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SHORT REPORT

Sex-specific interaction between *APOE* and *APOA5* variants and determination of plasma lipid levels

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The *APOA5* and *APOE* genes play an important role in determination of plasma levels of triglycerides (TG) and total cholesterol (TC). We have analyzed *APOA5* (T-1131>C and Ser19>Trp) and *APOE* (e2/e3/e4) variants in 2500 representatively selected Caucasians (1168 men, 1332 women). In female subjects (but not in male) an association between *APOE* polymorphism and TC was observed on the background of the common *APOA5* haplotype (TT-1131/SerSer19) – *APOE2* carriers have the lowest (5.12 (1.15) mmol/l) and the *APOE4* carriers have the highest (6.05 (1.06) mmol/l) levels of plasma TC ($P<0.001$). If at least one *APOA5* C-1131 or Trp19 allele was present, *APOE* exhibits no significant effect on plasma TC. *APOA5* did not affect plasma TG levels, if *APOE4* allele was present. In the presence of *APOE2* or *APOE3*, carriers of the *APOA5* alleles, C-1131 and/or Trp19, have higher TG levels (1.64 (1.05) mmol/l) than others (1.37 (0.75) mmol/l) ($P<0.01$). In male subjects, the same, but non-significant trend was observed.
In female subjects, we have detected an interaction between *APOE* and *APOA5* variants and plasma lipid levels.
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Introduction

Increased concentrations of plasma lipids (total cholesterol (TC) and triglycerides (TG) are a risk factor for myocardial infarction development,[1] and are known to be modified by both genetic and environmental factors.

Apolipoprotein E (APOE) is a structural component of TG-rich lipoproteins and it serves as a ligand for the lipoprotein receptors. Three common *APOE* isoforms (apoE4 (Cys112>Arg), apoE3 and apoE2 (Arg158>Cys)) are known. The *APOE4* allele is associated with high and the *APOE2* allele with low levels of TC.[2]

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Apolipoprotein A5 (APOA5) is located on TG-rich and on HDL-particles and plays a role in lipoprotein lipase activation.[3] In the *APOA5* gene, variants T-1131>C and Ser19>Trp are associated with differences in TG levels.[4,5]

The aim of our study was to evaluate if there is any interaction between the *APOE* and *APOA5* gene variations and plasma lipids in a large Caucasian population.

Materials and methods

Subjects

The 1168 men and 1332 women (response rate of 84%) represent a 3-year cohort of a selected 1% Czech Caucasian population sample. The individuals were recruited from nine districts in 1997–1998 and re-invited in 2000–2001 according to the WHO protocol (*MONICA Project*. Manual WHO/MNC 82.2, November 1983). Written informed

consent was obtained from the study participants and the local ethics committee approved the design of the study.

Genetic and biochemical analysis

DNA was isolated[6] and *APOE*[7] and *APOA5*[8] were genotyped as described before. The lipoprotein parameters were measured by the WHO Regional Lipid Reference Centre, IKEM, Prague on a Roche COBAS–MIRA autoanalyser, using reagents from Boehringer Mannheim Diagnostics and Hoffmann-La Roche.

Statistical analysis

Analysis of variance for repeated measures was used for the statistical analysis. The individuals with *APOE4/E2* genotypes and individuals, where not all genotypes were available' were excluded. According to *APOA5* genotypes, the individuals were separated into two subgroups; the carriers of the TT-1131/SerSer19 haplotype and the carriers of at least one allele C-1131 or Trp19. Within each group, the individuals were further separated as *APOE2* carriers, *APOE3E3* homozygotes and *APOE4* carriers. Values are given like mean (SD).

Results

Study population

The basic characteristics are summarized in Table 1. The distributions of *APOE* alleles (male/female: *APOE2* = 7.6/7.7%, *APOE3* = 82.6/82.5%, *APOE4* = 9.8/9.8%) and *APOA5*[8] genotypes are similar to the frequencies described for other Caucasians.[9] The distribution of combinations of *APOE* and *APOA5* genotypes is summarized in Table 2.

Effects of the individual *APOE* and *APOA5* genotypes

Both in male subjects and female subjects, *APOE2* carriers have the lower levels of TC and *APOE4* carriers have higher levels of TC in comparison with the *APOE3E3* homozygotes (Table 3a; $P<0.01$).

Also the *APOA5* gene has the usual effect on TG levels – individuals carrying the Trp19 and C-1131 alleles have higher levels of TG ($P<0.001$) than the others.[8]

Interaction between *APOE* and *APOA5* genotypes and plasma lipid levels

Total cholesterol and TG levels have been affected significantly by combinations of *APOE* genotypes and *APOA5* haplotypes in female subjects ($P<0.05$ for interaction, F = 3.95, d.f. 2; 1324). The effect of *APOE* was detectable only in individuals with the most common haplotype T-1131T/Ser19Ser ($P<0.001$ for trend; Table 3a). In individuals with at least one *APOA5* allele C-1131 and/or Trp19, *APOE* has no effect on TC levels (Table 3b). Additionally, if *APOE4* allele is present; there was no effect of *APOA5* on TG levels (Table 3b). TG levels are elevated in carriers of at least one *APOA5* allele C-1131 and/or Trp19 only if *APOE2* or *APOE3* alleles are present ($P<0.01$; $P<0.05$ for interaction, F = 4.02, d.f. 2; 1338).

Combinations of APOA5 and APOE variants showed no significant effects on the plasma lipids in male subjects.

Plasma TC were significantly lower in *APOE2* allele carriers in comparison with the *APOE4* carriers ($P<0.05$) regardless of the *APOA5* haplotype (Table 3a). Similarly, the carriers of the less common *APOA5* alleles have elevated TG levels in comparison to others.[8] We have detected no significant differences in TG levels after dividing these subgroups according the *APOE* genotypes despite the fact that a non-significant trend, similar to the results observed in female, is present (Table 3b).

Table 1 Basic characteristics of the analyzed individuals

	Male	*Female*
N	1168	1332
Age (years)	49.1 (10.9)	48.8 (10.5)
Cholesterol (mmol/l)	5.73 (1.04)	5.87 (1.15)
Triglycerides (mmol/l)	2.14 (1.59)	1.50 (0.38)
HDL-cholesterol (mmol/l)	1.24 (0.34)	1.51 (0.37)
BMI (kg/m^2)	28.2 (4.0)	27.4 (5.4)
Smoking prevalence (%)	32.7	25.4

Values are expressed as mean (SD).

Table 2 Distribution of the *APOA5* haplotypes and the groups of *APOE* genotypes in the Czech population

	APOA5							
	Male				*Female*			
	TT-1131/SerSer19		*'Others'*		*TT-1131/SerSer19*		*'Others'*	
APOE	N	%	N	%	N	%	N	%
+E2	111	9.5	40	3.4	129	9.7	52	3.9
E3E3	596	51.0	224	19.2	636	47.8	282	21.2
+E4	148	12.7	48	4.1	166	12.5	67	5.0

In *APOA5* subgroup 'Others' are individuals with at least one less common *APOA5* allele (C-1131 and/or Trp19).

Discussion

The final levels of plasma lipids are under polygenic control, with more variants in a couple of genes contributing in the total effect. These genes will affect plasma lipids together with external/environmental (smoking, dietary habits, menopausal status, physical activity, etc.) factors. This makes the discovery of gene–gene–environment interaction very complicated.

In our study, we have detected that the effect of interactions between variants in the *APOA5* and *APOE* genes on plasma lipids is more pronounced in female than in male subjects.

In women, carriers of at least one *APOA5* allele C-1131 or Trp19, *APOE* polymorphism has no effect on plasma TC.

Table 3 Plasma levels of total cholesterol (a) and triglycerides (b) according the *APOA5* haplotypes and the groups of *APOE* genotypes in the Czech population

a

	APOA5					
		Male			*Female*	
APOE	*Total*	*TT-1131/SerSer19*	*Others*	*Total*	*TT-1131/SerSer19*	*Others*
+E2	5.28 (0.99)	5.22 (1.09)	5.40 (1.12)	5.22 (1.04)	5.11 (1.15)	5.47 (1.02)
E3E3	5.82 (1.06)	5.78 (1.07)	5.90 (1.13)	5.74 (1.14)	5.69 (1.15)	5.88 (1.22)
+E4	5.85 (1.02)	5.81 (1.09)	5.95 (1.13)	5.96 (1.02)	6.03 (1.07)	5.79 (1.14)
P (*APOE*)	0.01	0.05	0.05	0.01	0.001	n.s.
P (*APOE/APOA5*)		n.s.			0.05	

b

	APOE					
		Male			*Female*	
APOA5	*+E2*	*E3E3*	*+E4*	*+E2*	*E3E3*	*+E4*
TT-1131/SerSer19	1.95 (1.40)	1.96 (1.22)	2.23 (2.05)	1.37 (0.74)	1.37 (0.75)	1.47 (0.77)
'Others'	2.26 (1.33)	2.24 (1.67)	2.16 (1.09)	1.68 (1.17)	1.62 (0.97)	1.44 (0.67)
P (*APOE/APOA5*)		n.s.			0.01	n.s.

In *APOA5* subgroup 'Others' are individuals with at least one less common *APOA5* allele (C-1131 and/or Trp19). Values are expressed as mean (SD).

APOE exhibit the effect on TC only in individuals with commonest *APOA5* haplotype T-1131T/Ser19Ser. Further, the effect of *APOA5* variants on TG levels was detectable in individuals with *APOE2E2*, *APOE3E3* or *APOE3E2* genotypes, but not if the *APOE4* was present. In men, a similar, non-significant trend was visible.

The number of published studies analyzing gene–gene interaction in connection to atherosclerosis is not high, but some interesting examples could be selected.

For example, an interaction between *APOE4* allele and peroxisome proliferator-activated receptor (*PPAR*; nuclear transcription factor) CT genotype and plasma TC and the risk of coronary heart disease was detected.[10] Furthermore, the combinations of the *APOE* and cholesterol ester transfer protein alleles have significant effect on plasma HDL-cholesterol levels.[11]

Finally, the *APOE–APOA5* interaction was analyzed in hypertriglyceridemic individuals. Schaefer *et al*[12] found six *APOE2/E2-APOA5Trp19* carriers out of 170 individuals with TG levels over 2.3 mmol/l and this combination was not detected in healthy normolipidemic individuals. We did not confirm this interaction, but in 111 patients with extreme TG levels (>10 mmol/l) the combination *APOE4–APOA5*Trp19 was overrepresented ($P<0.005$) in comparison with the healthy population ($N=2559$).[13]

Nothing is known about the possible mechanism of interaction between *APOE* and *APOA5* variants in genetic determination of plasma lipids. *APOE* negotiates the interaction of lipoprotein particles with lipoprotein particles and *APOA5* plays a role in the activation/stabilisation of lipoprotein lipase, but their role in lipid metabolism is still not completely understood. As both proteins are located on TG-rich particles, we can speculate about the possibility that lipoprotein particles in individuals with common *APOA5* haplotype have a slightly delayed half-life in plasma and thus the effect of *APOE* variants could be more efficient. Why this effect is more manifested in women than in men remains questionable.

It is evident that the effect of *APOE* genotype on lipid parameters may be modified by other genes. We conclude that there could be a sex-specific interaction between variants in *APOE* and *APOA5* genes, which may play a role in genetic determination of plasma levels of lipids in female subjects, but not in male subjects. The mechanism of the interaction needs to be analyzed in detail.

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