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Gene polymorphisms association with conventional risk factors and cardiovascular complications

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Summary

The aim of this study was to evaluate the gene polymorphisms association with ***conventional*** *risk factors and cardiovascular complications. The case control study was conducted in 2007-2011 and included 405 patients with coronary artery disease (CAD) and acute ischemic episodes admitted to the Municipal Clinical Hospital "Sfânta Treime", Chisinău. Insertion/deletion (I/D) genotypes of angiotensin-converting enzyme (ACE) and A1166C polymorphism of angiotensin II type 1 receptor gene, Asp298Glu (A/G) genotypes of the endothelial nitric oxide synthase (eNOS) and PlA1/2 (A_1/A_2) genotypes of A_2/A_2 genotype* ***of glycoprotein (GP) IIb/IIIa*** *receptor gene (GPIIb/IIIa) receptor polymorphisms were identified by amplified polymerase chain reaction and restricted fragment length polymorphism. The authors concluded that the carrier of D/D genotype and D allele in ACE gene, being positively correlated with the risk C/C polymorphic variant of angiotensin II type 1 receptor gene, was associated with hypertension and cardiovascular death. A_2/A_2 genotype* ***of GP IIb/IIIa*** *receptor gene was associated with susceptibility to CAD and high frequency of myocardial infarction and dyslipidemia, particularly in smokers. The impact of eNOS polymorphic markers for CAD proved to be hypertension-mediated.*

Keywords

Gene polymorphisms, conventional risk factors, cardiovascular complications

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Background

Detection of the genetic factors that cause or predispose to CAD remains the topic of many scientific papers in this field. They have been investigated separately or in association within the European population, but the genetic complexity of this CAD was not foreseen and new approaches are needed [2,6].

Studies aimed at identifying the genes responsible for the heritability of CAD have uncovered several candidate genes with different roles in vascular biology that are believed to be involved in the pathogenesis of CAD. Of these, the more important are the renin-angiotensin system, endothelial dysfunction and homeostasis genes: ACE gene and angiotensin II type 1 receptor gene (AGT_1R), Asp298Glu (A/G) of eNOS and of platelets (PlA1/2) GPIIb/IIIa receptor polymorphisms. Polymorphisms within these system genes have been extensively studied in relation to CAD, however findings are conflicting [1,3,5,6,7]. To clarify these data, we studied the association of genes polymorphism with conventional risk factors and cardiovascular complications.

The aim of the current study was to assess the association of gene polymorphisms with conventional risk factors and cardiovascular complications in patients with CAD.

Material and methods

The case control study was conducted in 2007-2011 and included 405 patients with acute coronary episodes admitted to the *Municipal Clinical Hospital "Sfânta Treime"*, Chisinău. The control group consisted of 290 matched persons without CAD (data used for matching were age, sex, residence and professional activity). Sex-distribution in the study group was uniform, male/female ratio being 2:1, which was two times more males ($P<0.001$). Mean age was 57.93 ± 0.34 years, with insignificant variation in the control group ($P>0.05$).

The study was bicentric, case-control, approved by the *National Ethics Committee for Clinical Trials* and *Drug Development of Ministry of Health of the Republic of Moldova* (Nr.331, 03.06.2010). All subjects were native-born citizens and residents of the Republic of Moldova, had comparable socio-economic status, and were ethnically matched.

Patients were included in the study in the order of their hospital admission, after clinical and enzyme stabilization and informed consent obtained. This method of patient selection ensured randomness of the study group.

Criteria for inclusion in the study were the clinical diagnosis of acute Q wave and non-Q-wave myocardial infarction, unstable or exercise angina pectoris, consistent with recommendations of the *European Society of Cardiology* [4].

Exclusion criteria: hypercholesterolemia (total cholesterol ⩾8mmol/L) and secondary hypertriglyceridemia; pacemaker implant with evidence of ventricular preexcitation; atrioventricular conduction blocks (2nd or 3rd degree sinoatrial or atrioventricular block); active liver disease; acute gastrointestinal diseases; severe kidney disease; and, associated diseases that influence life expectancy.

Standard questionnaires were used to collect data on past and current medical history, examination results, and also personal and demographic data, cardiovascular risk factors, family history of CAD, hemodinamic data; lipidogram, blood glucose level, cardiac enzymes, instrumental investigations- ECG and echocardiography.

Polymorphism of renin-angiotensin system: I/D of ACE gene and A1166C genotype (cytosine or adenine variants, A/C) of AGT_1R gene, Asp298Glu (A/G) of eNOS gene and PlA1/2 (A_1/A_2) genotypes of GPIIb/IIIa receptor gene were identified by amplified polymerase chain reaction and restricted fragment length polymorphism in *the Institute of Genetics and Plant Physiology, a branch of the Academy of Sciences of Moldova* [3,5]**.**

Data were computer processed by variation, association and descriptive analysis methods. The relationships between the studied phenomena were determined by using simple linear regression, quantitatively expressed by the correlation coefficient "r". For estimating genetic frequencies we used POPULATION GENETIC ANALYSIS by Nei Masatoshi, Director of the *Institute of Molecular Evolutionary Genetics*; and, the Diploid Data Set at *the Genetics Center*, New York University, Langone. Frequency of studied genes loci was calculated with the help of the Hardy-Weinberg equilibrium.

Results

Stratification of coronary patients according to ACE I/D polymorphism confirmed the prevalence of homozygous individuals with risk deletion/deletion (D/D) genotype as compared with the controls (19.64% vs. 11.03%, respectively, x2=8.77, $P<0.05$), while genotype I/I was present in the control group (33.11% vs. 19. 64%, x2 13.31, $P<0.01$). There were no significant differences in the number of heterozygous I/D in both groups (60.72% vs. 55.86%, respectively, $P>0.05$). ACE I/D polymorphism genotyping and the estimation of the allele frequency revealed signif-

icant differences in the presence of risk D allele in patients with CAD compared with controls (78.65% vs. 61.24%, OR=1.29, x2=8.77, *P*<0.05). Compared with those in which this was not present (I/I), the analysis of the risk factors and clinical manifestations showed that ACE D/D homozygous or ACE I/D heterozygous genotypes in patients with CAD was associated with increased prevalence of hypertension (90.91% and 88.24% vs. 78.18 %), systolic blood pressure (155.32 ± 1.46 mm Hg and 140.5 ± 1.31 mm Hg vs. 125.42 ± 1.36 mm Hg), diastolic blood pressure (95.42 ± 1.35 mm Hg and 90.6 ± 1.28 mm Hg vs. 80.5 ± 1.84 mm Hg) and recurrent angina pectoris (40.00% vs. 34.11% vs. 23.64%, respectively, *P*<0.01).

No statistically significant differences were found between carriers of genotypes I/I, D/D or I/D in terms of degrees of hypertention. Considering the spectrum of risk factors and the clinical presentation according to ACE gene polymorphism recorded in this study, it appears that the presence of D allele and, in particular, homozygous D/D state are associated with blood pressure values exceeding the optimal level [r_{xy}=0.81, $P_{(D/D-I/I)}$<0.01]. The carrier of D allele and heterozygous I/D state was associated with recurrence of angina symptoms [r_{xy} =0.42, $P_{(I/D-I/I)}$<0.05] and a significantly higher risk of cardiovascular death [r_{xy}= 0.27, $P_{(I/D-I/I)}$<0.05].

Genotype frequencies of AGT_1R cytosine or adenine variants (A/C) in the group of patients with CAD were: A/A genotype was detected in 72 (25.74%) of the patients, C/C – in 47 (16.78%) and A/C – in 161 (59.28%). In the control group genotype frequencies were: 31(10.69%) C/C carriers, 162 (55.86%) A/C and 97 (33. 40%) A/A carriers. No significant differences in the presence of the studied genotypes were found (*P*>0.05).

Genotyping AGT_1R A/C polymorphism showed no conclusive differences between the presence of the risk allele C in CAD patients (72.83% vs. 70.71%, *P*>0.05), or non-risk allele A frequency (27.17% vs. 29.29%, *P*>0.05), compared with controls.

Comparative analysis of the characteristics of CAD patients grouped according to A/C polymorphism of AGT_1R gene, revealed the association of homozygous C/C state or heterozygous A/C state with increased prevalence of hypertension (95.49% and 89.44% vs. 68.33%, *P*<0.05).

Estimation of the association between clinical determinants and A/C polymorphism of AGTR gene showed that the presence of the risk C/C genotype in the coronary patients is associated with increased prevalence of hypertension [r_{xy}= 0.88, $P_{(C/C-A/A)}$<0.01] compared with homozygous A/A genotype. Analysis of association indices in patients with CAD certify that between the carrier of the D risk allele of ACE gene and the C risk allele of the gene AGT_1R was a moderate positive correlation (r_{xy} = 0.58, x^2= 35.30, *P*<0.001).

The distribution of Asp298Glu eNOS gene polymorphism frequencies in CAD patients showed no differences between them and control in terms of frequency of A/G genotype (53.21% vs. 57.93%, *P*>0.05) and risk allele A/A frequency (63% vs. 79%, *P*>0.05). No significant age-related differences were found, but there was a tendency towards accumulation in women (37.84% vs. 24.27%, *P*=0.06).

Comparative analysis of the characteristics of CAD patients grouped according to Asp298Glu eNOS gene polymorphism, revealed that homozygous state with risk genotype A/A or heterozygous A/G state are associated with increased prevalence of hypertension (96.00% and 87.91% vs. 69.64%, *P*<0.05), with no clear difference in terms of obesity (57.33% and 44.96% vs. 37.50%, *P*>0.05).

Analysis of clinical manifestations shows that almost 89.33% of the A/A genotype carriers had arterial hypertension grade II-III, while such levels of hypertension were found in only 69.64% of the G/G carriers and in 85.23% of A/G carriers. Analysis of echocardiographic findings showed reduced ejection fraction <50% in more than half of A/G genotype (57.05%), the same being also found in patients with genotypes G/G and A/A (42.86% vs. 46.67%, respectively).

Estimation of the association between clinical determinants and Asp298Glu eNOS gene polymorphism has shown that, compared with non-carrier individuals (G/G), homozygous A/A state and heterozygous carriers (A/G) in coronary patients are associated with increased prevalence of arterial hypertension [r_{xy} = 0.84, $P_{(AA-GG)}$<0.01].

Analyzing the frequencies of PlA GPIIb/IIIa receptor genotypes according to the polymorphism detected by MspI enzyme digestion we found that risk haplotype A_2/A_2 was detected in 63 (22.50%) of the patients and 28 (9.66%) controls, the difference being statisticaly significant (x2=16.28, *P*<0.001). Significant age-group differences were not found, but a trend of male prevalence (53.39% vs. 43.24%, *P*=0.06).

Analysis of A_1/A_2 GPIIb/IIIa polymorphism genotyping revealed that mutant A2 allele tents to be more common in the CAD patients compared with controls (72.85% vs. 70.71%, *P*=0.06). At the same time, the frequency of recessive A1 alleles in the coronary patients was lower than in the controls.

Platelet membrane glycoproteins play an important role in platelet adhesion and aggregation. The

allelic variants for GPIIb/IIIa bind to fibrinogen being the key reaction in the process of platelet aggregation. The presence of PlA_2 allele leads to increased functional activity of receptors and is associated with intense adenosine diphosphate induced platelet aggregation *in vitro*.

The analysis of the relationship between the carrier-state of different genotypes and risk factors revealed a significant difference between genotypes A_1/A_1, A_1/A_2 and A_2/A_2 carriers and the prevalence of smoking (48.68% and 53.90% vs. 69.84 %, respectively, $P<0.01$), and mixed dyslipidemia (59.21% and 75.17% vs. 63.49%, $P<0.05$). Of note was the statistically significant difference between groups in terms of the share of old myocardial infarction in the history of the study patients: A_2/A_2 genotype was detected more frequency than A_1/A_1 (20.63% vs. 9.21%, respectively, $P<0.05$).

Analysis of biochemical characteristics in relation with A_1/A_2 GP IIb/IIIa gene polymorphism showed that A_2/A_2 genotype was associated with higher prothrombin levels as compared to A_1/A_1 and A_1/A_2 variants (106.96 ± 0.52% vs. 90.83 ± 0.59% vs. 80.00 ± 1.05%, $P<0.05$). Signs of grade II and III heart failure were present in 25.53% of A_1/A_2 genotype carriers , 15.87% of A_2/A_2 and 14.47% of A_1/A_1 ($P>0.05$) genotype.

It is noteworthy that one fourth of the risk A_2/A_2 and A_1/A_2 genotype carriers presented Q wave acute myocardial infarction, compared with A_1/A_1 carriers (28.36%, 22.22% vs. 19.73%, respectively, $P<0.05$).

It can be said that the presence of the A_2 allele and homozygous state A_2/A_2 were associated with the presence of dyslipidemia [r_{xy}= 0.53, $P_{(A2/A2\text{-}A1/A1)}<0.05$], smoking [$r_{xy}$ = 0.64, $P_{(A2/A2\text{-}A1/A1)}<0.01$], as risk factors and a high frequency of previous myocardial infarction.

Conclusion

Carrier state of D/D genotype and D allele in ACE gene is a marker of increased risk for CAD and is associated with a high frequency of hypertension and cardiovascular death, being positively correlated with the risk C/C polymorphic variant of AGTR1 gene. The A_2/A_2 genotype of GP IIb/IIIa receptor gene is associated with susceptibility to CAD and high frequency of myocardial infarction and dyslipidemia, particularly in smokers. The impact of eNOS polymorphic markers for CAD was proved to be hypertension-mediated.

Conflict of interest: None declared

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