

Discussion

Plasma APOA5 and TG

In contrast to the prediction from the APOA5 mouse models of an inverse relationship between APOA5 and TG levels [1], the major finding of this study is the positive correlation between plasma APOA5 and TG. Circulating APOA5 levels are approximately 300-fold lower than APOC3, but if, as suggested, APOA5 recycles between particles [8], these low levels would be sufficient for its suggested catalytic role in TG metabolism. Thus total plasma APOA5 might not be as critical as the relative distribution of APOA5 between HDL and VLDL particles. The reason for the positive correlation between APOA5 and TG in healthy men and the negative correlation between the two in mouse models is unclear, but it suggests that in this case extrapolation from animal models to man should be made with caution.

APOA5 genotypes and APOA5 levels

The functionality of S19W has been demonstrated in vitro, with the W19-signal peptide showing 50% lower secretion of a reporter protein than the S19-signal peptide [9]. It is thus surprising that, compared with S19 homozygotes, W19 carriers had significantly higher APOA5 levels. Circulating mature APOA5 no longer has a signal sequence and will be identical whether the signal peptide originally had a serine or a tryptophan at residue 19. In order to explain the discrepancy between predicted and observed plasma levels of APOA5 by genotype, these data suggest that APOA5 of W19 origin is less efficiently degraded or cleared. One possibility is that APOA5 of S19 or W19 origin could be associated with different types of lipoprotein particles of different metabolic fates. This concept is supported by the different lipoprotein profiles of people with different S19W genotypes [10].

Table 1 Rare allele frequencies of *APOA5* S19W and −1131T>C and hazard ratios (HR) in subjects who went on to develop type 2 diabetes and those who did not, in the Northwick Park Heart Study II

	Non-diabetic	Diabetic	*p*-value for difference	HR[a] (95% CI)	HR[b] (95% CI)
S19W					
SS	2,027	133	0.58	SS 1.00	1.00
SW	248	12	–	W+0.67 (0.36–1.23)	0.62 (0.33–1.15)
WW	11	0	–	*p*=0.20	*p*=0.13
Allele frequency (95% CI)	0.059 (0.05–0.07)	0.041 (0.02–0.06)	0.21	–	–
−1131T>C					
TT	2,067	131	0.38	TT 1.00	1.00
TC	267	11	–	C+0.56 (0.29–1.07)	0.51 (0.27–0.98)
CC	14	0	–	*p*=0.08	*p*=0.04
Allele frequency (95% CI)	0.063 (0.06–0.07)	0.039 (0.02–0.06)	0.10	–	–

[a] Adjusted for age and recruitment practice
[b] Adjusted for age, recruitment practice and TGs

The −1131T>C SNP was not associated with differences in APOA5 levels, although the −1131C allele was associated with higher TG levels [2, 3]. Published molecular analysis does not support the functionality of 1131T>C or other SNPs that define haplotype *APOA5*2* [9]. Haplotype analysis predicts that −1131T>C acts as a genetic marker for variants within the closely linked *APOC3* gene, due to strong linkage disequilibrium [3], thus the lack of association with plasma APOA5 levels is not unexpected.

APOA5 genotype and type 2 diabetes risk

This study had 80% power at the 5% level to detect a relative risk of 1.77 for diabetes for 19W+vs 19SS (or relative risk of 0.37 if association is negative), and a relative risk of 1.74 (or 0.38) for −1131C+vs −1131TT. This limited power to detect an effect of *APOA5* genotypes on future risk of type 2 diabetes suggests that any such risk would be less than 1.77. Since numbers of affected men who carried the rare alleles of either *APOA5* SNP in this study were low, these results need to be confirmed in a larger study, and in addition in women. One of the limitations of this study is the absence of any fasting glucose or insulin measures at the baseline assessment or subsequent follow-up visits to confirm diagnosis. Identification of the men with type 2 diabetes by the medical record search is unlikely to include any false-positive diagnosis but in the absence of a full recall for fasting glucose testing some subjects may have been missed.

Most of what we know about APOA5 is inferred from animal and in vitro studies, which suggested that the potential actions of APOA5 are intracellular inhibition of hepatic VLDL secretion and the promotion of catabolism of TG-rich plasma lipoproteins [11]. These present results highlight the need for additional in vivo and in vitro studies to clarify the role of APOA5 in human TG metabolism.

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Duality of interest The authors have stated that there was no duality of interest associated with this study.

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