

ROS1 Asp2213Asn polymorphism is not associated with coronary artery disease in a Greek case-control study

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Abstract

Background: Rs619203 (Cys2229Ser) and rs529038 (Asp2213Asn) polymorphisms in the *ROS1* gene have been studied in relation to myocardial infarction (MI) yielding inconsistent results. We investigated the role of ROS1 rs529038 polymorphism in coronary artery disease (CAD) in Greeks using a case-control study.

Methods: Genotyping for rs529038 polymorphism was performed using a multiplex PCR technique in patients with CAD (n=294) and controls (n=311). Logistic regression analysis was used to calculate crude and adjusted odds ratios (ORs).

Results: Logistic regression analysis did not show any statistically significant effect of ROS1 polymorphism in the occurrence of CAD (AG vs. AA, OR: 1.08, p=0.635; GG vs. AA, OR: 0.62, p=0.220). Adjustment for confounding factors gave similar results, irrespective of the type of disease (i.e., stable coronary artery disease vs. acute coronary syndrome).

Conclusions: Our findings do not support the hypothesis that ROS1 rs529038 polymorphism is an important contributing factor in the etiology of CAD in the Greek population.

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Keywords: acute coronary syndrome; atherosclerosis; coronary artery disease; growth factor receptor; non-synonymous polymorphism.

Introduction

V-ros UR2 sarcoma virus oncogene homolog 1 (*ROS1*) gene is located in 6q22 and encodes for a proto-oncogene. This product is also a type I integral membrane protein with tyrosine kinase activity that may function as a growth or differentiation factor receptor and is expressed in most tissues (1, 2).

The *ROS1* gene was initially implicated in the etiology of myocardial infarction (MI) in a genome-wide association study. This study demonstrated that non-synonymous rs529038 and rs619203 polymorphisms, which are almost in complete linkage disequilibrium, were important factors for risk of disease (3). To our knowledge, there are only two additional studies that attempted to replicate these findings. These studies showed inconsistent results in Caucasians of non-European origin (4, 5).

These particular polymorphisms also have been shown to increase the risk for restenosis following coronary stenting, as well as for hypertension and atherothrombotic cerebral infarction in Japanese (6–8).

The aim of the present study was to investigate whether ROS1 rs529038 polymorphism is a significant contributor to coronary atherosclerosis in a Greek population. We employed a case-control study design using 294 patients with coronary artery disease (CAD) and 311 control subjects.

Materials and methods

Subjects

Study participants were unrelated individuals, exclusively of Greek origin, and were selected consecutively from hospitals in the Athens area. Subjects presenting with either acute coronary syndrome (ACS) or stable CAD were recruited as study cases. CAD was defined as >50% stenosis in at least one of the three main coronary vessels, as assessed by coronary angiography. ACS was defined as: (1) acute MI, or (2) unstable angina corresponding to class III of the Braunwald classification (9). Controls were subjects with negative findings following coronary angiography, or negative stress test, or subjects without symptoms of disease who were admitted to the same hospitals as cases and were free of cardiovascular disease, cancer, or inflammatory diseases. The Institutional Ethics and Research Committee approved the research protocol and all participants gave their informed consent before enrollment in the study.

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Risk factor definition

Subjects defined as hypercholesterolemic had either total cholesterol levels >200 mg/dL (>5.128 mmol/L), or were under treatment for hyperlipidemia. Subjects whose blood pressure was $\geq 140/90$ mm Hg, or who were taking antihypertensive medication were classified as having hypertension. Individuals with a fasting blood glucose >125 mg/dL (>5.277 mmol/L), or those requiring a special diet or treatment were classified as diabetics. Positive family history was defined as the presence of premature MI among first-degree relatives (<55 years for male relatives and <65 for female relatives). Smokers and non-smokers were grouped together.

Genotyping

Genomic DNA was extracted from whole blood using the salting-out method (10). Since the above single nucleotide polymorphisms (SNPs) are in almost complete linkage disequilibrium, we genotyped for rs529038 polymorphism only. Rs529038 was detected among other SNPs, using an arrayed primer extension-based genotyping method (APEX-2). This method allows multiplex DNA amplification and detection of SNPs on microarrays using four-color single-base primer extensions (11). The specific primers for rs529038 polymorphism were:

Left: GCTATTTAAGAAAAATTTCTGAATAACTGAAGTTGGT
Right: CGACCAAAGACCTACTTTTCATAGAATTCAG

Statistical analysis

The χ^2 -test was used to compare the observed numbers of each genotype with the values expected for a population in Hardy-Weinberg equilibrium. Continuous variables are shown as mean \pm SD, while categorical variables are presented as percentages. Differences in genotype distributions between patients and controls were evaluated using the χ^2 -test. Odds ratios (ORs) were calculated using logistic regression. Multivariate logistic regression analysis was used to

calculate the adjusted OR. Variables used included age, sex, and the prevalence of smoking, diabetes, hypertension, hypercholesterolemia and family history of MI. Two-sided tests were performed and a p -value ≤ 0.05 was used for statistical significance. Statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Power analysis was performed using QUANTO 1.2 software under the assumption of the additive model of inheritance.

Results

Demographics of participants are presented in Table 1. Mean age and the percentage of male subjects were slightly higher in patients compared to controls. As expected, patients exhibited a higher prevalence of diabetes, hypercholesterolemia, hypertension, family history of MI and smoking compared to controls. Body mass index (BMI) did not differ significantly between the two study groups.

The distribution of ROS1 rs529038 polymorphism in controls was comparable with the Hardy Weinberg equilibrium ($p=0.93$). Table 2 shows the distribution of genotypes between patients and controls and results of the logistic regression analysis that assessed the relationship between ROS1 rs529038 polymorphism and the likelihood of having CAD (presence or absence of ACS). The latter analysis was performed both before and after controlling for the effect of several potential confounders such as age, sex, and prevalence of smoking, diabetes, hypertension, hypercholesterolemia and family history of MI. There was no statistically significant effect of rs529038 polymorphism on the occurrence of CAD. In addition, the lack of association was not influenced by the type of disease (stable CAD vs. ACS) (p for

Table 1 Characteristics of the study subjects.

Subject characteristics	Cases (n=294)	Controls (n=311)	p-Value
Age, years	63.8 \pm 10.8	60.7 \pm 14.9	0.002
Male sex, %	81.7	70.2	0.001
BMI, kg/m ²	28.0 \pm 3.9	28.2 \pm 4.6	0.580
Hypercholesterolemia, %	78.1	59.0	<0.001
Diabetes, %	30.2	15.1	<0.001
Hypertension, %	73.5	58.8	<0.001
Family history of MI, %	27.9	16.7	0.002
Current or former smoking, %	74.8	61.0	<0.001

BMI, body mass index; MI, myocardial infarction.

Table 2 Logistic regression analysis for the effect of ROS1 rs529038 polymorphism in coronary artery disease occurrence (presence or absence of acute coronary syndrome).

SNP	Genotype	Cases (n=294)	Controls (n=311)	Crude OR; 95% CI; p-value	Adjusted OR; 95% CI; p-value
ROS1 rs529038	AA	57.1%	57.6%	Referent	Referent
	AG	39.1%	36.3%	1.08; 0.78–1.52; 0.635	0.96; 0.65–1.41; 0.818
	GG	3.7%	6.1%	0.61; 0.28–1.34; 0.220	0.51; 0.20–1.31; 0.160

Age, sex, prevalence of smoking, diabetes, hypertension, hypercholesterolemia and family history for myocardial infarction were used as covariates. Unadjusted and adjusted odds ratios (ORs), 95% confidence intervals (CI) and p -values were calculated using AA as referent genotype.

interaction between type of disease and genotype = 0.9).

Discussion

The aim of the present study was to investigate the role of ROS1 rs529038 polymorphism in the development of CAD in Greek adults. We did not observe any statistically significant effect of the polymorphism on the occurrence of disease, irrespective of the presence of ACS.

The minor allele frequency in the control group is 0.242, which is in accordance with the frequencies reported in previous studies (0.21–0.27) (3–5).

Rs529038 and rs619203 polymorphisms, along with four more variants in other genes, were first reported to be associated with MI in a genome-wide association study (3). Among these reported variants, only the association of ROS1 polymorphisms was replicated in the unadjusted analysis of a second study that utilized a prospective study design. However, the observed association disappeared after adjustments were made for potential covariates (4). A third case-control study did not show any significant association of ROS1 polymorphisms with MI (5).

The studies mentioned above, along with ours, represent another example of failure to replicate previous reports of polymorphisms associated with disease. This may be attributed either to false positive initial associations, or to genuine population diversity. It is possible that for some diseases, the genetic effects of some variants are important in some populations, but not in others (12).

Our study is limited by the small sample size. Thus, we cannot exclude the possibility of a modest effect of rs529038 in disease, which might be apparent in a larger sample size. However, our sample size is similar to that used by Shiffman et al., who initially found the ROS1 polymorphism to be a MI risk factor (3). A power analysis showed that the power of our study to detect the unadjusted OR of 1.75 and the adjusted OR of 1.54 in the Shiffman study is 98% and 91%, respectively (at a significance level of 0.05 and assuming the additive model of inheritance).

We cannot exclude the possibility of misclassification of subjects with silent CAD in the control group. Some of these individuals were not subjected to coronary angiography or stress testing, and reported the absence of symptoms of disease. Also, cases with fatal MI were not included. Thus, we cannot rule out the possibility that this polymorphism may predispose individuals to more severe disease.

In conclusion, our study did not replicate the findings of Shiffman et al. (3). In the Greek population studied, ROS1 rs529038 polymorphism does not seem to play a major role in the etiology of CAD.

Studies using a larger sample size are needed in order to come up with more definitive conclusions regarding the role of ROS1 rs529038 polymorphism in CAD.

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