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Sex-specific effect of *APOAV* variant (Val153>Met) on plasma levels of high-density lipoprotein cholesterol

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Abstract

The importance of the *APOAV* gene for the determination of plasma triglyceride levels has been suggested by creations of transgenic and knockout mice and confirmed in population studies. We examined whether the newly detected *APOAV* variant is associated with plasma lipid levels and risk of myocardial infarction (MI). *APOAV* polymorphism (Val153>Met) was genotyped in 1191 males and 1368 females representatively selected from the Czech population. Lipid levels were analyzed in 1997 and 2001 in all individuals. Subsequently, we have analyzed the genotype frequencies of *APOAV* polymorphism in 435 male patients with MI. Val153>Met variation in the *APOAV* gene affects the plasma high-density lipoprotein cholesterol levels showing a higher level in Val/Val homozygotes than in Met carriers in both years (1.51 ± 0.36 and 1.52 ± 0.37 mmol/L compared with 1.42 ± 0.33 and 1.39 ± 0.35 mmol/L, $P < .01$). This association has been observed in females but not in males. Other analyzed lipid parameters (total cholesterol, low-density lipoprotein cholesterol, and triglycerides) have not been associated with *APOAV* Val153>Met variant. In a group of patients with MI, the frequency of the Met153 carriers was not significantly different from the male population sample (6.5% vs 6.4%). Val153>Met variation in the *APOAV* gene plays a sex-specific role in genetic determination of plasma high-density lipoprotein cholesterol levels, but does not influence risk of MI in males.

1. Introduction

High plasma triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) levels have been suggested as independent risk factors of cardiovascular disease development [1,2]. Similar to the other risk factors, it is estimated that the contribution of genetic and environmental factors to plasma lipid levels is approximately the same.

The apolipoprotein *AV* gene, a new member of the *APOAI/CIII/AIV* gene cluster, has been identified by comparative sequencing of human and mouse DNA by Pennacchio et al [3-5]. The human *APOAV* gene consists of 4 exons and codes for a 369–amino acid protein, expressed only in the liver. Generations of transgenic and knockout mice assessed the importance of this gene for plasma TG determination. The transgenic mice exhibited diminished levels of plasma TG, and the knockout mice exhibited elevated levels of plasma TG, although the plasma cholesterol levels were not influenced significantly.

Among the *APOAV* gene variants described, 2 (T-1131>C and Ser19>Trp) have been repetitively associated with plasma TG levels in studies with a different design.

In the first published study, an association was found between T-1131>C polymorphism and plasma levels of TG on random, high-fat as well as on low-fat diets in healthy nonsmokers [3]. The similar effect was found subsequently in population-based studies [6-11].

In 2 studies, C-1131 allele was found to be associated with extreme levels of plasma TG [12,13].

The second common *APOAV* variant, Ser19>Trp, was described shortly after detection of the *APOAV* gene [6,7]. In this study, it was also suggested that Trp19 carriers have significantly higher plasma levels of TGs, and this association was observed in males and females from different ethnic groups [6,7,10,11,14].

Another *APOAV* variant (Val153>Met) was described [15] in the Chinese population, but no association between this variant and plasma TG levels was detected [15].

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Table 1
Basic characteristics of the individuals involved in the study (control data are from 2001)

	Controls		Patients
	Males	Females	Males
n	1189	1368	435
Age (y)	49.2 ± 10.8	48.8 ± 10.6	55.1 ± 7.6
Cholesterol (mmol/L)	5.75 ± 1.06	5.80 ± 1.15	6.02 ± 1.26
TGs (mmol/L)	1.98 ± 1.28	1.46 ± 0.85	ND
HDL-C (mmol/L)	1.26 ± 0.33	1.50 ± 0.36	ND
BMI (kg/m^2)	28.2 ± 4.0	27.6 ± 5.5	28.0 ± 3.7
Diabetes	72 (6.0)	60 (4.4)	109 (25.0)
Hypertension	489 (41.1)	457 (33.4)	187 (43.0)
Smoking prevalence	389 (32.7)	348 (25.4)	113 (26.0)

Data are given as mean ± SD or n (%). ND indicates not determined.

Because of the important roles that *APOAV* variants play in plasma TG level determination, the aim of this study was to evaluate the putative association of the new common *APOAV* variation (Val153>Met) with plasma lipid levels in a large population-based study with white ethnicity and to analyze the genotype frequencies of this polymorphism in myocardial infarction (MI) survivors.

2. Subjects and methods

2.1. Subjects

The 2557 unrelated whites (1189 males and 1368 females, aged 28 to 67 years, response rate of 84%) included in this study represented a 3-year cohort of the selected sample of 1% of the Czech population [10,11,18]. The individuals were recruited from 9 Czech districts in 1997 to 1998 and reinvited in 2000 to 2001 according to the protocol used previously for the MONICA study [16]. The TG levels, total cholesterol, and HDL-C are available for all individuals in both years. Written informed consent was obtained from the study participants and the local ethic committee approved the design of the study. Basic characteristics of the controls are summarized in Table 1.

Four hundred thirty-five males younger than 65 years (average age, 55.1 ± 7.6 years) who had survived their first MI were also analyzed [10,11]. The blood samples for genetic and biochemical analyses were obtained within 48 hours of admission to coronary care units. As expected, patients with MI are older and have higher plasma cholesterol levels and diabetes prevalence, but do not differ in body mass index (BMI) levels, smoking prevalence (expressed as current smokers), and hypertension prevalence (Table 1).

2.2. DNA and biochemical analysis

Three milliliters of blood collected into EDTA tubes for DNA isolation were stored at −20°C. DNA was isolated by the standard method [17].

To genotype the Val153>Met (G457>A) polymorphism of the *APOAV* gene, oppositely oriented oligonucleotides AV153-F 5′ TGA TGG AGC AGG TGG CCC TGC GAG TGC AG and AV153-R 5′ TCA CCA GGC TCT CGG CGT ATG GGT GG and restriction enzyme *Bsh*1236I (Fermentas, Vilnius, Lithuania) were used as described in detail elsewhere [18].

Blood samples for lipid analysis were obtained after overnight fasting. The lipoprotein parameters were measured enzymatically by the World Health Organization Regional Lipid Reference Centre, IKEM Prague, on the Roche COBAS MIRA autoanalyzer (Basel, Switzerland), using conventional enzymatic methods with reagents from Hoffmann-La Roche (Basel, Switzerland). The BMI was calculated as the weight in kilograms divided by the square of height in meters.

2.3. Statistical analysis

Statistical analysis was performed using analysis of variance. Triglycerides were logarithmically transformed before the analysis to obtain the normal distribution of data.

Analysis of the association between the *APOAV* polymorphisms and plasma levels of cholesterol and TGs has been done separately for data from 1997 to 1998 and from 2000 to 2001.

3. Results

3.1. Population frequency of the Val153>Met alleles and genotypes

Distributions of the *APOAV* Val153>Met polymorphism genotypes are summarized in Table 2. The frequencies of the alleles and genotypes of the polymorphism are not different between males and females. We have detected 6.5% of the heterozygotes. Met153Met homozygotes are very rare—we have detected just one carrier of this combination in the entire population.

In the same population, *APOAV* T-1131>C and Ser19>Trp have also been analyzed [10,11]. No linkage disequilibrium of these variants with Val153>Met has been detected.

3.2. Val153>Met alleles and genotypes and plasma lipid levels

No significant association has been found between plasma total cholesterol or TG levels and this polymorphism either in the first or second survey, in either males or females.

In both years, the female *APOAV* Val153Val homozygotes had higher plasma HDL-C than Met carriers (1.51 ± 0.36 vs

Table 2
Distribution of the genotypes of the Val153>Met polymorphism in the *APOAV* gene in the Czech population and in patients with MI

	Males	Females	Patients with MI
Val/Val	1113 (93.6)	1280 (93.6)	407 (93.5)
Val/Met	76 (6.4)	87 (6.3)	26 (6.0)
Met/Met	0 (0)	1 (0.1)	2 (0.5)

Data are given as n (%).

Table 3
Effect of the Val153>Met polymorphism in the *APOAV* gene on plasma HDL-C levels (mmol/L)

	Male		Female	
	Val/Val (n = 1113)	+Met (n = 76)	Val/Val (n = 1280)	+Met (n = 88)
HDL (1998)	1.27 ± 0.33	1.25 ± 0.28	1.51 ± 0.36*	1.42 ± 0.33
HDL (2001)	1.23 ± 0.34	1.20 ± 0.33	1.52 ± 0.37*	1.39 ± 0.35
LDL-C (1998)	5.74 ± 1.16	5.99 ± 1.04	5.81 ± 1.16	5.69 ± 1.08
LDL-C (2001)	5.75 ± 1.05	5.80 ± 1.12	5.62 ± 1.21	5.54 ± 1.22
TG (1998)	2.10 ± 1.65	2.21 ± 1.47	1.44 ± 0.80	1.51 ± 0.86
TG (2001)	1.97 ± 1.39	2.11 ± 1.28	1.45 ± 0.85	1.56 ± 0.97

The effect of the polymorphism was observed just in females, but was not dependent on year of the analysis. Data are given as mean ± SD.

* $P < .01$ for each year.

1.42 ± 0.33 mmol/L in 1997/1998, $P < .01$, and 1.52 ± 0.37 vs 1.39 ± 0.35 mmol/L in 2000/2001, $P < .01$). BMI, smoking status, or menopausal status did not influence the effects of the analyzed variant on HDL-C significantly.

This association was not observed in the male subpopulation (Table 3).

No appreciable differences were detected in any of the associations when BMI, smoking status, and in females, menopausal status were also entered as covariates.

3.3. Val153>Met polymorphism and MI

The distribution of individual genotypes did not differ significantly between patients with MI and controls—the frequency of the Met153 carriers was 6.4% in the male population and 6.5% in patients with MI.

4. Discussion

The present study was performed to analyze the role of the new common variant of *APOAV* gene (Val153>Met) in the genetic determination of plasma total cholesterol, HDL-C, and TGs. The results of most of the associations' studies undertaken are not consistent and reproducible every time. The causes of the differences are insufficient numbers of individuals, and differences in sex, ethnicity, age, BMI, diets, and so on. To minimize the chance of false-positive or false-negative results, we have used the protocol of the MONICA study for selection of the examined individuals. This protocol was prepared for World Health Organization monitoring of cardiovascular risks and is accepted as one of the best possible selective criteria for preparation of a representative population sample. In addition, the selection of the regions in the Czech Republic uniformly covers geographic distribution of districts.

More than 2500 unrelated white individuals (equally distributed between both sexes, wide age range) whose lipid levels have been analyzed independently in 2 different years were included in this study. Thus, we believe that the design of the presented study reduces the chance of false-positive or false-negative results to a minimum.

The present investigation indicated that a recently described Val153>Met polymorphism in the *APOAV* gene is associated with plasma levels of HDL in a large group of white females, and that this association is not modulated by BMI, smoking, and menopausal status. In contrast, the effect of this variant on HDL levels was not detected in males, and no association between this variant and plasma levels of TG was detected.

Recently, it was described that apoA-V has an effect on lipid metabolism through activation of lipoprotein lipase [19]. It is not known how the apoA-V could affect the metabolism of HDL particles. In addition, we have no data regarding whether this variant (Val153Met) could alter the structure and/or function of *APOAV*.

Two previously detected *APOAV* polymorphisms (T-1131>C and Ser19>Trp) and their effect on plasma lipids have been analyzed in large population studies. The study of Talmud et al [7] included 2808 white males, and associations between rare alleles of both *APOAV* polymorphisms and higher plasma TG were observed. Pennacchio et al [6] analyzed males and females from different ethnic groups (848 whites, 1392 African Americans, and 420 Hispanics). Here, polymorphism Ser19>Trp was associated with plasma TG levels in whites and African Americans, but not in Hispanics. On the other hand, the impact of a second polymorphism (T-1131>C) was more pronounced in Hispanics than in whites and was not detectable in African Americans.

We have previously described a similar effect of both polymorphisms in the Czech whites. Although the absolute impact varied, we have found associations between TG levels and rare alleles of both *APOAV* polymorphisms in both sexes and in both years [10,11]. Furthermore, although plasma levels of TG are substantially changed after MI, we also suggest the effect of 1 *APOAV* polymorphism (Ser19>Trp) on TG levels in these patients [10]. In addition, we have found that the presence of the homozygosity of at least one less common *APOAV* allele is almost 4 times higher in patients with MI if compared with the population [10,11]. Other authors described higher frequency of the C-1131 carriers in patients with coronary artery disease indicated for bypass surgery [20].

Our results indicate that female *APOAV* Val153Val homozygotes expressed a beneficial lipoprotein profile—they have significantly higher plasma levels of HDL-C and they could be at lower risk of coronary heart disease than Met15 carriers. This hypothesis needs to be confirmed in a large case-control study of a group of females affected by MI.

It is well known that plasma levels of TG and HDL-C are inversely related. Interestingly, if summarized from some so far unexplained reasons, the T-1131C and Ser19Trp variants in *APOAV* have an effect on TG level, but not on HDL-C; inversely, according to our results, Val153Met has an effect on HDL, but not on TG levels. In the Czech Central European population, the frequency of the rare allele was much lower if compared with the Chinese population (~20%), where this allele was first analyzed [15]. In contrast to whites, Chinese

individuals exhibited no association between this variation and plasma lipid levels (TG, total cholesterol, and HDL-C).

Finally, we have found no difference in Met153 carriers' prevalence between male patients with MI and a population group of the same sex. This negative result is not surprising, as no effect between this variant and plasma lipids was observed in males.

We summarize that in contrast to other *APOAV* variants in a large population cohort, Val153>Met variation at the *APOAV* loci was not found to be playing a role in the determination of plasma TG levels either in males or females. In females, Val/Val homozygotes have significantly higher levels of HDL-C in both years of evaluation. Thus, the *APOAV* gene may not only be important in the genetic determinant of plasma TG levels, but it can also influence HDL concentration, at least in females as well.

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