

POLYMORPHISMS IN THE APOLIPOPROTEIN L1 GENE AND THEIR EFFECTS ON BLOOD LIPID AND GLUCOSE LEVELS IN MIDDLE AGE MALES

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[Received May 11, 2006; Accepted July 7, 2006]

ABSTRACT: *Apolipoprotein L1 in plasma is associated with high-density lipoprotein. Novel APOL1 polymorphisms are investigated along with the association of two common haplotypes (Lys166Glu, Ile244Met, Lys271Arg) with circulating lipid and glucose levels. Although the amino acid substitutions occur in the amphipathic alpha helices region involved in lipid binding, these substitutions were found not to independently account for variability in circulating lipid and glucose levels in 149 middle age males.*

KEY WORDS: Apolipoprotein L, Blood Lipids, Glucose, Polymorphisms

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INTRODUCTION

Apolipoprotein L1 (APOL1) was first identified and characterised in 1997 (Duchateau et al., 1997) and in 2001, we reported the cloning of five further apolipoprotein L (APOL) proteins (Page et al., 2001) yielding a new gene family consisting of six proteins (APOL1 to 6) (Page et al., 2001; Duchateau et al., 2001). Although the precise functions of APOL proteins have not as yet been fully described, each APOL protein shares significant identity within their predicted amphipathic alpha helices (Page et al., 2001) in areas which is suggestive of a role in lipid binding (Duchateau et al., 1997; Page et al., 2001). In the circulation APOL1 is found associated with large APOA1 containing high-density lipoprotein (HDL) particles representing a distinct marker of this HDL sub-population (Duchateau et al., 1997). Moreover, four clustered polymorphisms in APOL1 which exist in linkage disequilibrium have been described, three of which cause amino acid substitutions (Lys166Glu A/G, Ile244Met A/G, Lys271Arg A/G) (Duchateau et al., 2001) in these amphipathic regions, while the other is silent (Ala218Ala A/C).

In the current paper, we report the identification of two more polymorphisms (Pro234Ala C/G and Arg336Arg A/G) in the APOL1 gene and study the association between the two common haplotypes of APOL1 on circulating lipid and glucose levels.

MATERIALS AND METHODS

One hundred and forty nine normolipidaemic and mildly hyperlipidaemic (total cholesterol, 3.8-8.0mmol/l; triglycerides (TAG)0.7-3.6mmol/l) adult males, aged 35-70y, were recruited. Individuals on hypolipidaemic therapy or other medication known to interfere with lipid metabolism were excluded. The study was approved by the University of Reading Ethics Committee and each participant provided written consent prior to participation. Venous blood samples were collected in potassium-EDTA tubes, centrifuged at 1600 g for 10 min and plasma collected for analysis. HDL-cholesterol (HDL-C) was determined after precipitation of the APOB containing lipoproteins in a sub-sample of plasma using dextran sulphate and magnesium chloride and cholesterol levels measured in the supernatant. Samples were analysed for TAG, cholesterol and glucose using a Monarch automatic analyser and enzymatic colorimetric kits (Instrumentation Laboratories Ltd., Warrington, UK). Low-density lipoprotein cholesterol (LDL-C) levels were computed using the Friedewald formula (Friedewald and Levy, 1972). Age, body mass index (BMI) and blood pressure (systolic SBP and diastolic DBP) were also obtained for each subject (Table 1). Genomic DNA was extracted from buffy coats using the QIAamp DNA Blood Midi Kit (Qiagen Ltd., Crawley, UK). PCR primers (5'-GGCACGATAAAGGCCAGCAGTACAGAAACTG-3' and 5'-GCCCCTGCCAGGCATATCTCTCCTGGT-3' were designed to APOL1 (AF305224) (Page et al., 2001). PCR was performed, products purified and cloned before sequencing as previously described (Page et al., 2001). Restriction length fragment polymorphism (RLFP) analysis was also performed on PCR products by digestion with either *Nco*I or *Hind*III (Promega, Southampton, UK). All data was tested for normality and skewed data sets were log transformed (\log_{10}) where necessary. Independent

student t-tests and the Mann Whitney tests were used to determine the significance of any inter-group differences. Stepwise multiple regression was performed, using a P-in of 0.05 and P-out of 0.10 in order to establish the independence of associations of the metabolic variables. A P-value < 0.05 was considered significant. All statistical analysis was performed using the SPSS statistical package (Version 10.0, Chicago, USA).

Table 1. Anthropometric and fasting lipid and glucose levels of the group overall (n=149) and according to APOL1 genotype.

VARIABLE	ALL (N=149)	APOL1 HH(N=83)	APOL1 Hh(N=62)	P'
age (years)	50(1)	50(1)	50(2)	0.810
BMI (kg/m ²)	27.5(0.3)	27.3(0.4)	27.8(0.4)	0.193
TC (mmol/l)	5.55(0.07)	5.53(0.10)	5.58(0.11)	0.522
LDL-C (mmol/l)	3.58(0.06)	3.56(0.09)	3.60(0.10)	0.714
HDL-C (mmol/l)	1.22(0.02)	1.22(0.03)	1.24(0.04)	0.594
TAG (mmol/l)	1.65(0.05)	1.65(0.07)	1.63(0.08)	0.792
Glucose (mmol/l)	5.33(0.10)	5.35(0.18)	5.29(0.08)	0.503
SBP (mmHg)	136(2)	134(2)	138(3)	0.367
DBP (mmHg)	82(1)	81(1)	82(1)	0.979

APOL1 - HH homozygous haplotype; APOL1 - Hh heterozygous haplotype. Abbreviations; BMI- body mass index; TC- total cholesterol; HDL-C- high-density lipoprotein cholesterol; LDL-C- low-density lipoprotein cholesterol; TAG- triglycerides; SBP- systolic blood pressure; DBP- diastolic blood pressure. The inter-APOL1 group differences were analysed by Independent student tests and the Mann-Whitney tests; P<0.05.

RESULTS

DNA sequencing of the 149 adult males led to the confirmation of a cluster of six single nucleotide polymorphisms within a 513 bp region of exon 6. Four of these polymorphisms were already known (Duchateau et al., 2001), three representing conserved amino acid substitutions (Lys/Glu166 AAG/GAG, Ile/Met244 ATA/ATG, Lys/Arg271 AAG/AGG), the fourth being silent (Ala/Ala234 GCA/GCC). These occurred all at the same allele frequency of 0.75/0.25, respectively confirming the results of Duchateau et al. (Duchateau et al., 2001) that all are found in linkage disequilibrium. The two novel polymorphisms were Pro234Ala (a non-conserved substitution) and Arg336Arg (silent). The former represents a nucleotide substitution from C to G in the first position of the codon for amino acid 234 (*i.e.* Pro/Ala234, CCA/GCA). This represented a rare polymorphism only found in two of the men occurring at a frequency of 0.013/0.987 within the 149 males genotyped. It was also found only associated with the more frequent haplotype (Lys166, Ile244, Lys271). The sixth polymorphism Arg336Arg (AGA/AGG) was found to occur at the same frequency as the already known four polymorphisms (0.75/0.25). All five polymorphisms were found to be in strong linkage disequilibrium, extending the region of the two known haplotypes present in APOL1. From our sequencing and RLFP data we typed all five common polymorphisms in the 149 subjects and calculated the frequencies of the homozygous and heterozygous occurrence of each of the common and rare haplotypes. Individuals were classified as either homozygous for the common Lys166/Ile244/Lys271

haplotype (HH, n = 83, 55.7%), or homozygous for the rare Glu166/Met244/Arg271 haplotype (hh, n = 4, 2.6%) or heterozygous if they possessed both of these haplotypes, (Hh, n = 62, 41.6%). The mean (SEM) age, BMI, TC, LDL-C, HDL-C, TAG, glucose, SBP and DBP of the group overall and according to APOL1 genotype were calculated (Table 1). A mean (SEM) age, BMI, TC and SBP/DBP of 50(1)y, 27.5(0.3)kg/m², 5.55(0.07)mmol/l and 136(2)/82(1) mmHg was observed for the group as a whole. Statistical analysis focussed on the common homozygous haplotype (HH) and the heterozygous haplotype (Hh), due to the small group number in the rare homozygous haplotype group (hh)(n=4). Age and BMI were found to be comparable in the two main APOL1 subgroups (Table 1), and there was no significant effect of the APOL1 polymorphisms on any of the lipid, BP or glucose parameters measured. Using stepwise multiple regressions only age (P=0.00) and BMI (P=0.00) emerged as being independent determinants of fasting TG levels, with an r² of 0.239 when both variables were included in the model. Only BMI and TG emerged as being independently associated with HDL-C, together accounting for 22.6% of the variability in this outcome measure. These associations between TG, HDL-C advancing age and increasing body mass has been previously reported.

DISCUSSION

Previously, observed associations between APOL1 levels and the HDL particle, its structural attributes and the presence of a steroid response element (SRE), are suggestive that APOL1 may act as a catalyst in lipid exchange between HDL and a variety of cells and triglyceride rich lipoproteins (Duchateau et al., 1997; Page et al., 2001; Duchateau et al., 2001; Monajemi et al., 2002; Duchateau et al., 2000). It is likely that APOL1 may act as a mediator of the activity of one or more of the enzymes involved in lipid exchange (Rye et al., 1999). Thus, it was speculated that mutations in this APOL gene could have an impact on the formation, hydrolysis and receptor mediated removal of HDL from the circulation and therefore overall HDL levels. However no impact of the described haplotypes in exon 6 on either circulating TG or HDL-C was observed. This suggests that such polymorphisms in the APOL1 gene may only cause subtle changes in protein structure which maybe consistent with the relatively conserved resultant amino acid substitutions. Indeed, within the APOL protein family the observed amino acid residue substitutions at each APOL1 polymorphism equate to similar amino acid residues as found previously (Page et al., 2001) at each site for the other members. For example, the corresponding residues at Lys166Glu in APOL1 are Glu (APOL2), Lys (APOL3, 4), Glu (APOL5) and Asp (APOL6). At Ile228Met in APOL1, Val (APOL2 to 4) and Leu (APOL5, 6) and at Lys271Arg in APOL1, Lys (APOL2, 3), Arg (APOL4), Gly (APOL5) and Glu (APOL6).

In the current investigation 2 novel APOL1 polymorphisms are reported. However no association between 5 common

polymorphisms in the APOLI gene, which is predicted to encode for the lipophilic region of the protein, and circulating lipid and glucose levels was evident. A limitation of the current study is the relatively small number of participants included, and additional investigations examining the impact of the haplotype described here and variations at other gene regions, on circulating APOLI and lipid and lipoprotein profile, are merited. Such information could help unravel the specific functions of this apolipoprotein subgroup and whether they have a protective role in cardiovascular disease.

ACKNOWLEDGEMENTS

The Medical Research Council (UK) supported this work. We would also like to thank Jan Luff for her technical assistance. There are no conflicts of interest.

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