

# **Association between polymorphisms of genes encoding the antioxidant enzymes and cardiovascular disease development in Kazakhstan population**

Skvortsova Liliya Aleksandrovna, submitted in fulfillment of the requirements of the degree of Doctor of Philosophy (6D060700 Biology)

## **ABSTRACT**

**General description.** The dissertation work is devoted to the study of the mechanisms of antioxidant protection in the development of cardiovascular pathology. The work presents the data of model experiments to assess the expression of a number of antioxidant proteins in the development of atherosclerosis, as well as the results of a molecular epidemiological "case-control" study of the role of polymorphisms of genes encoding antioxidant protection proteins in the formation of hereditary predisposition to ischemic heart disease in the Kazakhstan population.

**Relevance of the topic of the study.** According to statistical data, cardiovascular diseases remain the most significant health problem in the world. Kazakhstan is no exception to the global "cardiovascular" trends. Experts from the World Health Organization predict a further increase in morbidity and mortality from cardiovascular diseases, mainly in developed and developing countries.

Achievements of modern science and medicine in knowing the basics of cardiovascular disease and the establishment of the major "classic" risk factors has largely reduced and brought under control mortality from cardiac pathologies in many developed countries. However, to further strive to reduce these parameters, more sensitive and individual markers of cardiovascular pathologies need to be investigated.

Studies in the field of molecular genetic predisposition to cardiovascular diseases attract close attention and interest of both physicians and scientists around the world. The body's response to various risk factors is determined by the interaction between individual genotypes and the action of environmental factors. The analysis of individual genetic characteristics allows us to identify mutations and polymorphisms in the key genes of CVD pathogenesis, which, along with the well-known environmental factors, already occupy a firm position among the risk factors for cardiovascular pathologies.

For Kazakhstan, the most significant disease of the circulatory system is coronary heart disease. By 2019, this pathology accounted for two-thirds of all circulatory system pathologies, occupying the leading position in morbidity and mortality. The understanding of the molecular genetic basis of coronary heart disease has evolved rapidly in recent decades. Using the methods of molecular biology and Genome Wide Association Studies (GWAS), more than 600 genes associated with coronary heart disease have already been identified. These are genes whose protein products may be involved in the signaling pathways of coronary heart disease initiation, course, and progression: Ras and MAPK signaling systems; TNF- $\alpha$  and NF- $\kappa$ B; activation of the cascade of inflammatory

signaling pathways by endothelial cells in response to accumulation of modified lipoproteins; leukocyte activation, migration, and adhesion to the inflammatory focus; foam cell formation, and smooth muscle cell proliferation; and, antioxidant systems.

Oxidative stress and antioxidant defense are two interrelated processes that characterize many disease states. According to modern concepts, oxidative stress is of primary importance in the pathogenesis of atherosclerosis and concomitant coronary heart disease. High levels of oxidative stress and reduced antioxidant status can significantly aggravate the course of coronary heart disease. At the same time, the molecular and genetic status of these two processes may differently influence the course of atherosclerosis and coronary heart disease pathogenesis. This fact is of fundamental importance for the identification of additional intrinsic risk factors for coronary heart disease in the context of preventive medicine. Studies in this field are incomplete and highly contradictory, as they are at the peak of their development. In this connection the aim of this work was to investigate the role of genetic variations in antioxidant protection genes in the development of coronary heart disease for Kazakhstan population as well as to determine the dynamics of expression of the most important antioxidant protection proteins in the vascular wall during atherosclerosis using a model object.

**The object of the study:** 10 rabbits (control and experimental groups) of Chinchilla breed; cohorts of conditionally healthy people (341 people) and patients with established diagnosis of coronary heart disease (360 people).

**Subject of the study:** Expression of antioxidant proteins SOD3 and GPX1 in normal vascular wall and those affected by atherosclerosis; genetic variations in antioxidant defense genes GCLC -129C/T, GCLM -588C/T, GPX1 Ala5-7, SOD3 Ala58Thr, GPX4 Leu220= and coronary heart disease.

**Purpose of the study:**

To evaluate the expression pattern of a number of antioxidant proteins in the development of atherosclerosis and to identify the role of polymorphisms of genes encoding antioxidant proteins in the formation of hereditary predisposition to coronary heart disease.

**Study Objectives**

1. To determine the expression level of antioxidant proteins SOD3 and GPX1 in normal vascular wall and atherosclerosis-infected one in a Chinchilla rabbit model subject.

2. On a voluntary basis, to collect biomaterial from people with established diagnosis of coronary heart disease to create a cohort of patients with coronary heart disease. At the same time, to analyze the obtained questionnaire data of the participants and to select a control group according to age, sex and ethnicity.

3. Genotyping of Ala58Thr single-nucleotide polymorphism of the superoxide dismutase enzyme system gene - *SOD3*, conducting statistical data processing and assessing the relative risk of coronary heart disease development in representatives of different genotypes.

4. Genotyping of promoter polymorphisms of genes of glutathione antioxidant enzyme system - *GCLC* -129C/T and *GCLM* -588C/T, statistical processing of the data obtained and relative risk assessment of ischemic heart disease development for representatives with -129T alleles of *GCLC* gene and -588T alleles of *GCLM* gene.

5. To perform genotyping of polymorphic trinucleotide tandem repeat Ala5-7, of the glutathione antioxidant enzyme system gene - *GPX1* and to assess relative risk of coronary heart disease development in representatives of different genotypes taking into consideration the three allelic system.

6. To perform genotyping of single nucleotide polymorphism C718T, gene of glutathione antioxidant enzyme system - *GPX4*, to conduct statistical processing of the obtained data and assess the relative risk of coronary heart disease development in representatives with different genotypes.

7. To evaluate the contribution of the studied gene variants encoding antioxidant protection enzymes to the development of cardiovascular pathology.

#### **Methods of the research:**

Molecular and genetic, immunohistochemical, epidemiological methods as well as statistical data processing methods were used.

**Scientific novelty of the study.** In a model system of induced atherosclerosis in Chinchilla rabbits, the expression pattern of GPX1 and SOD3 proteins in normal aortic wall and atherosclerosis-affected wall was determined. It is shown that SOD3 expression is induced at early stages of atherosclerosis (lipid stain stage) in the medial layer of the vessel underlying directly the inner elastic membrane and intima. SOD3 protein expression depends on the degree of extracellular matrix degradation at the late stages of atherosclerosis development (atheroma stage). Intensive expression of intracellular antioxidant protein GPX1 is observed at early stages of atherosclerosis (lipid stain stage) in the adventitial layer of the vessel. GPX1 expression is significantly repressed in the late stages of atherosclerosis (atheroma stage) in the intimal layer of the vessel.

For the first time in the Kazakh population of Almaty the frequency distribution of allelic variants in the genes *SOD3*, *GCLC*, *GCLM*, *GPX1* and *GPX4* encoding antioxidant proteins was revealed and their association with the risk of coronary heart disease was studied. It was found that promoter polymorphisms of *GCLC* -129C/T and *GCLM* -588C/T genes could be the internal genetic risk factors of ischemic heart disease for Kazakh population. For the Kazakh ethnic group, an additional risk factor for coronary heart disease could be the polymorphism of the number of alanine repeats of the Ala5-7 gene *GPX1*. The studied polymorphic variants of *SOD3* and *GPX4* genes do not show association with the risk of coronary heart disease in Kazakh population.

**Theoretical and practical significance of the work.** The results of the dissertation work on the study of the expression of antioxidant proteins during the development of inflammation of the vascular wall on a model object complement the research in the field of revealing the mechanisms of atherosclerosis development as the main pathophysiological link of ischemic heart disease. The analysis and theoretical substantiation of the data obtained contribute to further

more detailed study of the molecular-genetic cause of coronary heart disease. The study of genetic factors of antioxidant proteins, allows to better understand the influence of genetic diversity of polymorphisms on the efficiency of antioxidant protection of the organism in the development of ischemic heart disease. Further research in this area will contribute to the identification of panels of genes involved in the pathogenesis of coronary heart disease, both universal and taking into account ethnic, sex and age characteristics of patients. This contributes to the development of the effectiveness of preventive medicine in the development of coronary heart disease, as well as optimal protocols for the management and treatment of patients at risk of coronary heart disease.

**Provisions made for defense:**

- Extracellular antioxidant defense protein SOD3 is expressed in the subintimal and adventitial layers of normal rabbit aorta. At the initial stages of induced atherosclerosis (lipid stain stage) there is primary activation of extracellular SOD3 protein expression in the medial layer underlying the intimal layer, which correlates with the presence of extracellular matrix at the atheroma stage.

- Background Expression of the antioxidant protein GPX1 is observed in the intimal, adventitial, and part of the medial layer of the normal rabbit aorta. Increased expression of GPX1 protein in induced atherosclerosis occurs in all layers at the stages of lipid spots and lipid bands; GPX1 protein expression is suppressed in the intimal layer at the stage of atheroma. Intense expression of GPX1 protein in the adventitial layer mediates the complex response of the vascular wall in the development of atherosclerosis.

- In a population of healthy residents of the city. Almaty (69,5% of Kazakhs, 20,82% of Russians, others - 9,68%) the frequency of polymorphic allele -588T of *GCLM* gene (0,139) corresponds more to the frequency typical for European population (0,152); the frequency of polymorphic allele -129T of *GCLC* gene (0,111) corresponds to the frequency determined for Asian population (0,133); the frequency of the Ala5 polymorphic allele of the *GPX1* gene (0.661) is much higher than the frequency reported for the global population (0.345-0.398); the frequency of the polymorphic allele 58Thr of the *SOD3* gene (0.462) is much higher than the frequencies typical for European (0.374-0.383) and Asian (0.1-0.279) populations; the frequency of the Leu220= allele of the *GPX4* gene (0.603) corresponds to the frequencies in Asian populations (0.558-0.606).

- High risk of coronary heart disease in Kazakh population was significantly associated with the presence of two alleles -588T of *GCLM* gene (OR=2,91; CI=0,93-9,10; p=0,009) and/or two alleles -129T of *GCLC* gene (OR=3,22; CI=0,88-11,80; p=0,03) in the genotype. With high confidence, there was a risk of coronary heart disease for ethnic Kazakhs with the -588TT genotype for the *GCLM* gene (OR=4.23; CI=0.89-20.16; p=0.04) and/or with the -129TT genotype for the *GCLC* gene (OR=4.79; CI=1.02-22.39; p=0.04).

- The risk of coronary heart disease in ethnic Kazakhs may be associated with the presence of five alanine Ala5 repeats in the *GPX1* gene in the homozygous state (Ala5/5: OR=1.88; 95%CI=1.30-2.71;  $\chi^2=16.902$ , p=0.005),
- The Ala58Thr polymorphism of the *SOD3* gene and the Leu220= polymorphism of the *GPX4* gene are not risk factors for coronary heart disease in the Kazakhstan population (p>0.05).

**Publications.** On the materials of the dissertation work published: 1 article in the journal and the impact factor; 3 articles in the ranking national publications recommended by KKSON; 2 abstracts in international conferences.

**Structure and scope of the thesis.** The thesis is written on 138 pages of typewritten text and includes: introduction, literature review, materials and methods, results and discussion, conclusion, list of references (314 titles) and 5 appendices. The thesis is illustrated with 19 figures and 14 tables.