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Z.A. BULENTAYEVA, B.O. BEKMANOV, L.B. DJANSUGUROVA, R.I. BERSIMBAYEV

POLYMORPHISM OF GST AND XRCC GENES IN SUSCEPTABILITY TO CORONARY ARTEY DISEASE

(Institute of General Genetics & Cytology,
Al-Farabi Kazakh National University, Almaty, Kazakhstan)

The given article dedicated to investigation of the role of xenobiotic detoxification (GSTM1 and GSTT1) and DNA repaire (XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) genes in the development of coronary artery disease.

Cardiovascular diseases remain one of the major causes of death worldwide. Two thirds of cardiovascular disease cases are comprised by coronary artery disease (CAD, or heart ischaemic disease, ischaemia) and approximately 200 000 people suffer from it every year in US only [1].

Nowadays in developed countries the 80% of all death cases from cardiovascular pathologies are due to different CAD forms and its complications. For instance, in Kazakhstan CAD cases comprise 33.9% of all morbidity cases, in Russia more than 500 000 people died from CAD in 1998. Mortality rates from CAD are high in Finland, North Ireland, USA, England, and Australia [2].

Due to high morbidity and mortality rates, complexities in etiological and pathogenetical issues of disease and lack of adequate therapy and prevention approaches CAD still one of the intensively investigated problems of both clinicians and molecular biology specialists. Well known CAD risk factors include obesity, cholesterol rich diet, diabetes and smoking. Recently obtained data revealed that xenobiotics detoxification and DNA repair genes polymorphism can be associated with susceptibility to CAD development.

Large body of evidence suggests that DNA damage plays crucial role in the development of different pathological conditions, such as cancerogenesis, ageing and mutagenesis. DNA damage can be caused by wide range of toxic substances: hydrolysis, exposure of reactive oxygen species and other toxic metabolites. The origin of toxic metabolites can be exogenous as well as endogenous nature. Thus potentially toxic chemicals after entering the organism can undergo number of transformation reactions and become even more toxic. Currently it is believed that frequency of endogenous DNA damage incidences are higher comparing with the frequency of DNA damage resulted from exposure of exogenous toxic substances. One of the potent inducers of cellular damage is reactive oxygen species (ROS), which are continuously generated by all living cells. Almost all known ROS types such as, superoxide, hydroxyl radical and hydrogen peroxide can induce several types of DNA damage including oxidized bases, single and double breaks and formation of DNA adducts. Thus increased levels of DNA adducts were observed in vascular and heart tissues. It was demonstrated that polycyclic aromatic hydrocarbons can stimulate development of atherosclerotic plaque and its content in the tissues significantly correlated with other atherogenic factors such as concentration of low density lipoproteins in the blood, number of cigarettes smoked per day and etc [3].

To prevent harmful influence of toxic agent's cells developed number of protecting mechanisms. One of them dedicated to protect cell by enzymatic reaction of detoxification of reactive metabolites, the functions of others directed to repair occurred DNA damage.

Glutathione S-transferase (GST) family members are very important participants of xenobiotics detoxification process. They are known to catalyze number of reactions, including the detoxification of environmental carcinogens, anticancer drugs and they inactivate reactive metabolites, produced during the oxidative damage of the cell, thus preventing the DNA damage. Common homozygote deletion of GSTM1 and GSTT1 genes is known to result in producing virtually inactive enzyme, thus increasing the susceptibility of individual to oxidative stress [4].

It is known that oxidative DNA damage predominantly repaired by base excision repair enzymes. X-ray repair cross complementing group (XRCC) is the family of DNA repair genes which

participate in DNA base damage and single strand breaks. Polymorphisms of the XRCC1 genes were extensively studied in different cancer types. Genetic polymorphism in the genes involved into the DNA repair system may modify the DNA repair system and increase susceptibility to different pathological conditions [5].

The association of polymorphisms in the glutathione S-transferase (GST) genes with coronary artery disease (CAD) and myocardial infarction has been the subject of many investigations. However no studies were conducted in order to identify the involvement of repair genes to formation of susceptibility to CAD.

Therefore we conducted preliminary analysis to determine the involvement of that polymorphisms type to CAD incidence.

MATERIALS AND METHODS

The patients with the coronary artery disease who suffered myocardial infarction were evaluated at the Institute of Cardiology and Internal diseases. Age matched controls were selected after detailed evaluation of history; to exclude the presence CAD all clinical characteristics were carefully evaluated. Controls were healthy volunteers and did not have the risk factors for CAD development.

DNA was extracted from EDTA containing peripheral blood samples using the phenol-chloroform method. GSTM1, GSTT1, XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) genes polymorphism was detected by amplification of the genomic DNA with the following primers: GSTT1(s) 5'-TTC CTT ACT GGT CCT CAC ATC TC-3', GSTT1 (as) 5'-TCA CCG GAT CAT GGC CAG CA-3'; GSTM1 (s) 5'-GAA CTC CCT GAA AAG CTA AAG C-3', GSTM1(as) 5'-GTT GGG CTC AAA TAT ACG GTG G-3', XRCC1 (Arg399Gln) (s) 5'-CAAGTACAGCCAGGTCCTAG-3', XRCC1 (Arg399Gln) (as) 5'-CCTTCCCTCATCTGGAAGTAC-3', XRCC3 (Thr241Met) (s) 5'-GCCTGGTGGTCATCGACTC-3', XRCC3 (Thr241Met) (as) 5'-ACAGGGCTCTGGAAGGCACTGCTCAGCTCACGCACC-3'. PCR performed in 20μL reaction mixture, which contained 10mM KCl, 100mM Tris-HCl pH 8.0, 10mM dNTP, 15 pmol of each primer, 05 units of DNA polymerase and 30-50ng DNA template. PCR conditions for GSTM1 and GSTT1 were 94°C - 5 min.; 94°C – 2 min., 59°C - 1 min, 72°C – 1 min – 35 cycles; last extension 72°C - 10 min. Amplified fragments length are GSTT1 480 bp, GSTM1 (s) 215 bp. PCR conditions for XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) were 94°C - 5 min.; 94°C – 15 sec., 55°C and 60 °C (for XRCC1 and XRCC3 respectively) – 30 sec., 72°C – 45 sec., – 35 cycles; last extension 72°C – 5 min. After amplification PCR products were subjected to restriction digestion with *Nci I* and *NcoI* (for XRCC1 and XRCC3, respectively). After restriction analysis XRCC1 gave following fragments: homozygous normal genotype – 89 and 159 bp fragments; heterozygous 248, 159 and 89 bp fragments; homozygous for mutant allele – 248 bp fragment. And XRCC3 was digested to – one 136 bp fragment for homozygous normal allele; heterozygous genotype was fragmented to three bands – 136, 97 and 39 bp; homozygous for mutant allele was presented as two fragments 97 and 39 bp. β-globin was used as internal control.

Allele frequencies were calculated by gene counting methods. Presence of the particular allele was designated as wild genotype and homozygous absence or deletion of the allelewas designated as null genotype.

Statistical analysis. Genotype frequencies in various groups were compared by Chi-square test. Binary logistic regression was used to examine the relationship between genotype and disease, incorporating other variables into the model.

RESULTS AND DISCUSSION

Analysis was conducted on DNA samples extracted from peripheral blood obtained from 96 individuals with CAD and the same number of control individuals. We used PCR based genotyping assay to examine a polimorphism of GSTT1, GSTM1, XRCC1 and XRCC3 genes in CAD susceptibility. The genotypic results for each gene can be seen in Table 1-2.

As it is seen from the Table 1 the frequencies of the GSTT1 null and positive alleles are almost equal in case and control groups. However frequency of GSTM1 null genotype among CAD affected population is almost two times greater than in control population. Analyzing few studies dedicated to investigation the role of xenobiotic detoxification genes in the etiology of CAD it was revealed that obtained data is quite controversial. Thus M.H.Wilson et al. demonstrated that GSTM1 null genotype reduces the risk of myocardial infarction [6]. At the

Table 1. **Frequency of GSTM1 and GSTT1 positive and null genotypes in CAD affected population and control people, ORs and 95% CIs**

Genotypes	CAD affected population, (n=96), count %	Control population, (n=96), count %	OR	CI (95%)	χ^2	p
GSTT1(+/±)	65 (68.4%)	67 (70.5%)	1.10	0.60-2.048	0.099	0.75
GSTT1(-/-)	30 (31.6%)	28 (29.5%)				
GSTM1(+/±)	44 (46.3%)	69 (72.6%)	3.07	1.68-5.633	13.674	0.0002
GSTM1(-/-)	51 (53.7%)	26 (27.4%)				

Notice: OR – odds ratio; CI (95%) – 95% confidence intervals; P – probability value.

Table 2. **Frequency of XRCC1 Arg399Gln and XRCC3 Thr241Met genotypes, ORs and 95% CIs in CAD affected population and control people**

Genotypes	CAD affected population, (n=96), count %	Control population, (n=96), count %	OR	CI (95%)	χ^2	p
XRCC1 399 Arg/Arg	43 (45.3%)	44 (46.3%)	1.043	0.59-1.846	0.021	0.88
XRCC1 399 Arg/Gln; Gln/Gln	55 (54.7%)	51 (53.7%)				
XRCC3 241 Thr/Thr	78 (82.1%)	75 (78.9%)	0.817	0.40-1.679	0.302	0.58
XRCC3 241 Thr/Met Met/Met	78 (82.1%)	20 (21.1%)				

same time Ramesh and collegues investigating the role of polymorphism of GSTM1 and GSTT1 in northindian population revealed protective role of GSTT1 null genotype in CAD development [7]. Controversily, Abu-Amero with coworkers showed that null allells of both GSTM1 and GSTT1 genes can be considered as CAD risk factor [8]. Also S.A. *Salama and W.W. Au demonstrated that GSTT1 null* genotype is somehow associated with increased risk for atherosclerosis, as well as GSTM1 null genotype in combination with other metabolizing genes. The same authors have revealed that GSTM1 null genotype significantly increases frequency of chromosomal aberrations in patients with atherosclerosis [9].

Relying on the obtained data in was found that GSTM1 null genotype carriers are more susceptible to CAD development, however other genotypes are not associated with that pathology. Thus it was proposed that due to deletion in GSTM1 gene no active protein can be produced and therefore accumulation of toxic metabolites may occur. This phenomenon in combination with other CAD risk factors may accelerate the development of CAD and its complication such as myocardial infarction [10].

Very few studies have investigated the role of DNA repair genes polymorphism in the development of cardiovascular disease and none has investigated the role of XRCC1 and XRCC3 in CAD. As it can be seen in Table 2.

We found that neither XRCC1 Arg399Gln nor XRCC3 Thr241Met were not associated with CAD risk. It was proposed that given genotypes do not play important role in aetiology of cardiovascular diseases.

X-ray repair cross complementing group 1 (XRCC1) is the family of DNA repair genes which participate in repairing the DNA base damage and single strand breaks. Polymorphisms of the XRCC1 genes were extensively studied in different cancer type, however no CAD associated studies were conducted before. Thus number of epidemiological studied conducted in different studies to reveal association between genotype and cancer risk. A recent meta-analysis including 7385 cases and 9381 controls showed that 399Gln/Gln genotype was associated with an increased risk of lung cancer among Asians but not among Caucasians. A

multicenter study conducted in Europe concluded that this polymorphism was not ssociated with lung cancer risk. XRCC3 participates in DNA double-strand break via homologous recombinational repair, presents a non-conservative Thr241Met substitution in exon 7. Until now, there are several conflicting reports on the association between this polymorphism and lung cancer risk in the Caucasian population [11].

Taking into the account the fact that no association between XRCC 1 and XRCC 3 was found in our study it was proposed that DNA repair system is not tightly involved into the pathogenesis of CAD. However found association between GSTM1 null genotype and CAD can be evidence that detofication of many chemicals including environmental pollutants is very important and can comprise risk factors for development of cardiovascular pathologies. In addition to the well known functions, GST enzymes also inactivate endogenous unsaturated aldehydes, quinines, epoxides and hydroperoxides formed as secondary metabolites during the oxidative stress. Thus malfunctioning of the detoxification system may result in accumulation of toxic metabolites in circulatory system and may affect blood vessel structure and participate in atherosclerotic plaque formation. It was revealed that GST enzymes also play a key role in protecting blood vessels against endogenous oxidants [12]. This indicates that the lack of active GST enzymes may compromise one's capabilities for detoxification of different endogenous and exogenous oxidants and ultimately put one at higher risk of developing CAD.

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Резюме

Жүректің коронарлы артериясы ауруының дамуында ДНҚ молекуласының репарациясына ((XRCC1 (Arg399Gln) және XRCC3 (Thr241Met)) және ксенобиотиктер детоксикациясына (GSTM1 и GSTT1) қатысатын гендердің полиморфизмі зерттелді.

Резюме

В работе исследованы роли полиморфизма генов детоксификации ксенобиотиков (GSTM1 и GSTT1) и репарации ДНК (XRCC1 (Arg399Gln) и XRCC3 (Thr241Met) в развитии болезни коронарной артерии.