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NEWS

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**ASSOCIATION OF GCG REPEAT POLYMORPHISM
OF THE *GPXI* GENE CODING SELENIUM DEPENDENT
ANTIOXIDANT ENZYME WITH ISCHEMIC HEART DISEASE
DEVELOPMENT IN KAZAKHSTAN POPULATION**

Abstract. Human selenium dependent antioxidant protein GPX1 has been shown having a strong protective role in cardiovascular disorders. The *GPXI* gene has a unique GCG repeat polymorphism from four to six, resulting in the protein with 5, 6 or 7 numbers of alanine repeats and has been noted in associations with various disorders in different populations. The aim of this study was to evaluate whether this polymorphism is associated with ischemic heart disease (IHD) development in population from Kazakhstan. We evaluated 360 patients with IHD and 341 control volunteers. For detection of GCG repeats a real-time based, high resolution melting (HRM) analysis was applied. Multiple logistic regression analysis was applied to assess the risk for different genotypes obtained. There were no statistically significant associations of *GPXI*Ala 5-7 alleles and IHD development for general ethnically mixed group. Separate analysis of the main ethnic sub-groups showed that the presence of *Ala5* allele significantly increased the risk of IHD among Kazakhs (OR=1.73; 95% CI=1.31-2.29; $\chi^2 = 17.248$, $p = 0.005$). For Russian ethnic sub-group statistically significant associations for *Ala* 5-7 alleles were not observed. But there is a higher risk tendency for *GPXI*Ala7 allele (OR=1.37) and less for *Ala6* (OR=1.20). The presence of at least one *Ala5* allele of *GPXI* gene in genotype is associated with an increased tendency of IHD among Kazakhs.

Key words: oxidative stress, antioxidant system, glutathione peroxidase, Ischemic heart disease.

Inflammation accompanying all stages of ischemic heart disease development is characterized by the formation of an excessive amount of reactive oxygen species (ROS). Having a high reactivity and generated in a large amount, they can potentially interact with molecules that are not participants of vital signaling pathways and lead to destruction of cell components. Human glutathione peroxidase 1 (*hgpx1*) catalyzes the reduction of hydrogen peroxide and lipid hydroperoxides molecules which take place at high levels in the IHD conditions [1]. Low levels of red-cell GPX1 protein were observed as an independent risk factor for cardiovascular events [2]. Studies in mice show significant destruction of endothelial, vascular and cardiac tissue structure in heterozygous *GPXI*(+/-) carriers [3], [4], [5]. Regulation of *GPXI* expression and its protein activity is complex and many outer and inner gene factors can potentially be involved. In the *GPXI* gene protein coding region, it was identified several polymorphisms affecting GPX1 protein activity individually or in linkage disequilibrium [6]. Specific GCG repeat polymorphism located in the exon1 region has been noted to change the GPX1 activity and shown in associations with various disorders in different populations.

In this study we examined the hypothesis that *GPXI* in-frame GCG repeat polymorphism, resulting in the GPX1 protein with 5, 6 or 7 numbers of alanine repeats, may play a role in the pathogenesis of IHD in Kazakhstan population.

Materials and methods. Study subject. Place of collection of biomaterials and participants personal data was «City Clinical Hospital №1» (Almaty, Kazakhstan), cardiology department and «Kazakh-Russian Medical University». The study was approved by the ethics Committee («Kazakh-Russian Medical University» local ethics Committee, Almaty (protocol No. 36 of January 5, 2016)). All biological samples and participant data were collected only on a voluntary basis, after signing an informed consent to participate in the research.

Peripheral blood samples were collected from 360 patients with stable forms of angina pectoris (angina pectoris of I–IIY classes according to the Canadian classification of cardiologists, Association of Cardiologists of the Republic of Kazakhstan [7]), with chronic heart failure of II-III classes [8] and postinfarction atherosclerosis. Diagnosis of IHD was based on WHO criteria and was established after diagnostic stress tests or coronary angiography, evaluation of the presence of a chest pain syndrome, confirmed by ECG data and cardiospecific enzymes (troponin, myoglobin, creatine kinase).

Control blood samples were obtained from 341 healthy donors without clinical manifestations of IHD, without family history of atherosclerosis and ischemic events at ECG, oncology, autoimmune diseases, any hereditary diseases and acute/chronic inflammatory diseases. Control and IHD patients groups were selected to each other according to the questionnaire personal data. Questionnaires contained detailed information about ethnicity, demographic status, blood pressure, habits (tobacco, alcohol, diet) and previous illnesses, other concomitant illnesses.

DNA isolation. Genomic DNA was extracted from EDTA-treated peripheral blood samples using «Genomic DNA Purification Kit» (*Thermo Scientific*, USA). Qualitative and quantitative characteristics of the DNA samples were estimated by spectrophotometry (*Eppendorf Biophotometer plus*, Germany). Isolated DNA samples were stored at -20°C .

GCG repeat genotyping. Genotyping of all samples were performed using a real-time based high resolution melting (HRM) analysis. Samples with the known genotypes were used as control standards for identification of unknown samples.

Amplicon size detection of samples as control standards. Genotypes of samples used in HRM as the control standards were identified by usual PCR amplification of *GPXI* region containing target GCG repeat. 20 μl of PCR mixture contained 50 ng of target genomic DNA, 10 μl 2 \times PCR Master Mix (0.05 U/ μL Taq DNA polymerase, reaction buffer, 3.5mM MgCl_2 , 0.4 mM of each dNTP (*Thermo Scientific*)) and 5pM of each specific primer (*GPXI_F*: 5'-GAA ACT GCC TGT GCC ACG TGA CC-3'; *GPXI_R*: 5'-CGA GAA GGC ATA CAC CGA CTG GGC-3'). PCR optimized conditions: initial pre-incubation 3 min at 95°C , 35 cycles of denaturation 30 sec at 95°C , annealing for 30 sec at 60°C and extension for 1 min at 72°C . This resulted in production of three PCR products of 165, 168 and 171bp, representing the alleles with five, six or seven *Ala* repeats respectively. Allele sizes were determined by separation of the PCR products in 25% polyacrylamide gel electrophoresis (*Sigma-Aldrich*, USA) with the following visualization under UV light.

Amplicon size detection by high resolution melting analysis. The same high-purity primers were used for amplification of target GCG containing sequence of *GPXI* gene. PCR reaction conditions were highly optimized for HRM amplification. For each reaction previously diluted up to 5ng/ μl in TE buffer DNA was used. Reaction was performed in the mixture of 5 μl 2 \times PCR Master Mix (0.05 U/ μL Tbr DNA polymerase, SYBR Green I, optimized PCR buffer, 5mM MgCl_2 , 0.4 mM of each dNTP (*DyNAmo Flash SYBR Green qPCR Kit*, *Thermo Scientific*, USA), 3pM of each specific primer and 25ng of genomic DNA in a total volume of 10 μl . HRM-PCR was started with an initial activation of hot-start version of TbrDNA polymerase at 95°C for 7 min, then followed by 40 cycles of 95°C for 15s, and 60°C for 20s and 72°C for 1 min. To facilitate heteroduplex formation, reactions were heated slowly until 95°C during 30s and then cooled to 40°C for 1 min. A melting curve was detected by heating the reactions from 60°C to 98°C at $0.2^{\circ}\text{C}/\text{second}$. Amplification was done on *Piko Real time Thermal Cycler* (*Thermo Scientific*, USA) with subsequent detection and analysis of samples genotypes by using the supplied *Thermo Scientific Piko Real Software 2.2*.

Results were analyzed as fluorescence (*F*) versus temperature (*T*) graphs. The data were also analyzed using normalization, temperature shifting and difference plots. Genotyping of all samples was performed in three replicates and scored by different individuals to avoid genotyping error.

Statistical analysis. To compare the distribution of variables between case and control cohorts a Student's *t*-test was used. Allele frequencies were calculated according to the standard Hardy-Weinberg equilibrium (HWE) for 3 alleles. Estimation of the coefficient of relative risk was calculated by the method of «odds ratio» (odd ratio - OR) in conjunction with an estimate 95% confidence interval (95% CI) and the «Chi-square» test (χ^2) for 3 alleles (degrees of freedom = 2) and 6 genotypes (degrees of freedom = 5). We performed general model of multivariate analysis taking into account all alleles and genotypes separately. Each homozygous state allele effect was estimated in combinations with these allele heterozygous genotypes and other genotypes which are not included in this allele. Separate analyses were done for main ethnic groups (Kazakh and Russian). A *p*-value of <0.05 was considered statistically significant.

Results. Cohorts' characteristics. For genotyping and associative analysis we included all 360 patients and 341 healthy volunteers. The main characteristic data are summarized in Table 1. The average age and smoking status in two groups investigated were not statistically different (*p*>0.05). There were no detected differences in sex between men and women in two groups. As population studied is ethnically mixed, we distinguished three ethnic sub-groups found in our cohorts. The prevalent sub-group was Kazakhs, then Russians and other Asians (Koreans, Uighurs, and Kyrgyz) as among the control as among the IHD group. The ethnic's ratio in both groups was almost identical: 63.88% Kazakhs, 26.94% Russians, 8.21% other Asians in IHD cohort and 69.5% Kazakhs, 19.94% Russians, 9.68% Other Asians.

The total cholesterol level standard 5.02mmol/L was taken into account for both groups. In IHD group the total cholesterol level was more than 5.02mmol/L and in control group this level did not exceeded 5.02mmol/L.

Table 1 – Main characteristics of IHD group and control group

| Characteristics | | IHD group | Controlgroup | t st | pvalue |
|--------------------------|-------------|-----------------------------|-----------------------------|-----------------|--------|
| <i>n</i> | | 360 | 341 | | |
| Age (y) | | 1923 – 1970 (60 ± 12.04) | 1921 – 1970 (52 ± 13.23) | 0.51 | 0.699 |
| Gender, n (%) | Male | 91 (25.27) | 109 (31.96) | 1.628 | 0.350 |
| | Female | 269(74.72) | 232 (68.03) | 1.042 | 0.486 |
| Ethnicity, n (%) | Kazakhs | 230 (63.88) | 237 (69.5) | 0.908 | 0.530 |
| | OtherAsians | 28 (8.21) | 33 (9.68) | 0.840 | 0.555 |
| | Russians | 102 (28.32) | 71 (20.82) | 1.925 | 0.305 |
| Smokingstatus, n (%) | Smokers | 41(11.39) | 57 (16.71) | 1.796 | 0.323 |
| | Non-smokers | 319 (88.61) | 284 (83.28) | 0.758 | 0.587 |
| Totalcholesterol, mmol/L | | >5.02 (5.47±0,09) | <5.02 (4.01±0,06) | 13.498 | 0.047 |
| Bloodpressure, mmHg | | >140/90 | 120/60 | | |

GPXI HRM data results. GCG repeat polymorphism was successfully genotyped for all DNA samples isolated from blood of 360 IHD patients and 341 healthy people. 30 DNA samples were genotyped by separation of PCR products in 25% polyacrylamide gel electrophoresis for identification of 6 possible genotypes, used later on HRM genotyping as control standards with known genotypes.

For 671 other DNA samples, 659 samples were completely genotyped by HRM analysis (figure 1A). Absolute fluorescence varied from sample to sample. Melt curve raw data were plotted as fluorescence versus temperature. All samples had clear and high peaks on derivative melt curve plot without peaks of lower intensity represented nonspecific products (figure 1B). Genotypes results were better visualized using fluorescence-normalized melting curves (figure 2A) and difference plots (figure 2B). 12 samples were not genotyped by HRM as they gave the same but nonspecific melting curve distinct from melting curve of the known samples. They were repeatedly genotyped by separation in 25% polyacrylamide gel electrophoresis.

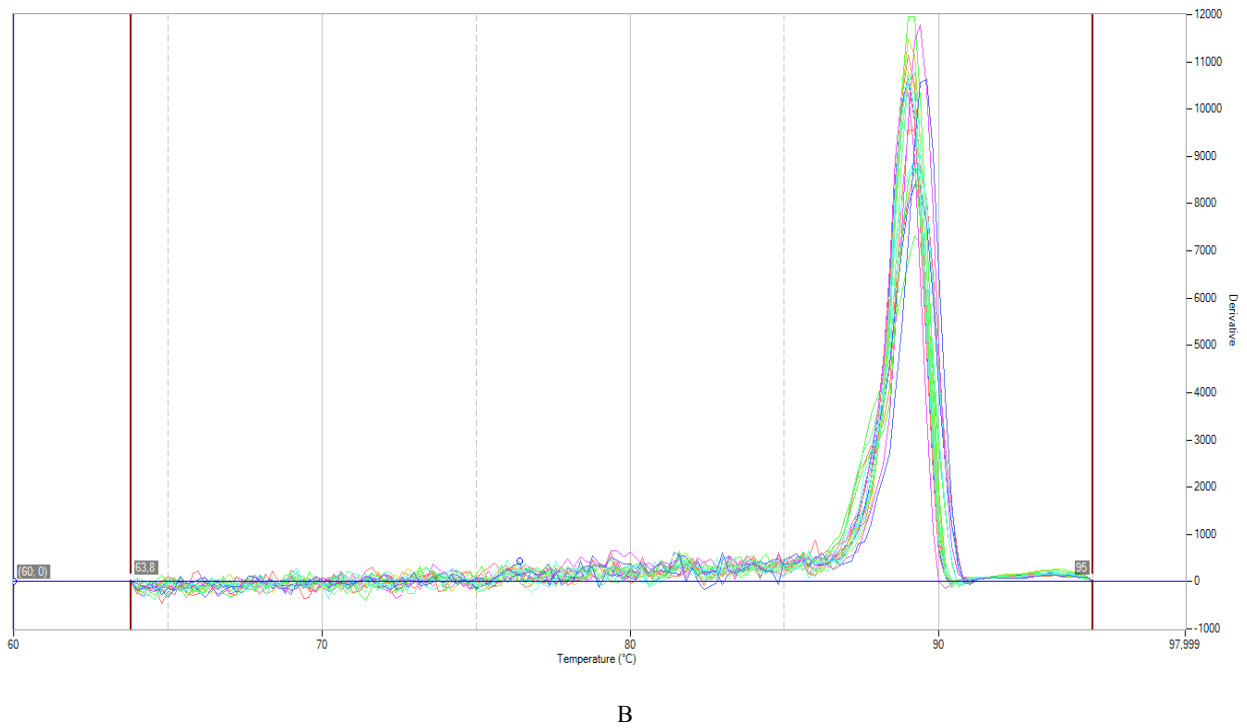
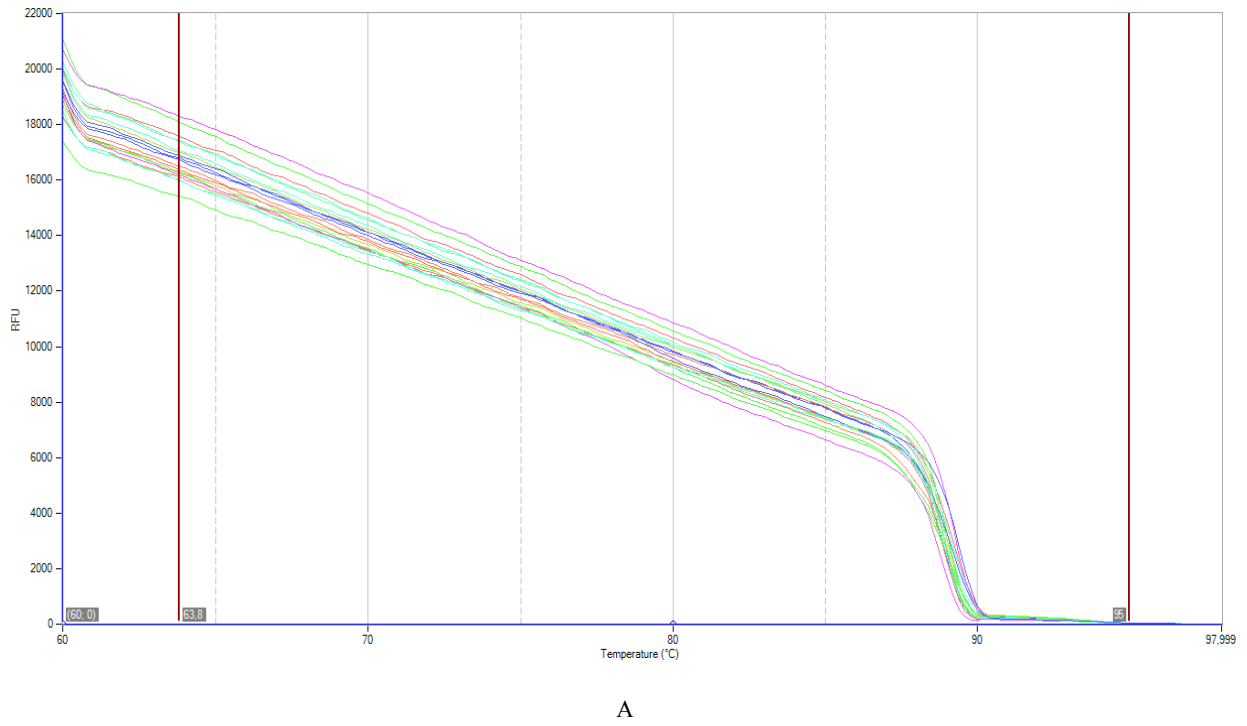
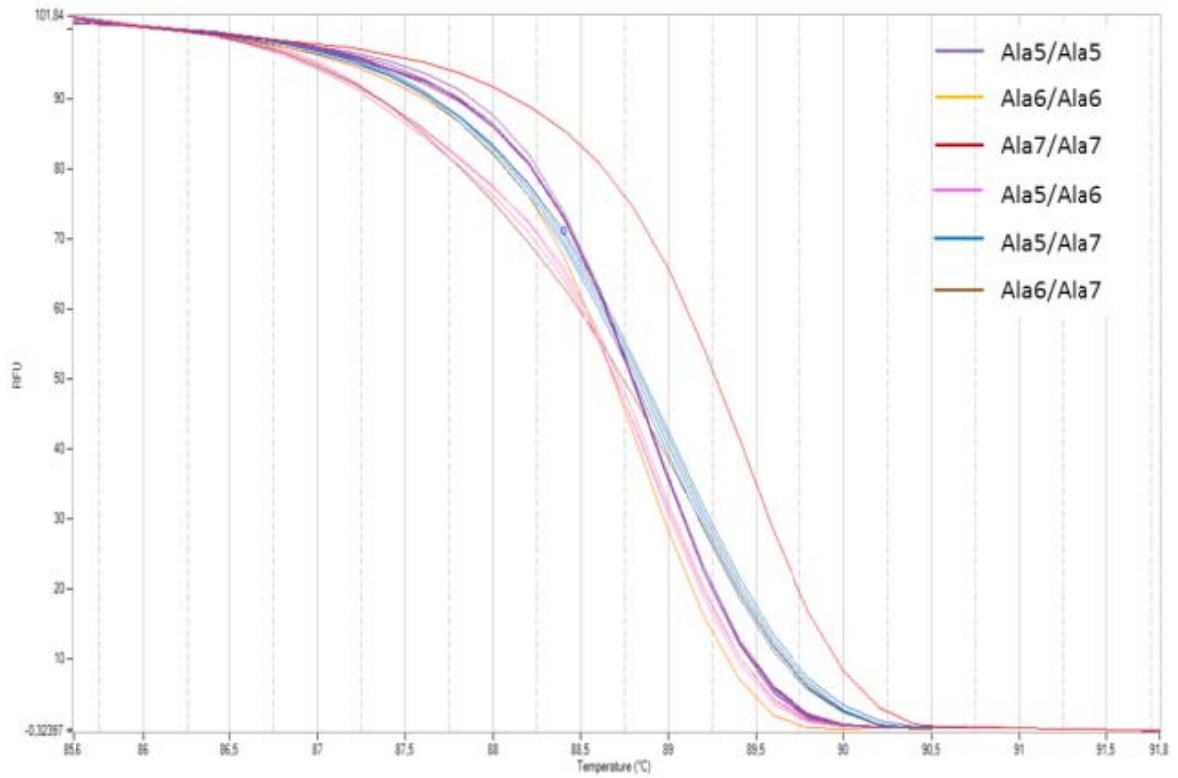
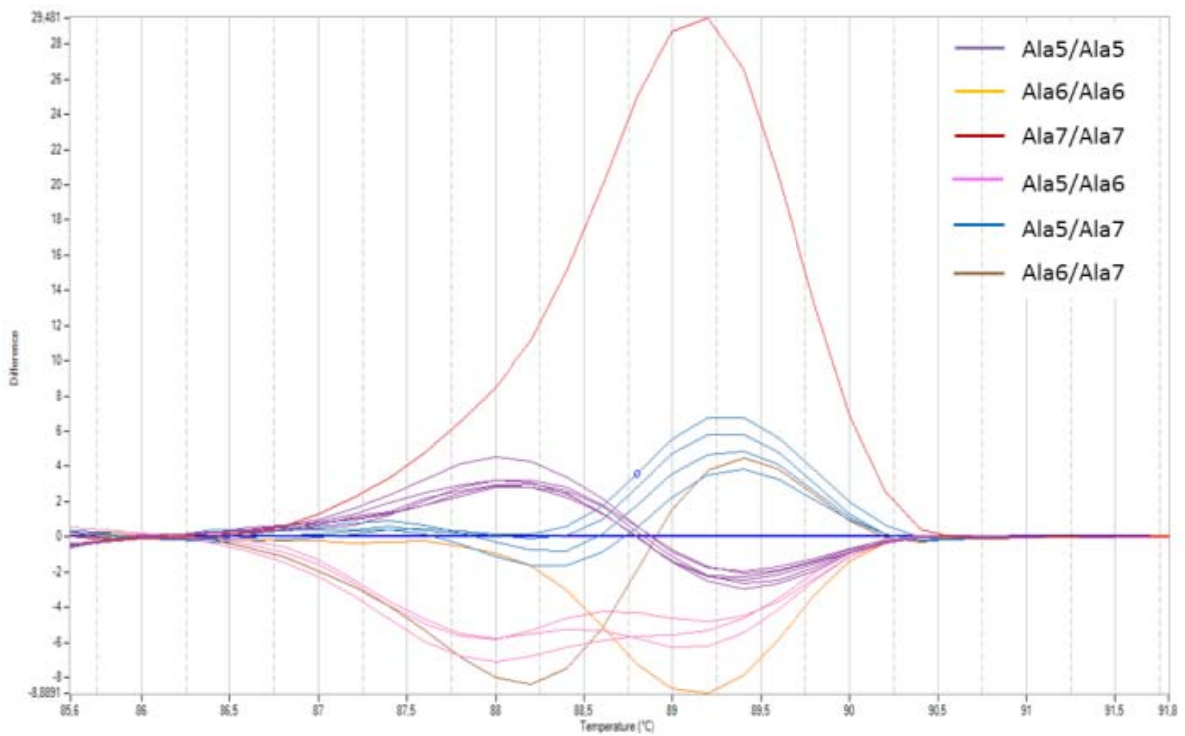


Figure 1 – Real-Time PCR data showing DNA Melt Profiles of the analyzed genotypes.
A: A standard melt curve plot. B: A derivative melt curve plot



A



B

Figure 2 – Detection of *GPX1* GCG repeat genotypes by high-resolution melting analysis. A: Melting curves after fluorescence-normalization. B: Fluorescence difference plots of the normalized data

GPXI GCG genotyping results. Genotyping of *GPXI* GCG repeat polymorphism for the general ethnic group showed distribution of genotypes frequencies in accordance with Hardy-Weinberg equilibrium: for the controls ($\chi^2=2.517$; $p=0.998$) and for the experimental group ($\chi^2=9.524$; $p=0.090$).

In the general population, we did not find statistically significant associations between *GPXI Ala* repeats and the risk of IHD. General model of inheritance showed a tendency for two genotypes to have risks properties: *Ala7/7* OR=1.67 and *Ala5/5* OR=1.27, $\chi^2=3.818$; $p=0.148$.

Statistically significant associations of *GPXI Ala* repeats with IHD were obtained for Kazakhs when a separate analysis of the ethnic sub-groups had been done (Table 2). The *Ala5* allele demonstrated high and significant risk of IHD (OR=1.73; 95%CI=1.31-2.29; $\chi^2 = 17.248$, $p = 0.000$), which is expressed in homozygous state (*Ala5/5* genotype: OR=1.88; 95%CI=1.30-2.71; $\chi^2 = 16.902$, $p = 0.005$), but not in heterozygotes: *Ala5/6* – OR=0.91, and *Ala5/7* – OR=0.54. *Ala6* (OR=0.69) and *Ala7* (OR=0.47) alleles had a protective properties which positive effect was more expressed in *Ala6/7* genotype (OR=0.33; 95%CI=0.12-0.92; $\chi^2 = 16.902$, $p = 0.005$).

Table 2 – Association between the *GPXI* GCG polymorphism and IHD in Kazakh ethnic group

| Alleles and alleles combinations | Controls | IHD patients | OR (95% CI) | χ^2 | p-value |
|----------------------------------|----------|--------------|--------------------|----------|---------|
| Multiplicative model (df=2) | | | | | |
| <i>Ala5</i> | 339 | 293 | 1.73 (1.31 – 2.29) | 17.248 | 0.000 |
| <i>Ala6</i> | 98 | 133 | 0.69 (0.51 – 0.94) | | |
| <i>Ala7</i> | 23 | 48 | 0.47 (0.28 – 0.78) | | |
| General model (df=5) | | | | | |
| <i>Ala5/5</i> | 126 | 93 | 1,88 (1.30 – 2.71) | 16.902 | 0.005 |
| <i>Ala5/6</i> | 71 | 78 | 0,91 (0.62 – 1.34) | | |
| <i>Ala6/6</i> | 11 | 20 | 0,54 (0.26 – 1.16) | | |
| <i>Ala5/7</i> | 16 | 29 | 0,54 (0.28 – 1.02) | | |
| <i>Ala6/7</i> | 5 | 15 | 0,33 (0.12 – 0.92) | | |
| <i>Ala7/7</i> | 1 | 2 | 0,51 (0.05 – 5.70) | | |

For Russians there was an opposite association of results of *GPXI* alleles and genotypes with IHD. *Ala5* allele had a nonsignificant protective effect according to multivariate model of inheritance (OR=0.67; $\chi^2 = 3.346$, $p = 0.188$), whereas *Ala6* and *Ala7* alleles had risk properties (*Ala6* OR=1.20; *Ala7* OR=1.37). General model of inheritance shows the risk for *Ala6/6* (OR=1.38; 95%CI=0.58-3.29) and *Ala7/7* (OR=2.16; 95%CI=0.42-11.00) genotypes, and protective role for *Ala5/5* genotype (OR=0.59; 95%CI=0.27-1.32). But these data also were not statistically significant ($\chi^2 = 3.398$, $p = 0.183$). There were no statistically significant associations with IHD among Other Asians.

Discussion. General and specific population characteristics are a key moment for further data processing and correct results obtaining. In our study, all individuals passed strong selection procedure to be included in the control or IHD group, to make groups that are the most appropriate to each other for minimization of undesirable impacts from other influencing factors. According to questionnaire data used, there were no statistically significant differences between case and control cohorts on the main population characteristics as ethnicity, age, sex, tobacco smoking and other disorders that may cause an oxidative stress and IHD. Average total cholesterol was statistically different between case and control. Individuals with only normal blood pressure (120/60 mmHg) were included in control group, whereas individuals with blood pressure more than 120/60mmHg with manifestation in current time were included in IHD group. In this study we did not account more detailed data on lipoprotein panel of blood (LDL, HDL, triglycerides) because they were not available for us.

For genotyping *GPXI* GCG repeat polymorphism (representing 5-7 alanine residues repeats in the GPX1 protein) we chose a real-time based HRM method as it had been demonstrated a suitable, fast and inexpensive method for short tandem repeats revealing [9], [10]. Another criterion for choosing this

method was its high accuracy for different mutations screening and polymorphisms genotyping [11], [12], [13], [14]. In our work, we confirm its high sensitivity in *GPX1* GCG repeat genotyping. For 701 DNA samples, 689 gave clearly visible genotype-specific melting profiles and were completely and successfully genotyped. 12 DNA samples gave the same but non-specific melting profiles. Genotyping by usual PCR in slow-timing PAGE gel electrophoresis (25%) revealed genotype-specific bands. Another non-genotype specific melt profiles may indicate the presence in amplified region an additional site-specific mutation and further sequencing analysis is needed. It is noteworthy that these melt profiles were fixed mainly among the IHD group: 10 samples in the IHD group versus 2 samples in the control.

Genotyping of *GPX1* GCG repeats in general population did not reveal any statistically significant associations with IHD. This is maybe due to the presence of three different ethnic groups: Kazakhs, Russians and other Asians, genetically and historically differ. Analysis of associations in these sub-groups separately shows opposite associative results for Kazakhs versus Russians. *Ala5* allele demonstrated a positive risk factor in IHD development for Kazakhs, whereas two other alleles *Ala6* and *Ala7* show strong protective effect (*Ala6* OR=0.69, *Ala7* OR=0.47). In Russians sub-group statistically significant data were not shown but there is a tendency of *Ala6* and *Ala7* alleles to risk effects. Russian group is two and a half times smaller than Kazakhs and for gene polymorphism with three alleles there should be a larger group to achieve a statistically significant data. Additional genotyping on larger Russian cohort is needed to obtain data for an unambiguous association conclusion. However, these data already show the importance of the genetic aspect of ethnicity in the development of IHD pathology. Lifestyle features historically entrenched among ethnic nations and genetically selected may have different effects on modern urban changes leading to health problems.

Despite the fact that little is known about the specific molecular mechanism of the effect of the GCG repeat on the functions of the GPX1 protein, the accumulated literary data, as well as the data obtained by us, clearly complement the idea of its important functional role in the body. Since Shen Q et al. [15] first published the data on the presence of an in-frame GCG repeat in *GPX1* gene and showed its high instability in vitro on the human myeloid leukemia cell line; little new data were obtained revealing the molecular mechanism of the effect of this polymorphism on GPX1 activity. In addition, it was identified additional single polymorphic sites in *GPX1* gene including promoter regions that may affect GPX1 protein activity in cells [16]. It has been shown the GCG repeat alleles in linkage disequilibrium with the *Pro198Leu* alleles [6] and -602A/G, -2C/T [17]. Hamanishi T. et al. indicated that the combination *Ala6/198Leu* had a 40% decrease in enzyme activity in vitro functional analysis. Moreover, he showed functional combinations of -602A/G, +2C/T, *5Ala/6Ala* and *Pro198Leu* in *GPX1* that are significantly associated with the intima-media thickness of carotid arteries and the risk of cardiovascular diseases in type 2 diabetes patients [6]. Winter J. et al. revealed a significant association between the *GPX16Ala* allele and the risk of coronary artery disease but not in co-segregation with *Pro198Leu* [18]. An interesting conclusion was done by Hardie J. et al. under observation of *8OHdG* (8-hydroxydeoxyguanosine) level changes in normal and tumor lung tissues [19]. Although there was no significant differences between *8OHdG* levels in tumour and normal lung tissue but there was a tendency to reduce the level of *8OHdG* in individuals possessing the *Ala6* alleles in normal tissue. Besides, loss of heterozygosity (LOH) in this region demonstrated an increase of *8OHdG* levels and reduce of *GPX1* activity in tumour lung tissues, comparing to normal. A case-control study had done by Kote-Jarai Z. et al. did not find any associations of GCG repeat polymorphism and young onset prostate cancer in Caucasian patients living in England [20]. Also, this GCG repeat polymorphism individually and in combination with *Pro198Leu* was noted to be associated with low bone mineral density (BMD) in Slovenian population [21]. Separately, it should be mentioned an event of LOH in the GCG repeat locus of *GPX1* gene widely observed in different cancer types development [22], [23], [24]. Allelic loss of alanine repeats region may significantly increase up to 42% of colorectal cancer events [25].

Data obtained in our research and controversial literature data show a complex and significant role of GCG repeat polymorphism in diseases development. This, undoubtedly, requires further epidemiological and molecular-genetic studies, paying attention to the ethnic component of the population because even in a relatively small population, ethnic differences in association are evident, which are apparently caused by lifestyle characteristics.

Conclusions. The present data demonstrate that an in-frame GCG repeat polymorphism in *GPXI* gene, resulting in the protein with 5, 6 or 7 numbers of alanine repeats is associated with IHD development for Kazakh ethnic group in ethnically mixed Kazakhstan population. For Russian and other Asians ethnic groups it is necessary to do additional case-control studies on larger groups of each ethnic cohort separately. Moreover, besides commonly used parameters for cardiovascular diseases such as main symptoms, blood pressure, ethnicity, age, gender, smoking status, it is necessary to obtain and consider detailed information on lipoprotein panel, fasting glucose, medical taking, diet, so as to obtain as much information as possible on all factors that may influence antioxidant homeostasis in the body.

REFERENCES

- [1] M.A. Reeves, P.R. Hoffmann, The human selenoproteome: Recent insights into functions and regulation, *Cell. Mol. Life Sci.* 66 (2009) 2457–2478. doi:10.1007/s00018-009-0032-4.
- [2] S. Blankenberg, H.J. Rupprecht, C. Bickel, M. Torzewski, G. Hafner, L. Tiret, M. Smieja, F. Cambien, J. Meyer, K.J. Lackner, Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease., *N. Engl. J. Med.* 349 (2003) 1605–1613. doi:10.1056/NEJMoa030535.
- [3] M.A. Forgione, N. Weiss, S. Heydrick, A. Cap, E.S. Klings, C. Bierl, R.T. Eberhardt, H.W. Farber, J. Loscalzo, Cellular glutathione peroxidase deficiency and endothelial dysfunction, *Am. J. Physiol. - Hear. Circ. Physiol.* 282 (2002) H1255–H1261. doi:10.1152/ajpheart.00598.2001.
- [4] P. Marc A. Forgione, MD; Andre Cap, PhD; Rongliu Liao, PhD; Nicanor I. Moldovan, M. Robert T. Eberhardt, MD; Chee Chew Lim, PhD; John Jones, P. Pascal J. Goldschmidt-Clermont, MD; Joseph Loscalzo, MD, Heterozygous Cellular Glutathione Peroxidase Deficiency in the Mouse, *Circulation*. 106 (2002). file:///C:/Users/emilio/Dropbox/Dharuma sol/forgione ma 2002.pdf.
- [5] M.A. Forgione, A. Cap, R. Liao, N.I. Moldovan, R.T. Eberhardt, C.C. Lim, J. Jones, P.J. Goldschmidt-Clermont, J. Loscalzo, Heterozygous cellular glutathione peroxidase deficiency in the mouse: Abnormalities in vascular and cardiac function and structure, *Circulation*. 106 (2002) 1154–1158. doi:10.1161/01.CIR.0000026820.87824.6A.
- [6] T. Hamanishi, H. Furuta, H. Kato, A. Doi, M. Tamai, H. Shimomura, S. Sakagashira, M. Nishi, H. Sasaki, T. Sanke, K. Nanjo, Functional variants in the glutathione peroxidase-1 (GPx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in Japanese type 2 diabetic patients, *Diabetes*. 53 (2004) 2455–2460. doi:10.2337/diabetes.53.9.2455.
- [7] L. Campeau, Letter: Grading of angina pectoris., *Circulation*. 54 (1976). <http://circ.ahajournals.org/content/54/3/522.2> (accessed 30 May 2017).
- [8] Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature., *Circulation*. 59 (1979) 607–9. <http://www.ncbi.nlm.nih.gov/pubmed/761341> (accessed 30 May 2017).
- [9] C.P. Vaughn, K.S.J. Elenitoba-Johnson, High-resolution melting analysis for detection of internal tandem duplications., *J. Mol. Diagn.* 6 (2004) 211–216. doi:10.1016/S1525-1578(10)60512-0.
- [10] A.Y.C. Tan, D.A. Westerman, D.A. Carney, J.F. Seymour, S. Juneja, A. Dobrovic, Detection of NPM1 exon 12 mutations and FLT3 - internal tandem duplications by high resolution melting analysis in normal karyotype acute myeloid leukemia., *J. Hematol. Oncol.* 1 (2008) 10. doi:10.1186/1756-8722-1-10.
- [11] H. Do, M. Krypuy, P.L. Mitchell, S.B. Fox, A. Dobrovic, High resolution melting analysis for rapid and sensitive EGFR and KRAS mutation detection in formalin fixed paraffin embedded biopsies., *BMC Cancer*. 8 (2008) 142. doi:10.1186/1471-2407-8-142.
- [12] J. Gonzalez-Bosquet, J. Calcei, J.S. Wei, M. Garcia-Closas, M.E. Sherman, S. Hewitt, J. Vockley, J. Lissowska, H.P. Yang, J. Khan, S. Chanock, Detection of somatic mutations by high-resolution DNA melting (HRM) analysis in multiple cancers, *PLoS One*. 6 (2011). doi:10.1371/journal.pone.0014522.
- [13] C.F. Taylor, Mutation scanning using high-resolution melting, *Biochem. Soc. Trans.* 37 (2009) 433–437. doi:10.1042/BST0370433.
- [14] G.H. Reed, C.T. Wittwer, Sensitivity and specificity of single-nucleotide polymorphism scanning by high-resolution melting analysis, *Clin. Chem.* 50 (2004) 1748–1754. doi:10.1373/clinchem.2003.029751.
- [15] Q. Shen, P.L. Townes, C. Padden, P.E. Newburger, An in-frame trinucleotide repeat in the coding region of the human cellular glutathione peroxidase (*GPXI*) gene: in vivo polymorphism and in vitro instability, *Genomics*. 23 (1994) 292–294. doi:10.1006/geno.1994.1499.
- [16] G. Ravn-Haren, A. Olsen, A. Tjønneland, L.O. Dragsted, B.A. Nexø, H. Wallin, K. Overvad, O. Raaschou-Nielsen, U. Vogel, Associations between *GPXI*Pro198Leu polymorphism, erythrocyte GPX activity, alcohol consumption and breast cancer risk in a prospective cohort study, *Carcinogenesis*. 27 (2006) 820–825. doi:10.1093/carcin/bgi267.

- [17] J. a Moscow, L. Schmidt, D.T. Ingram, J. Gnarra, B. Johnson, K.H. Cowan, Loss of heterozygosity of the human cytosolic glutathione peroxidase I gene in lung cancer., *Carcinogenesis*. 15 (1994) 2769–2773. doi:10.1016/0169-5002(95)98726-Q.
- [18] J.P. Winter, Y. Gong, P.J. Grant, C.P. Wild, Glutathione peroxidase 1 genotype is associated with an increased risk of coronary artery disease, *Coron Artery Dis*. 14 (2003) 149–153. doi:10.1097/01.mca.0000061618.36552.10.
- [19] L.J. Hardie, J.A. Briggs, L.A. Davidson, J.M. Allan, R.F. King, G.I. Williams, C.P. Wild, The effect of *hOGG1* and glutathione peroxidase I genotypes and 3p chromosomal loss on 8-hydroxydeoxyguanosine levels in lung cancer, *Carcinogenesis*. 21 (2000) 167–172. <http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/referer?http://carcin.oupjournals.org/cgi/content/full/21/2/167>.
- [20] Z. Kote-Jarai, F. Durocher, S.M. Edwards, R. Hamoudi, R.A. Jackson, A. Ardern-Jones, A. Murkin, D.P. Dearnaley, R. Kirby, R. Houlston, D.F. Easton, R. Eeles, Association between the GCG polymorphism of the selenium dependent *GPXI* gene and the risk of young onset prostate cancer, *Prostate Cancer Prostatic Dis*. 5 (2002) 189–192. doi:10.1038/sj.pcan.4500569.
- [21] S.J. Mlakar, J. Osredkar, J. Prezelj, J. Marc, The antioxidant enzyme *GPXI* gene polymorphisms are associated with low BMD and increased bone turnover markers, *Dis. Markers*. 29 (2010) 71–80. doi:10.3233/DMA-2010-0728.
- [22] Y.J. Hu, A.M. Diamond, Role of glutathione peroxidase 1 in breast cancer: Loss of heterozygosity and allelic differences in the response to selenium, *Cancer Res*. 63 (2003) 3347–3351.
- [23] Y.J. Hu, M.E. Dolan, R. Bae, H. Yee, M. Roy, R. Glickman, L. Kiremidjian-Schumacher, A.M. Diamond, Allelic loss at the GPx-1 locus in cancer of the head and neck, *Biol. Trace Elem. Res*. 101 (2004) 97–106. doi:10.1385/BTER:101:2:097.
- [24] M. Goldberg, D.S. Alberts, J. a Buckmeier, A.R. Prasad, R.S. Krouse, A.M. Diamond, Loss of heterozygosity at the glutathione peroxidase 1 locus is not an early event in colon carcinogenesis., *Genes Cancer*. 2 (2011) 910–3. doi:10.1177/1947601911431840.
- [25] Y. Hu, R. V Benya, R.E. Carroll, A.M. Diamond, Allelic loss of the gene for the *GPXI* selenium-containing protein is a common event in cancer., *J. Nutr*. 135 (2005) 3021S–3024S. doi:10.1385/BTER:101:2:097.
- [26] R.C. McKenzie, T. S. Rafferty, G.J. Beckett, Selenium: An essential element for immune function, *Immunol. Today*. 19 (1998) 342–345. doi:10.1016/S0167-5699(98)01294-8.

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ҚАЗАҚСТАН ПОПУЛЯЦИЯСЫНДА ЖҮРЕК ИШЕМИЯЛЫҚ АУРУЛАРЫНЫҢ ДАМУЫМЕН ЖАНАМАЛЫҚ СЕЛЕНДІ КОДТАЙТЫН АНТИОКСИДАНТТЫ ФЕРМЕНТИҢ GCG ҚАЙТАЛАНАТЫН *GPXI* ГЕНИ ПОЛИМОРФИЗМІНІҢ АССОЦИАЦИЯСЫ

Аннотация. Адамның селенийге тәуелді антиоксидантты протеині GPX1 жүрек-қан тамыр ақауларында ерекше қорғаныш рөліне ие. *GPXI* генінде төрттен алтыға дейін GCG бірегей қайталанатын, нәтижесінде 5, 6 немесе 7 қайталанбалы аланинді протеинге әкелетін және әртүрлі популяциялардағы түрлі бұзылыстармен байланысқан полиморфизмі болады.

Бұл зерттеудің негізгі мақсаты аталған полиморфизмнің Қазақстан популяциясында жүректің ишемиялық аурудың (ЖИА) дамуымен байланысын анықтау болды. Мұнда ЖИА бар 360 науқас пен 341 ерікті бақылау топ адамдары бағаланды. CGG қайталануын анықтау үшін «нақты-уақыт» жағдайындағы ПТР (HRM) талдауы қолданылды. Көп құрамды логистикалық регрессиялық талдау алынған әртүрлі генотиптердің әсерін бағалауда пайдаланылды.

Жалпы этникалық аралас топ үшін *GPXI Ala5-7* аллелдері мен ЖИА дамуы арасында статистикалық маңызды байланыс болмайтындығы анықталды. Негізгі этникалық кіші топтарды жеке талдау арқылы, қазақтар арасында *Ala5* аллелінің болуы ЖИА қауіпін едәуір арттыратыны көрсетілді (OR=1.73; 95%CI=1.31-2.29; $\chi^2 = 17.248$, $p = 0.005$). Ұлты орыс этникалық топтар үшін *Ala 5-7* аллелдері мен ЖИА арасында статистикалық маңызды байланыстар байқалмады. Алайда, *GPXI Ala7* аллелі үшін қауіптің жоғарылауы (OR = 1.37) және *Ala6* аллелі үшін қауіптің төмендеуі (OR = 1.20) тенденциялары байқалады.

GPXI генінің ең кем дегенде бір *Ala5* аллелінің генотипте болуы қазақтар арасында ЖИА өсу процесімен байланысты.

Түйін сөздер: тотығу стрессі, антиоксиданттық жүйе, глутатионпероксидаза, жүректің ишемиялық ауруы.

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**АССОЦИАЦИЯ ПОЛИМОРФИЗМА GCG, ГЕНА GPX1,
КОДИРУЮЩЕГО СЕЛЕН-ЗАВИСИМЫЙ АНТИОКСИДАНТНЫЙ БЕЛОК,
С РАЗВИТИЕМ ИШЕМИЧЕСКОЙ БОЛЕЗНИ СЕРДЦА В КАЗАХСТАНСКОЙ ПОПУЛЯЦИИ**

Аннотация. Неоднократно, было показано, что селен-зависимый антиоксидантный белок *gpx1* проявляет сильную защитную роль при сердечно-сосудистых расстройствах. Ген GPX1 имеет уникальный трехнуклеотидный полиморфизм GCG, от четырех до шести, в результате чего белок может содержать от 5, до 7 повторов аланина в кодирующей последовательности. Данный полиморфизм был отмечен в ассоциациях с различными заболеваниями в разных популяциях. Целью нашего исследования было оценить, связан ли этот полиморфизм с развитием ишемической болезни сердца (ИБС) у населения Казахстана.

Для исследования были отобраны 360 пациентов с ИБС и 341 условно здоровых людей. Для определения GCG повторов использовали метод реал-тайм ПЦР с применением высокоразрешающего анализа плавления ДНК (HRM). Для оценки риска полученных генотипов, был применен множественный логистический регрессионный анализ.

Для общей, этнически смешанной группы, статистически значимых ассоциаций аллелей GPX1 *Ala 5-7* и развития ИБС выявлено не было. Анализ основных этнических подгрупп в отдельности, показал, что наличие аллеля *Ala5* значительно увеличивает риск ИБС среди казахов (OR=1.73; 95%CI=1.31-2.29; $\chi^2 = 17.248$, $p = 0.005$). Для Русской этнической подгруппы статистически значимых ассоциаций для аллелей *Ala 5-7* не наблюдалось. Но можно отметить повышенный риск для аллеля GPX1 *Ala7* (OR = 1,37) и менее для *Ala6* (OR = 1,20).

Таким образом, наличие в генотипе хотябы одного аллеля *Ala5* гена GPX1, увеличивает риск развития ИБС у казахов.

Ключевые слова: оксидативный стресс, антиоксидантная система, глутатион пероксидаза, ишемическая болезнь сердца.

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