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Original Article

Association of the genetic markers for myocardial infarction with sudden cardiac death

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ABSTRACT

Objective: Investigate the association of rs17465637 gene *MIAF3* (1q41), rs1376251 gene *TAS2R50* (12p13), rs4804611 gene *ZNF627* (19p13), rs619203 gene *ROS1* (6q22), rs1333049 (9p21), rs10757278 (9p21), rs2549513 (16q23), rs499818 (6p24) associated with myocardial infarction available from the international genome-wide studies with sudden cardiac death (SCD) in a case–control study.
Methods: A sample of SCD cases ($n = 285$) was formed using the WHO criteria; the control sample ($n = 421$) was selected according to sex and age. DNA was isolated by phenol–chloroform extraction from the myocardial tissue of SCD cases and blood of control cases. The groups were genotyped for the selected SNPs by real-time PCR using TaqMan probes (Applied Biosystems, United States).
Results: No statistically significant differences in the genotype and allelic frequencies of studied single nucleotide polymorphisms between sudden cardiac death cases and control were detectable in general group. By separating the groups of sex and age differences in the genotype frequencies of rs1333049, rs10757278 and rs499818 are statistical significance. Genotypes CC of rs1333049 and GG of rs10757278 are associated with an increased sudden cardiac death risk in men ($p = 0.019$, OR = 1.7, 95% CI 1.1–2.8; $p = 0.011$, OR = 1.8, 95% CI 1.2–2.8, respectively). Genotype AG of rs499818 is associated with an increased sudden cardiac death risk in the women over 50 years old ($p = 0.009$, OR = 2.4, 95% CI 1.3–4.6).
Conclusion: Polymorphisms rs1333049 and rs10757278 are associated with SCD in men and rs499818 in the women aged over 50 years.

1. Introduction

Cardiovascular diseases are still leading causes of population mortality in many countries of the world. According to the WHO data as of 2010, 56.8% of all fatal cases in the Russian Federation are caused by cardiovascular diseases, with ischemic heart disease (IHD) accounting for 29.5%.[1] Sudden cardiac death (SCD) is one of the IHD forms in the WHO classification (International Classification of Diseases 10th revision); it can develop on the background of a current cardiovascular disease or be a solitary disease manifestation. SCD is a multifactorial state, contributors to which are both genetic and environmental factors. Development of the predictive component in modern medicine, on the one hand, and the absence of reliable and affordable tools for diagnosing predisposition for SCD development, on the other, encourage the research into genetic factors underlying development of this IHD from. Numerous current genome-wide association and case–control studies deal with the genetic predisposition for IHD and myocardial infarction (MI).[2–5] However, the studies on the associations of gene polymorphisms and mutations with SCD are still rather few and almost absent in the Russia. Recent genome-wide studies have detected the association of some single nucleotide polymorphisms (SNPs) with an increased risk of IHD and, in particular, myocardial infarction.[2,6] According to ICD-10, SCD does not include the death of myocardial infarction; however, the clinical picture and the mechanism of fatal outcome development in SCD and myocardial infarction are rather similar. We assumed that the SNPs associated with the development of myocardial infarction might also contribute to SCD development.

So the aim of this study is investigate the association of rs17465637 gene *MIAF3*, rs1376251 gene *TAS2R50*, rs4804611

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gene *ZNF627*, rs619203 gene *ROS1*, rs1333049, rs10757278, rs2549513, rs499818 associated with myocardial infarction available from the international genome-wide studies with sudden cardiac death.

The associations of rs619203 gene *ROS1*, rs1376251 gene *TAS2R50*, and rs4804611 gene *ZNF627* with MI were first detected in a multistage study involving 11,053 SNPs.[7] Rs499818 and rs2549513 were detected in the genome-wide Framingham Heart Study as most likely associated with widespread IHD outcomes, namely, MI, stroke, and fatal IHD outcome. The fatal IHD outcome was regarded as the death caused by IHD after exclusion of the remaining causes. Also, in Framingham Heart Study a 13-Kb locus of chromosome 9 (9p21) displayed the association of MI, stroke, and fatal IHD outcome; this locus contains several SNPs, including rs1333049 and rs10757278.[6] In the list of SNPs associated with MI, IHD was included rs17465637, which is based on the results of joint research analysis with a probability greater than 80% is associated with coronary artery disease (OR 1.20).[8]

2. Methods

The design of study is a case–control. The bank of SCD cases for 1999–2012 ($n = 285$; mean age, 53.2 ± 8.8 years; men, 69.9%; and women, 30.1%) was formed. The group included people who died suddenly in Oktyabr'skii district, Novosibirsk, Russia subject to forensic examination which was conducted according to standard protocol by a qualified medical examiner. Standard protocol includes examination of the corpse, an autopsy and examination of body cavities, extracting organocomplexes, the study of organs and tissues, taking material for histological studies. Taking into account a limited volume of information on the time of fatal event development, the formed group contains the fatal cases that developed during 1 h or no more than 24 h in the absence of witnesses and estimated as a death of cardiac genesis according to autopsy. The main postmortem diagnosis is acute coronary insufficiency and acute circulatory failure. The presence of morphological changes in the cardiac tissue typical for myocardial infarction or cardiomyopathies was the exclusion criteria. The persons in a state of alcoholic and drug intoxication identified during the chemical research were excluded from the group. According to the protocols of a forensic study 56.4% of SCD persons have signs of atherosclerosis (75.7% of them – signs of aortic atherosclerosis, 95.7% – signs of coronary atherosclerosis).

The control group was selected statistical comparable in sex and age of the group SCD at a ratio of 3 control individuals to 2 SCD case from the DNA bank of Health, Alcohol and Psychosocial factors In Eastern Europe (HAPIEE) international project that covered the same district of Novosibirsk ($n = 421$; mean age, 59.3 ± 6.5 years; men, 64.1%; and women, 35.9%). HAPIEE is a prospective cohort study to examine the effects of traditional and nontraditional risk factors, social and psychosocial factors in the development of cardiovascular and other noncommunicable diseases in Eastern Europe. Protocol of study included a questionnaire of social and economic life's conditions, history of chronic disease, the state of physical activity and health. The examination included the measurement of growth, body weight, blood pressure, heart rate, circumferences of chest, waist and hips. Also respiratory and cognitive functions were investigated. The control group included individuals without coronary artery disease and myocardial infarction in anamnesis. The individuals from control group did not have criteria of angina pectoris in the questionnaire of G.A. Rose. The ECG also showed no signs of myocardial ischemia, cicatricial changes of the myocardium.

DNA was isolated by phenol–chloroform extraction from the myocardial tissue of SCD cases and blood of control cases.[9]

The groups were genotyped for the selected SNPs rs17465637, rs2549513, rs1376251, rs499818, rs4804611, rs619203, rs1333049, rs10757278 by real-time PCR in an ABI 7900HT device using TaqMan probes (Applied Biosystems, United States).

The data were statistically processed with the help of SPSS 16.0 software package; the genotype and allelic frequencies of the studied SNPs were determined in the SCD and control groups. The cohorts were compared according to the genotype and allelic frequencies with the help of contingency tables using χ^2 test according to Pearson. In the case of fourfold tables, two-sided Fisher's exact test with Yates' correction for continuity was used. A relative SCD risk for particular allele or genotype was calculated as the ratio of changes using two-sided Fisher's exact test and Pearson's χ^2 test. The examination of linkage disequilibrium was performed using pair-wise r^2 and D' statistics in CubeX.

The study was approved by ethics committee of Federal State Budgetary of Scientific Institution "Institution of Internal and Preventive Medicine".

3. Results

Eight SNPs regarded as the markers associated with MI and IHD according to the data of international genome-wide studies have been tested for their potential association with SCD, namely, SNPs rs17465637, rs2549513, rs1376251, rs499818, rs4804611, rs619203, rs1333049, and rs10757278 (Table 1).

The frequencies of genotypes of rs17465637, rs2549513, rs1376251, rs499818, rs619203, in the control group are in Hardy–Weinberg equilibrium. The genotype frequencies of rs1333049, rs10757278, rs4804611 in the control group deviated from Hardy–Weinberg equilibrium ($\chi^2 = 5.22$, $p = 0.022$; $\chi^2 = 4.66$, $p = 0.031$; $\chi^2 = 3.91$, $p = 0.048$, respectively). Genotyping errors were excluded using PCR and subsequent restriction fragment length polymorphism (RFLP) analysis by original techniques. The results of real-time PCR are identical to the results of PCR-RFLP. In the age group under 50 years genotype frequencies of rs1333049, rs10757278 are in Hardy–Weinberg equilibrium, and in the group older than 50 years there are deviations from it. This may indicate a loss to some genotypes in a population with age, with a corresponding increase in the share of other genotypes in it.

No statistically significant differences were detectable when comparing the SCD and control cohorts according to the genotype and allelic frequencies of rs17465637, rs2549513, rs1376251, rs4804611, rs619203.

Found statistically significant differences in allele frequencies of rs1333049 in the SCD group and control group ($p = 0.024$). In the group of SCD homozygous genotype CC of rs1333049 and homozygous genotype GG of rs10757278 increase compared with the control group, but the difference did not statistical significance ($p = 0.052$ and $p = 0.059$, respectively). By separating the groups of sex these differences are statistical significance. The odd ratio found in the group of men who died SCD carrier GG genotype rs10757278 (26.4%) is 1.8 times higher than in the control group of men (17.2%) (95% CI 1.2–2.8, $p = 0.011$), and genotype CC rs1333049 (26.2%) 1.7 times higher in group of men who died suddenly than in the control group of men (16.9%) (95% CI 1.1–2.8, $p = 0.019$).

The group of women over 50 years old displayed statistically significant differences in the genotype frequencies of SNP rs499818. The share of carriers of AG genotype is 2.4-fold higher in SCD group of women over 50 years old (55.6%) as compared with the control group of women over 50 years old (34.1%) at 95% CI of 1.3–4.6 and $p = 0.009$.

In addition we examined the correlation among rs1333049 and rs10757278 (based on the literature) using pair-wise r^2 and D' statistics for linkage disequilibrium. In control group D' was −0.978 and r^2 was 0.9511.

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