

Variant Polymorphisms of GST and XRCC Genes and the Early Risk of Age Associated Disease in Kazakhstan

Zeinep A. Berkimbayeva, Almagul T. Mansharipova, Elmira M. Khussainova, and Leyla B. Djansugurova

Abstract—It is believed that DNA damaging toxic metabolites contributes to the development of different pathological conditions. To prevent harmful influence of toxic agents, cells developed number of protecting mechanisms, such as enzymatic reaction of detoxification of reactive metabolites and repair of DNA damage. The aim of the study was to examine the association between polymorphism of GSTT1/GSTM1 and XRCC1/3 genes and coronary artery disease (CAD) incidence. To examine a polymorphism of these genes in CAD susceptibility in patients and controls, PCR based genotyping assay was performed. For GST genes, frequency of GSTM1 null genotype among CAD affected group was significantly increased than in control group ($P < 0.001$). Frequencies of the GSTT1 null and positive alleles are almost equal in both groups ($P > 0.1$). We found that neither XRCC1 Arg399Gln nor XRCC3 Thr241Met were associated with CAD risk. Obtained data suggests that GSTM1 null genotype carriers are more susceptible to CAD development.

Keywords—Cardiovascular disease, DNA reparation, gene polymorphism, risk factors, xenobiotic detoxification.

I. INTRODUCTION

CARDIOVASCULAR diseases (CVD) remain one of the major causes of death worldwide [1]. According to World Health Organization' statistical data, 2005, the number of global deaths from CVDs comprised 17.5 million, which represents 30% of all lethal cases, then by 2015 heart disorders, mainly heart attacks and strokes, will be responsible for almost 20 million deaths worldwide [2]. Within the structural cardiovascular disease, coronary artery disease (CAD) comprises almost two thirds of the mortality cases. In 2005 it caused more than 400 000 deaths in US only and became the single most leading cause of death in North America [3].

Indeed, CAD alone is the most common cause of premature permanent disability and mortality in Kazakhstan with an average annual incidence of first-time CAD increasing significantly from 291.8 to 444.4 per 100 000 per year in the year between 2000 and 2008 [4].

Z. A. Berkimbayeva, E. M. Khussainova, L. B. Djansugurova are with the Laboratory of molecular genetics, Institute of General Genetics and Cytology, Almaty, Kazakhstan (phone: +7-727-269-45-88; e-mail: zikagirl@mail.ru; khussainova@mail.ru).

A. T. Mansharipova is with Kazakhstan-Russian Medical University, Almaty, Kazakhstan (phone: +7-727-250-83-73; e-mail: dralma@mail.ru).

In contrast incidences of myocardial infarction in one region of the republic only increased by 2.9 fold from 13.4 in 2000 to 38.6 per 100 000 per year in 2006 [5]. Currently in Kazakhstan, there is an increasing trend of young CAD patients in both men and women [3].

In Kazakhstan, CAD still remains one of the most intensely problematic conditions for clinicians due to its high morbidity and mortality rates and limited therapy and prevention approaches. The complexities in its etiology and pathogenetic origin of the disease have attracted a lot of attention from molecular biology specialists and geneticists. It is a product of the interaction between internal and external risk factors. Among the most frequently studied CAD risk factors include gender, heredity, obesity, cholesterol rich diet, diabetes and smoking. Extensive clinical studies demonstrated that men have greater risk to develop cardiovascular pathology in early ages. Moreover recent studies revealed that pathology of cardiovascular disorders have significant gender differences, which contribute diagnostics and treatment in female population [6], [7].

In recent times however it has been suggested that polymorphism within xenobiotics detoxification factors and DNA repair genes can also be associated with susceptibility to CAD development [8]. Published evidence suggests that DNA damage plays a crucial role in the development of different pathological conditions, such as carcinogenesis, ageing and mutagenesis [9]. Thus 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 of exogenous as well as endogenous nature [10]. Thus potentially toxic chemicals organism can induce a number of transformation reactions after entering the body and can then be modified to become even more toxic. Currently it is believed that frequency of endogenous DNA damage incidences are higher compared with the frequency of DNA damage resulting from exposure to exogenous toxic substances [10].

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 [11] and indeed increased levels of DNA adducts have been observed in vascular and heart tissues. It has been demonstrated previously that polycyclic aromatic

hydrocarbons can stimulate the development of atherosclerotic plaque and its content in the tissues significantly correlate with other atherogenic factors such as the concentration of low density lipoproteins in the blood, number of cigarettes smoked per day, for example [12].

To prevent harmful influence of toxic agents, cells developed a number of protecting mechanisms. Among these include dedicated systems for cell protection such as enzymatic detoxification reactions on reactive metabolites and others directed at repair to damaged DNA.

Glutathione S-transferase (GST) family members are very important participants of xenobiotics detoxification process. They are known to catalyze a number of reactions, including the detoxification of environmental carcinogens. As key components of anticancer drugs they inactivate reactive metabolites produced during the oxidative process of cell metabolism thus preventing the DNA damage. Common homozygote deletion of GSTM1 and GSTT1 genes are known to result in virtually inactive enzymes, thus increasing the susceptibility of the individual to oxidative stress [13].

During the last decade, the role of DNA damage in cardiovascular disease pathology has been extensively studied, although molecular mechanisms are still far from clear. The notion that DNA damage can be induced not only by exogenous factors such as intracellular toxic substances, but also by an endogenous metabolites detoxifying machinery find support from epidemiological studies. Thus Izzotti *et al* [14] for example demonstrated an association between metabolic polymorphisms and nucleotide alterations in atherosclerotic lesion cells. Moreover, it was suggested that DNA adducts may result in atherosclerotic plaque formation and CAD development. In addition, atherosclerotic lesion formation and cancer development may also have closely-related or common molecular origin and pathways [15]. Thus in the context of improper detoxification mechanisms DNA repair system gain special importance. It is hypothesized that combined malfunctioning of detoxification and DNA damage repair systems may lead to severe atherogenesis and therefore more severe heart pathology.

It is also known that oxidative DNA damage is predominantly repaired by the base excision repair enzymes. X-ray repair cross complementing group (XRCC) is the family of DNA repair genes which participate in the repair of DNA base damage and single strand breaks [12]. Polymorphisms of the XRCC1 genes have extensively been studied in different cancer types [13, 16]. Genetic polymorphism in the genes involved into the DNA repair system may modify the DNA repair system and increase susceptibility to different pathological conditions [17]. However no studies looking into the association with CAD have been conducted earlier. The main epidemiological studies have been conducted on the association between XRCC1 genotypes and cancer risk. A recent meta-analysis including 7385 cases and 9381 controls showed that 399Gln/Gln genotype is associated with an increased risk of lung cancer among Asians but not among Caucasians [18]. XRCC3 participates in DNA double-strand break via homologous recombinational repair and it manifests

as a non-conservative Thr241Met substitution in exon 7 [19]. Still data showing the association between this polymorphism and lung cancer risk even in Caucasian populations is still controversial [20, 21].

On the other hand, the associations between polymorphisms in the glutathione S-transferase (GST) genes with CAD and myocardial infarction in particular have been the subject of many investigations. However, so far no studies have shown any association between the polymorphism within the xenobiotic detoxification GSTM1/GSTT1 gene and the XRCC1/XRCC3 DNA repair gene with susceptibility to CAD. The aim of this study was to investigate the potential association between xenobiotic detoxification (GSTM1/GSTT1) and DNA repair gene (XRCC1/XRCC3) polymorphisms in CAD male patients of Kazakhstan origin. Male participants were selected because of their well described inherent higher risk of CAD.

II. SUBJECTS AND METHODS

A. Study Population

In this case-control study, 96 male patients suffering coronary artery disease, acute coronary syndrome (unstable angina, acute myocardial infarction (AMI) with and without Q wave), with heart failure, NYHA class I-II and stable angina were evaluated. Arterial hypertension and hypercholesterolemia were considered as risk factors for acute myocardial infarction development. The patients were admitted to hospital for treatment of heart failure within the department of Cardiology and Internal medicine disease Institute. All patients were inhabitants of southern-west regions of Kazakhstan (Almaty city and Almaty district). CAD patients with ongoing liver, pancreas pathologies, renal failure, blood diseases, oncological disorders, acute inflammatory diseases were excluded from the study group. The 96 age and ethnicity-matched healthy controls did not have the risk factors for CAD development i.e. a history of coronary heart disease in the family or hypertension. The exclusion criteria consisted of: diabetes mellitus status, local and systemic inflammatory disorders, allergic reactions, thrombophlebitis of lower extremities. Ethics committee of the Institute approved the study and informed consent was obtained from all patients.

B. DNA Isolation and Genotyping

DNA was extracted from EDTA containing peripheral blood samples using the phenol-chloroform method. Polymorphic sites of GSTM1, GSTT1, XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) genes were examined by PCR and PCR-RFLP analysis. The details of these methods have been previously described [22]. Allele frequencies were calculated by gene counting methods. In the case of GSTM1/GSTT1 genes the presence of the particular allele was designated as wild genotype and homozygous absence or deletion of the allele was designated as null genotype. After restriction analysis XRCC1 gave following fragments: homozygous normal genotype – 89 and 159 base pairs (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 39bp.

B. Statistics

Allele frequency differences were analyzed by using Pearson's chi-square and Fisher's exact test. The odd ratio calculations were calculated using the Cochran-Armitage test. P-value of 0.05 was considered statistically significant. Analysis was performed using the GraphPad InStat Software (V. 2.04. Ralf Stahlman, Purdue University) and "Case-Control Study Estimating Calculator" from Tapotili Company (Laboratory of Molecular Diagnostics and Genomic Dactiloscopy of "GosNII Genetika" State Scientific Centre of Russian Federation; <http://www.tapotili.ru>).

III. RESULTS

Analysis was conducted on DNA samples extracted from peripheral blood obtained from 96 individuals with CAD and 96 control volunteers. Characteristics of case and control groups are shown in Table I.

TABLE I
CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF CHD PATIENTS
(N=96) AND HEALTHY CONTROLS (N=96).

INDICES	CHD	healthy
Age (years)	* 49,6±7,3	*46.5 ± 10.5
Sex (M/F)	96/0	96/0
Ethnicity		
Kazakh	63 (65.6%)	62 (64.6%)
Russian and others	33 (34.4%)	34 (35.4%)
Cholesterol (mmol/L)	5.62±0.18	4.38±0.40 p>0.05
Angina Status (CCS)		
CCS 1	0	0
CCS 2	29 (30.2%)	
CCS 3	67 (69.8%)	
Dyspnoea Status (NYHA)		
NYHA 2	51 (53.1%)	0
NYHA 3	45 (46.9%)	
Left ventricular function		
>50%	40 (41.7%)	0
<50%	56 (58.3%)	
Hypertension	70 (72.9%)	0
S-T elevation Myocardial Infarction (at least 0.1 mV)	0	0

Diabetes Mellitus	0	0
Hypercholesterolemia	76 (79.2%)	0
Preoperative Myocardial Infarction	0	0
Preoperative medication Aspirin (%)	98.9% (95)	X
Nitrates (%)	71.9% (69)	X
Beta blockers (%)	85.4% (82)	X
Calcium antagonists (%)	38.5% (37)	X
Diuretics (%)	30.2% (29)	X
ACE inhibitors (%)	80.2% (77)	X

* Values are given as mean ± standard deviation.

X denotes data not relevant

As demonstrated on Table I, there was no significant difference between cases and controls in terms of age and ethnicity. We used PCR based genotyping assay to examine a polymorphism of GSTT1, GSTM1, XRCC1 and XRCC3 genes in CAD susceptibility. The genotyping results are summarized in Tables II-IV.

TABLE II
FREQUENCY OF GSTM1 AND GSTT1 POSITIVE AND NULL GENOTYPES IN
CAD AFFECTED POPULATION AND CONTROL PEOPLE

genotypes	patients (n, %)	control (n, %)			R	5%CI
GSTT1(+/-)	5 (67.7%)	8 (70.8%)				
			.22	.639	.16	.627- 2.140
GSTT1(-/-)	1 (32.3%)	8 (29.2%)				
GSTM1(+/-)	4 (45.8%)	0 (72.9%)				
			4.60	0.001	.18	.741- 5.816
GSTM1(-/-)	2 (54.2%)	6 (27.1%)				

Abbreviations: OR - odds ratio; 95% CI - 95% confidence interval.

* Statistically significant.

As shown on Table II the frequencies of the GSTT1 null and positive alleles were not significantly different between the cases and controls. GSTT1 (+/-) genotype distribution in case (67.7%) and control group (70.8%) were not significantly different. In addition, the GSTT1 (-/-) polymorphism in patients (32.3%) and healthy group (29.2%) was found not to be significantly different.

The frequency of GSTM1-positive alleles (GSTM1 +/-) genotype was significantly lower in the case group (45.8%) compared with the control group (72.9%). In contrast, the frequency of the null GSTM1 (-/-) deletion was significantly

higher in the case group (54.2%) as compared to the control group (27.1%). Indeed, the GSTM1 null genotype among CAD affected population was almost two times greater than in control population (OR=3.18; 95% CI: 1.741-5.816, $P<0.001$).

TABLE III
FREQUENCY OF XRCC1 ARG399GLN GENOTYPES AND
ASSOCIATION STATISTICS

enotype	G	attients (n, %)	ontrol (n, %)	2	R	5% CI
RCC1 399 Arg/Arg	X	3 (44.8%)	5 (46.9%)		.92	.52 – 1.62
RCC1 399 Arg/Gln	X	5 (46.9%)	5 (46.9%)	.22	.64	.57 – 1.76
RCC1 399 Gln/Gln	X	(8.3%)	(6.2%)		.36	.45 – 4.09

Interestingly, XRCC1 399 genotype distribution in CAD patients was Arg/Arg n=43 (44.8%), Arg/Gln n=45 (46.9%), Gln/Gln n=8 (8.3%) and in control group Arg/Arg n=45 (46.9%), Arg/Gln n=45 (46.9%), Gln/Gln n=6 (6.2%). The Gln/Gln genotype or the Arg/Gln genotypes were not associated with an increased risk of coronary artery disease (OR 1.00, 95% CI: 0.57–1.62 for the Arg/Gln genotype and OR 1.36, 95% CI: 0.45–4.09 for the Gln/Gln genotype).

TABLE IV
FREQUENCY OF XRCC3 THR241MET GENOTYPES AND
ASSOCIATION STATISTICS

enotype	G	attients (n, %)	ontrol (n, %)	2	R	5% CI
RCC3 241 hr/Thr	X	8 (81.2%)	5 (78.1%)		.21	.60 – 2.46
RCC3 241 hr/Met	X	6 16.7%)	8 18.8%)	.36	.55	.87
RCC3 241 et/Met	M	2.1%)	3.1%)		.66	.11 – 4.04

Frequency of XRCC3 241 in the case group was Thr/Thr n=78 (81.2%), Thr/Met n=16 (16.7%), Met/Met n=2 (2.1%) and in healthy group was Thr/Thr n=75 (78.1%), Thr/Met n=18 (18.8%), Met/Met n=3 (3.1%). No statistically significant associations were found between the haplotypes of XRCC3 gene and heart ischemic disease (OR 1.21, 95% CI: 0.60–2.46 for the Thr/Thr genotype; OR 0.87, 95% CI: 0.41–1.82 for the Thr/Met genotype and OR 0.66, 95% CI: 0.11–4.04 for the Met/Met genotype).

IV. DISCUSSION

In this study we hypothesized that GST gene null polymorphism was associated with the generation of functionally inactive GST enzyme that may contribute to the accumulation of toxic products in organism and overall DNA damage in cells. Interestingly, the results of our study indicated that indeed, there was a significantly higher frequency of GSTM1 null genotypes among CAD case group compared with the control group (OR=3.18; 95% CI: 1.741-5.816, $P<0.001$). In contrast, the frequencies of the GSTT1 null and positive alleles were not significantly case and control groups (OR=1.16; 95% CI: 0.627-2.140, $P>0.1$).

The few studies that have investigated the role of xenobiotic detoxification genes in the etiology of CAD have obtained contradictory data. On one hand, Wilson *et al.* [23] demonstrated that there was no significant association between GSTT1 null polymorphism and CAD incidence. In addition, the same authors [24] found that GSTM1 null genotype reduces the risk of myocardial infarction both in population of European origin and South Asian populations. According to the author's interpretations of obtained results GSTM deletion may lead to plaque stabilization rather than to its regression, and therefore resulting in relative decrease of AMI incidence. Moreover, GSTM-correlated risk is significantly associated with AMI rather than with CAD in general. In contrast, Girisha and colleagues while investigating the role of polymorphism of GSTM1 and GSTT1 in North Indian population demonstrated a significant association of GSTT1 null, but not GSTM1 genotype in CAD development [25]. Others have suggested that geographical origins of different populations may contribute the gene-environment interaction and should be taken into consideration when analysing epidemiological studies [26]. Abu-Amro *et al* [27] also reported that besides the impact of ethnic variability, the null alleles of both GSTM1 and GSTT1 genes can be considered as CAD risk factors independent of smoking status. Thus it was deduced that smoking status and genetic polymorphisms are independent risk factors for CAD development and that the genotype-smoking interaction does not increase CAD incidences [27]. In another study [28], the authors showed that GSTT1 null allele and different combinations of GSTM1 null genotypes with other metabolizing genes are associated with increased risk for atherosclerosis development. Indeed, the authors [28] demonstrated that frequency of chromosomal aberrations among smoking patients in comparison to smoking controls was significantly increased in individuals with GSTM1 null allele.

Interestingly, very few studies have investigated the role of DNA repair gene polymorphisms for XRCC1 and XRCC3 in the development of cardiovascular disease. In the current study we found that neither XRCC1 Arg399Gln nor XRCC3 Thr241Met, are associated with CAD risk. This contrasts the observation that XRCC3 TT genotype is significantly more frequent in myocardial infarction patients than in healthy controls [29] and an observation that suggested that these genotypes may increase the risk of myocardial infarction.

Guven and colleagues [29] observed an association between micronucleus frequency and CAD incidence. Although no direct association between XRCC1 A399G and CAD presence or its severity has so far been shown, some studies have demonstrated that patients with XRCC1 G399 allele had an elevated frequency of micronucleus [30]. Although it is still not clear whether the interactions between DNA damage and cardiovascular disease is that of causal or no-causal effect, the probability of their interaction is high and an observation that needs further precise investigations.

Given the fact that no association between XRCC 1 and XRCC 3 is found in our study we propose that the DNA repair system is not tightly in the pathogenesis pathway of CAD. However association between GSTM1 null genotype and CAD is a clear evidence that detoxification of some chemicals including environmental pollutants is very important and may 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 we hypothesize that 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 is reported that GST enzymes also play a key role in protecting blood vessels against endogenous oxidants [21]. This indicates that the lack of active GST enzymes may compromise one's capabilities for detoxification of different endogenous and exogenous oxidants and ultimately one is disposed to higher risk of developing CAD.

V. LIMITATIONS OF THE STUDY

CAD is a complex disease and in its development a plethora of risk factors and multitude of underlying molecular pathologies may play a role. Involvement of different components and multiple events into the process makes it difficult to show an association between genotype and disease. Indeed, this may be so in cases where there is an activation of different components that may trigger different pathways to produce similar disease outcome. In most cases small effects can be detected in larger sample size, therefore small number of subjects in both groups in our study might significantly limits the reliability of our findings. In addition, distribution of particular disease-associated alleles may vary in different ethnic and geographic populations [26]. Despite the fact that this study was conducted on individuals of Kazakh origin, there is still no clear data indicating how the GST and XRCC polymorphisms are distributed among Kazakhstani population in general and Kazakhs particularly. Moreover influences of different environmental and interethnic processes taking place in different parts of Kazakhstan may significantly affect the genetic polymorphism and therefore study outcome. Also the absence of data on risk exposure to environmental toxicants/radiation, cigarette smoke (both passive and active), alcohol consumption limits the interpretation. Also, the lack of a precise knowledge of the molecular mechanisms that attributes polymorphism to disease development and gene-environment interactions makes it difficult to make an

association with DNA repair and xenobiotic detoxification gene,. It is likely that various forms of gene polymorphisms participating in the same biochemical pathways, cancel each other's effect [26].

VI. CONCLUSION

Based on our results we suggest that GSTM1 null genotype carriers are more susceptible to CAD development. We recommend that a careful investigation on DNA repair gene polymorphism involving a larger sample size is needed in order to establish whether there is a stronger statistically relationship in the absence of other confounding factors.

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