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Gene variants at *FTO*, *9p21*, and *2q36.3* are age-independently associated with myocardial infarction in Czech men

J.A. Hubacek [a,*], M. Vrablik [b], D. Dlouha [a], V. Stanek [c], M. Gebauerova [c], V. Adamkova [d], R. Ceska [b], G. Dostálová [e], A. Linhart [e], L. Vitek [f], J. Pitha [a]

[a] *Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic*
[b] *3rd Department of Medicine, 1st Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic*
[c] *Cardiology Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic*
[d] *Preventive Cardiology Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic*
[e] *2rd Department of Medicine, 1st Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic*
[f] *Institute of Medical Chemistry and Laboratory Diagnostics and the 4th Department of Internal Medicine, 1st Faculty of Medicine, Charles University in Prague, Czech Republic*

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ABSTRACT

Aim: Cardiovascular disease (CVD) is a major cause of morbidity and mortality in developed countries. This study aimed to confirm the effect of common putative CVD-associated gene variants (*FTO* rs17817449, *KIF6* rs20455, *9p21* rs10757274 and *2q36.3* rs2943634) on CVD manifestation, and determine whether this effect differs between younger (< 50 years) and older CVD patients.
Methods: 1191 controls and 1889 MI patients were analyzed. All participants were Caucasian Czech males aged <65 years (532 were <50 years) who were examined at cardiology clinics in Prague, Czech Republic. Variants of *FTO*, *9p21*, *2q36.3*, and *KIF-6* were genotyped using PCR-RFLP or TaqMan assay.
Results: Variants of *FTO* (OR 1.48; 95% CI, 1.19–1.84 in a TT vs. GG comparison, p = 0.0005); *9p21* (OR 1.74; 95% CI, 1.41–2.14 in an AA vs. GG comparison, p = 0.0001); and *2q36.3* (OR 1.34; 95%CI, 1.09–1.65 in an AA vs. +C comparison, p = 0.006) were significantly associated with MI in the male Czech population. In contrast, genotype frequencies of *KIF-6* (rs20455) were the same in patients and controls (P = 1.00). Nearly identical results were observed when a subset of young MI patients (N = 532, aged <50 years) was analyzed.
Conclusion: We confirmed the importance of determining *FTO*, *9p21*, and *2q36.3* variants as part of the genetic determination of MI risk in the Czech male population.

1. Introduction

Cardiovascular diseases (CVDs) (e.g. myocardial infarction (MI) and cerebrovascular disease) are the most common causes of death in industrially developed countries and their incidence is increasing in other parts of the world [1].

For decades, there have been 5 major modifiable CVD risk factors (smoking, diabetes, abdominal obesity, hypertension, and dyslipidemia). In reality, however, these risk factors (even when combined) only accounted for a portion of CVD cases and the cause of CVD is not apparent in a substantial number of patients [2].

An important factor in the determination of CVD risk is associated with heritability/genetic predisposition. Studies of twins have shown that the probability of CVD death is several times higher in siblings of twins already affected by CVD, compared to siblings of unaffected twins [3]. However, despite intensive efforts in recent decades, knowledge of risk variants for particular genes still leaves much to be desired [4]. One reason for this may be due to research having been particularly concentrated on screening variants associated with the 5 major risk factors (which are also partially determined by environmental factors). As expected, analyses of these genes (e.g. apolipoproteins E and A5, lipoprotein lipase, PCSK9, etc.) [5–11] have shown certain contributions to CVD/CVD risk factor determination, but the contribution of risk size estimation (and its improvement) was relatively low and usually did not exceed 10%.

The new direction (using whole-genome scans) is the identification of genes associated with CVD via yet unknown mechanisms, or genes that remain associated with CVD after adjustments for traditional risk factors. There are already several suitable genetic markers that do not conform to traditional thinking regarding the cause of CVD [12].

Most of these markers are at the *9p21* locus on chromosome 9 [13]. Clinically significant variants are located in a gene-free region and the mechanism of how these variants affect CVD remains to be elucidated.

* Corresponding author at: Videnska 1958/9, 140 21 Prague 4, Czech Republic.
E-mail addresses: jahb@ikem.cz (J.A. Hubacek), vrablikm@seznam.cz (M. Vrablik), dadl@ikem.cz (D. Dlouha), vlst@ikem.cz (V. Stanek), mage@ikem.cz (M. Gebauerova), vead@ikem.cz (V. Adamkova), richard.ceska@vfn.cz (R. Ceska), Gabriela.Dostalova@vfn.cz (G. Dostálová), alinh@lf1.cuni.cz (A. Linhart), vitek@cesnet.cz (L. Vitek), japi@ikem.cz (J. Pitha).

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Another CVD risk candidate is the *FTO* (fat mass and obesity-associated) gene. It has primarily been described in relation to obesity [14], but other studies have shown that (after adjusting for BMI) *FTO* variants are also associated with diabetes [15]. Nevertheless, our pilot study found a relationship between *FTO* and CVD risk in non-diabetics [16]. Another suitable CVD risk candidate seems to be single nucleotide polymorphism (SNP) rs2943634, located in a gene-free region at 2q36.3 [17]. The last major candidate is the gene for kinesin-like protein 6, *KIF6* (rs20455), which has also been described (independently of traditional risk factors) as a risk factor for CVD development [18].

Despite the relatively high power of the first genome wide association studies, not all associations have been replicated in different populations (for example [19,20]). This is also true for Central and Eastern European Slavic populations, where some differences (i.e. environmental factors, or genetic background) could be expected [21–23].

Our study aimed to: 1) confirm/exclude the previously described associations between the abovementioned genetic variants and CVD risk in the Czech Slavic population; and 2) determine whether an association between these polymorphisms and CVD disease is more expressed in younger MI survivors.

2. Materials and methods

2.1. Samples

1889 male patients (self-reported Czech origin, aged 54.42 ± 8.14 years) who had survived their first myocardial infarction at an age < 65 years were analyzed [16,24,25]. Patients were routinely examined between April 2006 and August 2015 at the respective Cardiology departments of the Institute for Clinical and Experimental Medicine and General University Hospital in Prague, Czech Republic [26]. Within this group, 532 patients <50 years of age were identified.

As a controls, sample of 1191 males (49.0 ± 10.7 years) from the Czech Post-MONICA study [27–29] have been genotyped. These individuals were recruited between 2000 and 2001.

2.2. DNA analyses

DNA was isolated from frozen EDTA-blood using standard salting-out method. Polymorphisms of *FTO* [30,31] and *9p21* [24] were analyzed using PCR and restriction analysis, as described in details before.

Variant rs2943634 at *2q36.3* was examined via PCR-RFLP analysis. Genomic DNA amplifications were performed in a 25-μL volume using the primers 5′ aaa gca agc aca tct gtg gct gta c and 5′ tac act tga aaa ttg tag ttg ctc c. The PCR product (150 bp) was cut with 5 units of Bsp 1407I restriction enzyme. Restriction fragments of 26 bp and 124 bp represent the minor A allele, whereas the presence of an uncut product represents the major C allele.

The last variant, *KIF6* (rs20455), was genotyped using the TaqMan RT-PCR assay (Applied Biosystems, Life Technologies, Prague, Czech Republic; ID No.: C___3054799_10).

2.3. Risk factor analysis and definitions

Traditional risk factors were defined in the same manner as in our previous studies [24–26]. Specifically, i) current and/or past smokers; ii) dyslipidemia as total cholesterol over 5 mmol/L, and/or triacylglycerol over 2 mmol/L, or self-reported lipid-lowering treatment; iii) BMI ≥ 30 kg/m^2; iv) hypertension (self-reported treatment or blood pressure > 139 mm/89 mm); and v) diabetes as self-reported diabetes, and/or fasting glucose >7 mmol/L, and/or antidiabetic treatment.

Body weight was measured with an electronic weight scale (to the nearest 0.1 kg) and height was measured with a stadiometer (to the nearest 0.5 cm).

Additional data for controls and patients was obtained through personal questionnaires that were completed under the supervision of a trained nurse. Lipoprotein parameters in fasting plasma samples (drawn from patients the morning after admission to the coronary unit) were assessed using conventional enzymatic methods (reagents from Boehringer Mannheim Diagnostics and Hoffmann-La Roche) in a CDC Atlanta accredited laboratory.

Diastolic and systolic blood pressures were measured after 10 min in a sitting position with an automatic sphygmomanometer (BP-203 NA, Nippon Colin, Japan) and reported as an average of 3 readings on the right arm.

2.4. Statistical analyses

Patients and controls were primarily compared as an entire group. An additional subgroup of younger patients (<50 years) was also created and a subset of older unhealthy controls was used for comparison in an effort to increase the power to detect the differences in genetic predispositions. This subgroup included men >40 years who had a minimum of 3 CVD risk factors (i.e. smokers and diabetics with hypertension, dyslipidemia, and obesity) without (self-reported) CVD.

ANOVA was used to identify the potential effects of the analyzed variants on individual traditional CVD risk factors. The values are reported as means ± standard deviations. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for each genotype and genotypes used as a reference category were given a value of 1.00. Dominant, co-dominant, and recessive models were used for the analysis of the genotype differences between the examined groups.

Due to the large number of analyses, only P values < 0.01 were considered significant.

2.5. Ethics approval

Written informed consent was provided by all study participants. The study was approved by the respective institutions' ethics committees and was conducted according to Good Clinical Practice guidelines. The study protocol conformed to all ethical guidelines from the 1975 Declaration of Helsinki and was approved by the respective institution's Ethics Committees.

3. Results

Call rates for the individual polymorphisms were between 97.9% and 99.0% in patients, and between 96.0% and 98.9% in controls. The genotype frequencies of all analyzed SNPs were similar to those in previously studied Caucasian populations [13,14,17].

The basic anthropometric and biochemical characteristics for all study participants are summarized in Table 1.

As expected, the patient group had a significantly higher number of smokers, diabetics, and hypertensives. Obesity and dyslipidemia did not appear to be major CVD risk factors in these individuals.

In the control group, *KIF6* (rs20455), *9p21* (rs10757274), and *2q36.3* (rs2943634) were not associated with traditional CVD risk factors (data not shown in details).

Analyses of the individually studied genotypes confirmed that CVD risk is associated with some, but not all, variants (Table 2).

As was previously suggested by our pilot study [24], *9p21* (rs10757274) is the strongest genetic predictor of MI in the Czech population identified to date. Our extended study showed that carriers of at least one risky G allele had a 1.51 greater MI risk than AA homozygotes (95% CI, 1.28–1.79, P = 0.00001) and each G allele individually increased MI risk by approximately 35% (see Table 2 for further details).

We also confirmed a significant MI risk associated with the GG genotype of the rs17817449 polymorphism of the *FTO* gene with OR 1.48 (95% CI, 1.19–1.84, P = 0.0005) for TT vs. GG comparison.

Finally, a recessive analysis model detected a significantly lower MI risk in minor AA homozygotes for *2q36.3* (rs2943634) compared with major C allele carriers (OR 0.75, 95% CI, 0.61–0.92, P = 0.006).