INTERNATIONAL JOURNAL OF SYSTEMS APPLICATIONS, ENGINEERING & DEVELOPMENT
DOI: 10.46300/91015.2022.16.8 Volume 16, 2022

Lack of an association between a rs9818870 marker at the MRAS gene locus and acute coronary syndrome in Czech males

MRAS and ACS in Czechs

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Abstract:- Acute coronary syndrome (ACS) including myocardial infarction is one of the leading and preventable causes of death in industrialized countries. Conventional cardiovascular risk factors are responsible for approximately 50% of cases. Attention is therefore focused also on genetic variants that are not strongly associated with conventional risk factors. One of them is the rs9818870 marker within the MRAS gene (muscle RAS oncogen homolog-gene, OMIM 608435, 3q22.3), which was recognized as a risk factor for cardiovascular events in Western populations. We analyzed the relationship between the rs9818870 variant and the risk of ACS in the Czech population. Methods: Rs9818870 (C→T) variant was successfully analyzed by PCR-RFLP at the 1,122 control males, younger than 65 years (post-MONICA study) and 1,190 males, consecutive patients with ACS (younger than 65 years). ANOVA and chi-square were used for statistical analyses. Results: Rs9818870 polymorphism was not associated with conventional risk factors (plasma lipids, blood pressure, obesity, smoking, diabetes mellitus) in the control group. We have not detected any association between the DNA marker and ACS (controls – CC = 70.8%, CT = 26.4%, TT = 2.8% vs. patients – CC = 69.7%, CT= 28.2%, TT= 2.1%; P = 0.34). Conclusion: Rs9818870 variant within the MRAS gene region is not robust risk factor for ACS development in the Czech Slavonic males.

Keywords—MRAS; polymorphism, acute coronary syndrome, males, Slavonic

I. INTRODUCTION

Coronary artery disease (CAD) and particularly acute coronary syndromes (ACS) rank among the most frequent causes of death in developed countries. However, a significant proportion of patients lack conventional cardiovascular risk factors. Genetic markers might be used to further improve identification of individuals at high risk of cardiovascular events.

Interestingly, the risk associated with variants detected through the genome wide association studies (GWAs) is often partially (for example the *FTO*) [1] or completely (e.g. the marker within the 9p21 locus) [2] independent on the conventional cardiovascular risk factors.

One of the CAD susceptibility loci was detected at the 3´UTR of *MRAS* gene (muscle RAS oncogen homolog-gene, OMIM 608435, 3q22.3) and rs9818870 SNP (which clusters with three additional SNPs) was associated with elevated risk of CAD with odds ratio of 1.15. [3] As this locus was not associated with conventional cardiovascular risk factors in the original study (2) the mechanism causing CAD development is unclear. The association of this locus with CAD has not been replicated so far. However, recently, other GWAs have detected an association of the same locus with coronary artery calcification [4]. Finally, Ellis et al. [5] identified rs9818870 as a predictor of CAD risk in individuals free of overt heart disease from the Canterbury Healthy Volunteer study.

To confirm the original finding, we have analysed if rs9818870 variant at *MRAS* locus is associated with the risk of ACS in the Czech Slavonic population.

II. MATERIAL AND METHODS

A. Analysed individuals

In our study, data from 1,223 male patients with ACS younger than 65 years (55.1 ± 7.7), consequently admitted to coronary care unit [1,6] and 1,139 control males (49.0 ± 10.7 years); selected according to the WHO MONICA Project protocol [7] were analyzed. All participants were of Caucasian ethnicity and signed the informed consent. The study was

approved by the institutional Ethics committee and conducted according to the Good Clinical Practice guidelines.

B. Laboratory and clinical analyses

DNA was extracted from peripheral blood white cells. Rs9818870 was genotyped using the polymerase chain reaction and restriction fragment length polymorphism analysis using the oligonucleotides 5´ tta ctt tga cgt gtc agt gta tac and 5´tca gac cgt atg ggt taa gtt ctc tgc and restriction enzyme Eco105I. All chemicals were purchased from Fermentas (Burlington, Ontario, Canada). Common C allele was represented by restriction fragments of 120 and 24 bp, while the presence of uncut product (144 bp) represented the minor T allele.

Lipoprotein parameters (assessed in plasma after an overnight fast) were measured using standard enzymatic methods in CDC Atlanta accredited laboratory.

In controls, systolic and diastolic blood pressures were measured 3 times after 10 minutes of rest in a sitting position on the right arm with an automated sphygmomanometer BP-203 (Nippon Colin co., Ltd.). The mean of the second and the third measurement was used for analyses. In patients with ACS, because of acute settings during examination, only history of hypertension including the use of antihypertensive drugs was analyzed.

In controls, risk factors were defined as follows i/ self-reported current smoking ii/ dyslipidemia as plasma total-cholesterol over 5.2 mmol/L, and/or triglycerides over 2 mmol/L or self-reported lipid-lowering treatment; iii/ body mass index (BMI) equal or higher than 30 kg/m^2; iv/ hypertension (self reported antihypertensive treatment or seated systolic/diastolic blood pressure higher than 139/89mmHg (measured in control group only) and finally v/ diabetes mellitus as self reported diabetes and/or fasting glucose over 7 mmol/L and/or antidiabetic medication. The self-reported data were obtained through personal questionnaires filled up under supervision of a trained nurse.

The Hardy-Weinberg test (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls) was applied to confirm the independent segregation of the alleles. Chi-square tests and odds ratio (95% CI) were calculated according the http://www.physics.csbsju.edu/cgi-bin/stats/contingency_form.sh?nrow=2&ncolumn=3, and http://www.hutchon.net/ConfidOR.htm. P-values less than 0.05 were considered to be significant.

III. Results and Discussions

Total plasma cholesterol was significantly higher in controls than in patients with ACS ($p < 0.001$) and no significant difference was found in plasma triglycerides and BMI values. Prevalence of ever smokers (67% vs. 33%), diabetics (37% vs. 9%) and hypertensives (86% vs. 41%) were as expected higher in ACS patients than in controls (all $p < 0.0001$).

The genotyping call rate was 98.5% for ACS patients and 97.3% for healthy controls. The frequencies of individual genotypes (Table 1) were within the Hardy Weinberg equilibrium ($p = 0.18$ for patients and $p = 0.49$ for controls) and the minor allele frequency is almost identical with the frequencies described in the Western populations (0.16 in Czechs, ~ 0.15 in Western Europeans [3]).

TABLE I.

Frequencies of the rs9818870 genotypes in healthy controls and acute coronary syndrome (ACS) patients. P* value for codominant model of analysis is given.

Males	Controls		ACS patients		OR (95%CI)	P	P*
	N	%	N	%	Crude		
CC	794	70.8	829	69.7	1.0		
CT	296	26.4	336	28.2	1.08 (0.90-1.10)	0.37	0.34
TT	32	2.8	25	2.1	0.75 (0.44-1.27)	0.28	

In the entire study, we have not confirmed the association between the rs9818870 marker and ACS (30.3% vs. 29.2% carriers of the minor T allele [recessive model], $p = 0.565$; OR 1.05; 95% CI 0.88 – 1.26).

Similarly negative results were obtained if dominant (carriers of the C allele vs TT homozygotes; $p = 0.24$) or codominant (CC vs CT vs TT; $p = 0.34$) models were used. Further, the numbers of alleles did not differ between the groups ($p = 0.87$).

As in the original study, we were not able to detect significant associations between the rs9818870 marker and conventional risk factors of ACS in controls (dyslipidemia, $p = 0.37$; hypertension, $p = 0.72$; BMI, $p = 0.51$; smoking, $p = 0.29$; diabetes mellitus, $p = 0.41$).

Till recently, three studies with different designs suggest a possible association between the rs9818870 DNA variant at *MRAS* (3q22.3) locus and cardiovascular risk [2-4]. In contrast to these studies, we failed to detect this variant as a risk factor for ACS in the Czech Slavonic males. The reasons for this discrepancy remain speculative. Original studies were all performed in West European Caucasians, and some differences both in the genetic background and/or lifestyle could be supposed.

In summary, we have not confirmed the originally described association between DNA marker rs9818870 on chromosome 3 (3q22.3) and acute coronary syndrome in the Czech/Slavonic males. Further studies are needed to examine the effect of this variant in females.

This work was supported by project NT 12217-5 (IGA, MH, CR).

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