ONLINE MUTATION REPORT

Ser19→Trp polymorphism within the apolipoprotein *AV* gene in hypertriglyceridaemic people

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Cardiovascular disease (CVD) is the most common cause of death in industrialised countries. Raised plasma triglycerides (TGs) have been shown to be an independent risk factor for CVD.[1]

Recently, a new gene designated *APOAV* has been identified in the *APOAI/APOCIII/APOAIV* gene cluster by comparative sequencing by Pennacchio *et al*.[2] The human *APOAV* gene consists of four exons and codes for a 369 amino acid protein, which is only expressed in the liver. Generation of transgenic and knockout mice assessed the importance of this gene for plasma TG determination. The transgenic mice show decreased and the knockout mice increased concentrations of plasma TGs, whereas the plasma cholesterol concentrations are not influenced significantly.

In the human *APOAV* gene, T-1131C (originally referred to as SNP3) and Ser19→Trp polymorphisms have been detected.[2–4] Associations between these polymorphisms and TG concentrations have been found in healthy, non-smoking subjects not receiving lipid lowering medication as well as in different population samples. The C allele of the T-1131C polymorphism was found to be associated with extreme concentrations of plasma TGs.[5 6]

The aim of this study was to evaluate the putative association of a common *APOAV* variation (Ser19→Trp) in those with extreme plasma TG concentrations.

Key points

- A new apolipoprotein A*V* gene has been identified, which is expressed just in the liver. Generation of transgenic and knockout mice assessed the importance of apoAV for plasma triglyceride determination. Associations between T-1131→C and Ser19→Trp polymorphisms and plasma triglycerides have been found in population samples.
- This prompted us to study the Ser19→Trp polymorphism in 83 unrelated patients with extreme lipid indices (triglycerides of 20.4 (SD 12.8) mmol/l and total cholesterol of 10.4 (SD 3.7) mmol/l) and in a control group consisting of 2559 unrelated white people.
- In patients, the frequency of carriers of the Ser/Trp and Trp/Trp genotypes was much higher (30.1% *v* 14.1%, p<0.0001) compared to the population sample. This suggested a strong association between the Ser19→Trp polymorphism in the *APOAV* gene and extreme concentrations of plasma triglycerides.

SUBJECTS AND METHODS

The patients were selected from the database of the Prague Lipid Clinic of the 3rd Internal Department, which actively follows almost 2500 patients and has more than 30 years' experience in the diagnosis and treatment of lipid metabolism disorders. The group of patients consisted of 83 unrelated people (67 men and 16 women) aged 50.2 (SD 9.3) years with extreme lipid indices (TGs of 20.4 (SD 12.8) mmol/l and total cholesterol of 10.4 (SD 3.7) mmol/l). For inclusion in the study, initial lipid concentrations measured without any lipid lowering medication were considered.

In all patients the lipoprotein lipase (*LPL*) gene has been screened with heteroduplex analysis in an effort to detect some mutations.

A control group consisted of 2559 unrelated white people (1191 men and 1368 women, aged 28–67 years) selected as a 1% representative Czech population sample recruited as a follow up to the MONICA study. Their plasma lipid concentrations were as follows: TGs 2.0 (SD 1.3) mmol/l (men) and 1.5 (SD 0.8) mmol/l (women); and total cholesterol 5.8 (SD 1.0) mmol/l (men) and 5.8 (SD 1.2) mmol/l (women). Body mass index and smoking prevalence in the control group were comparable with the patient group, but there were more diabetic people among the patients (39.0% *v* 5.2%). Written informed consent was obtained from the study participants and the local ethics committee approved the design of the study.

DNA was isolated by a standard salting out method.[7] Oligonucleotides AV1-F 5′ TGC TCA CCT GGG CTC TGG CTC TTC and AV1-R 5′ CCA GAA GCC TTT CCG TGC CTG GGC GGC were used to amplify an *APOAV* fragment as described previously.[5 6] The polymerase chain reaction product (10 µl) was digested with 10 U of *Eco*52I (Fermentas) at 37°C overnight. The restriction fragments were analysed by 10% polyacrylamide microtitre array diagonal gel electrophoresis,[8] stained with ethidium bromide, and visualised on a UV transilluminator.

The lipoprotein indices were measured enzymatically in the Regional Lipid Reference Centre, IKEM, Prague, with a Roche COBAS MIRA autoanalyser, using conventional enzymatic methods. Body mass index was calculated as weight in kg divided by height in metres squared.

Statistical analysis was performed using the χ^2 test with Yates's correction.

RESULTS AND DISCUSSION

Heteroduplex analysis of the *LPL* gene did not detect any mutation in the 83 hypertriglyceridaemic patients; neither in the population nor in patient groups was *APOAV* polymorphism associated with diabetes.

The pattern of distribution of *APOAV* genotypes is summarised in table 1. The frequency of carriers of the Ser/Trp and Trp/Trp genotypes was much higher (p<0.0001) in patients with extreme TG concentrations compared to the population sample. Although not all hypertriglyceridaemic patients have the Trp allele, this result supports the importance of the *APOAV* gene in genetic determination of plasma TG concentrations.

Abbreviations: *APOAV*, apolipoprotein *AV*; CVD, cardiovascular disease; LPL, lipoprotein lipase; TGs, triglycerides;

Table 1 Allele distribution of the Ser19→Trp polymorphism in the *APOAV* gene in hypertriglyceridaemic patients and in controls (p<0.0001)

	Ser19→Trp polymorphism			
	Controls (2559)		Patients (83)	
	No	%	No	%
Ser/Ser	2198	85.9	58	69.9
Ser/Trp	352	13.8	22	26.5
Trp/Trp	9	0.3	3	3.6

Pennacchio *et al*[4] have found a higher frequency (23% *v* 9.5%) of Trp19 carriers in 82 male patients with plasma TG >90% compared to 82 patients from the opposite end of the distribution curve (TG <10%). A similar association was found in the groups of 50 and 50 females selected. A similar association was found in the groups of 50 and 50 females selected according to the same criteria (22% *v* 0%).

In the same groups of 83 patients and 2559 controls, we have previously detected[6] a strong association (p<0.0001) between T-1131C and C-1131C genotypes and extreme concentrations of plasma TGs. There is no linkage disequilibrium between rare alleles of both *APOAV* polymorphisms, thus the effect of both polymorphisms is independent.

Although the exact mechanism by which *APOAV* influences the plasma concentrations of TGs is unknown, the present results support the notion that this newly described gene is one of the most important genetic determinants of plasma triglycerides detected so far.

Our study included 83 unrelated hypertriglyceridaemic patients and 2559 representatively selected controls from the same population and suggested a strong association between the Ser19→Trp polymorphism in the *APOAV* gene and extreme concentrations of plasma TGs.

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