«MEDICAL UNIVERSITY KARAGANDA» NON-COMMERCIAL JOINT-STOCK COMPANY

# ANNOTATION

Dissertation work for the PhD degree specialty 6D110100 "Medicine"

# Topic: “ Association of PON1, CYP2C19 gene polymorphism with risk of coronary artery restenosis ”

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# Relevance of the research topic:

The incidence of coronary heart disease in the Republic of Kazakhstan has recently tended to steadily increase. In 2011, 51,974 new cases of coronary heart disease were registered in Kazakhstan.

Ischemic (coronary) heart disease (CHD) is a disease caused by insufficient blood supply to the heart muscle. The development of modern medicine has led to the emergence of new, unique methods of treatment of coronary artery disease – such as angioplasty and stenting of coronary vessels.

Endovascular methods of treatment, such as coronary artery stenting, have become widespread in the treatment of coronary artery disease, however, restenosis in the stent is the main limitation of the effectiveness of this method, and even the use of drug-coated stents has not completely solved the problem.

Given the widespread use of coronary artery stenting in the treatment of coronary artery disease, it is undoubtedly relevant to search for new predictors of the development of restenosis in the stent. There are reasons to believe that genetic factors, such as polymorphism of genes encoding certain enzymes and receptors, play a role in increasing the risk of developing restenosis. Currently, there are a number of large-scale programs GENDER (Genetic Determinants of Restenosis), CAPARES (Coronary AngioPlasty Amlodipine REStenosis Study), RESEARCH, ISAR-STEREO-2 (Strut thickness effect on restenosis outcome) aimed at studying the genetics of restenosis. The development of restenosis in the stent may also indirectly depend on polymorphisms in the genes of the hemostasis system, the renin-angiotensin system and the antioxidant system.

The PON1 gene is localized on the long arm of chromosome 7 (7q21.3–q 22.1), it consists of 27 thousand pairs of nucleotides and contains nine exons. Currently, 198 single-nucleotide substitutions have been identified, including 7 in the promoter and 5 in the coding regions of the PON1 gene.

Of all the polymorphic variants of the PON1 gene, the polymorphisms L54M (rs854560) and Q192R (rs662) have the greatest clinical significance.

The CYP2C19 gene has nine exons and is highly polymorphic, with more than 25 allele variants (they are marked with asterisks \*) currently registered with the cytochrome P450 Allele Nomenclature Committee (<http://www.cypalleles.ki.se/CYP2C19.htm>).

Additionally, other sites were analyzed (clinvar, dbsnp, pharmgkb, drugbank, etc.) Thus, an analysis was carried out using an algorithm in the R statistics environment of the SNPedia database for the presence of variations of the PON1, CYP2C19 gene as a predictor of the risk of coronary artery disease and coronary artery restenosis. Distribution data were obtained in various populations of the following alleles - L54M (rs854560) and Q192R (rs662) rs12248560,

rs41291556, rs41291556, rs72552267, rs4986893, rs4244285, rs72558186 (A) represents the SNP CYP2C19, rs56337013.

It is known that the frequency of occurrence of heterozygous genotypes is influenced by ethnicity. Thus, there is evidence that the frequency of occurrence of heterozygous genotypes of CYP1C19\*2GA alleles (\*1/\*2) in the Asian population, according to various authors, it ranges from 28% to 60%. As a result of numerous scientific works of domestic and foreign researchers, it was revealed that there is a difference in the prevalence of polymorphism of the CYP2C19 isoenzyme in different ethnic groups. The frequency of occurrence of this allele is about 12% in Europeans, 12% in African Americans and 29-35% in Asians. According to other sources, the prevalence of the allele with the CYP2C19\*2 genotype is about 25-30% in Europeans and 50-60% in Asians.

There are isolated studies in the Republic of Kazakhstan that reflect certain aspects of the problem under study. Within the framework of the master's thesis, Visternichan O.A. (2014) determined that polymorphism of the FGB gene (rs1800790) and polymorphism of the RBD gene in patients with coronary artery restenosis after stenting can be considered as possible genetic predictors of the development of restenosis in Kazakh men.

In the study of Kulmyrzayeva N.K. (2016), it was shown that in patients with ACS, the frequency of distribution of allele \*2 (GA and AA) by polymorphic marker G681A of the CYP2C19 gene in Kazakh nationality living in the territory of the Aktobe region was 27% and 2%, respectively, while the frequency of distribution by allele \*3 (GA and AA) was - 9% and 1%, respectively.

In this research work, the association of polymorphism of the PON1, CYP2C19 genes with the risk of coronary artery restenosis was studied. In addition, the association of the polymorphism of the PON1, CYP2C19 genes with the risk of coronary artery restenosis in patients with chronic coronary artery disease, in patients with coronary artery disease with stenting, in patients with coronary artery disease with restenosis after stent placement in comparison with practically healthy patients was investigated.

# The purpose of the study:

To evaluate the association of polymorphism of PON1, CYP2C19 genes with the risk of coronary artery restenosis in the Kazakh population.

# Research objectives:

* To analyze the relationship of vascular growth factors (PDGF-АА, FGFs) with the risk of coronary artery restenosis.
* To evaluate polymorphism of genes encoding biotransformation enzymes in coronary artery restenosis: PON1 L54M (rs854560), PON1 Q192R (rs662) and CYP2C19 (CYP2C19\*2 - rs4244285, CYP2C19\*3-rs4986893 and CYP2C19\*17-rs12248560).
* To analyze the frequency of occurrence of genetic polymorphism depending on the type of inheritance.

-To evaluate correlations between risk factors of coronary heart disease, quantitative indicators of coronary angiography and gene polymorphism.

# Scientific novelty:

* Diagnostic signs of the development of restenosis and vascular-inflammatory activity after coronary artery stenting in acute atherothrombotic events and coronary heart disease were established.
* The association of polymorphism of PON1, CYP2C19 genes with the risk of coronary artery restenosis was revealed for the first time.
* For the first time, predictors of restenosis development were determined based on the association of polymorphism of PON1, CYP2C19 genes and a comprehensive assessment of clinical and genetic parameters of vascular growth factors.

# Practical significance of the conducted research:

An increase in the value of vascular growth factors (PDGF-АА, FGFs) in coronary artery stenosis allows us to recommend them as markers of myocardial destabilization.

* Early genetic testing makes it possible to identify a group of patients at risk of developing an unstable type of coronary artery disease and a potential risk of developing coronary artery restenosis.
* The establishment of a correlation between the polymorphism of genes with a set of risk factors, contributes to the construction of criteria that determine the indications for early molecular genetic diagnosis.
* The results of the study were introduced into the clinical work of the cardiology department of the KGP "Multidisciplinary Hospital No. 1" in Karaganda, into the practical work of the Department of Internal Diseases of the "Medical University of Karaganda" NC JSC.

The dissertation work was carried out within the framework of Program-targeted funding of the Ministry of Education and Science of the Republic of Kazakhstan BR05236771-OT-18 “Personalized approach to the management of a number of important diseases”.

# The main provisions of the dissertation submitted for defense:

1. Patients with restenosis inside a previously installed stent have changes in laboratory tests, they have higher rates of chronic inflammation, female patients are more likely to have restenosis inside the stent when diagnosed with chronic forms of coronary heart disease.
2. The state of platelet growth factor and the carriage of polymorphisms PON1, CYP2C19 are predictors of the development of adverse outcomes in patients after percutaneous coronary intervention.
3. Progression of coronary atherosclerosis 12 months after coronary artery stenting in patients with stable angina is associated with the concentration of LDL cholesterol in the blood 12 months after the intervention. Patients with LDL cholesterol in the blood below 1.8 mmol/ l 12 months after stenting are significantly less likely to demonstrate the progression of coronary atherosclerosis in comparison with patients with a higher LDL cholesterol content in the blood.
4. Identification of genetic polymorphisms in patients after coronary artery stenting is recommended as promising predictors of the risk of restenosis after stent placement.

# Implementation into practice

Based on the materials of the dissertation, 2 certificate of registration of rights to the copyright object KZ No. 13260 was obtained. Certificate of entry of information into the state register of rights to objects protected by copyright. November 17, 2020. "Questionnaire for assessing the risk of complications against the background of double antiplatelet therapy in cardiac patients", authors Bodaubai R., Taizhanova D.Zh. Visternichan O.A., Kalimbetova A.B. (Appendix A).

KZ №13249. Certificate of entry of information into the state register of rights to objects protected by copyright. November 16, 2020. "Questionnaire for assessing risk factors for atrial fibrillation" Taizhanova D.Zh. Bazarova N.K., Bodaubai R., Kalimbetova A.B. (Appendix B).

There are acts of implementation of the results of research work in the practical activities of the cardiology departments of the KGP "Multidisciplinary Hospital No. 1" of the Karaganda region (Appendix B, D).

# Approbation of the work

The main provisions and results of the dissertation work were reported and discussed at international conferences: XI Congress of Cardiologists of the Republic of Kazakhstan. June 5-7, 2019, Almaty. Kazakhstan; International Conference Modern Molecular-Biochemical Markers in Clinical and Experimental Medicine-2019, 07-09 November 2019, Prague, Czech Republic; ESC Heart & Stroke 2020.Barcelona, 24-25 January 2020; Russian National Cardiology Congress in Kazan, September 29-October 1, 2020; St. Petersburg Cardiologists of the Russian National Congress, October 21-23, 2020; Russian National Congress of Cardiologists, St. Petersburg, October 21-23, 2020.

# Publications

Based on the materials of the dissertation, 28 scientific papers were published, of which 3 works were recommended by the Committee for Control in the field of education and Science of the

Ministry of Education and Science of the Republic of Kazakhstan, 4 works were published in publications that do not have zero Impact Factor and are included in the Scopus database, including 3 articles (1 article was accepted for publication), 1 thesis was published in The Clarivates Analytics database is in the journal. 2 copyright certificate.

# Materials and methods of research Study design: case-control

The research work was partially carried out on the program-system financing "personalized approach to the management of a number of important diseases" with registration number BR05236771-OT-18, conducted as part of the research work of the "Medical University of Karaganda" NC JSC The scientific study was made by the decision of the Bioethics Committee of the "Medical University of Karaganda" NC JSC (protocol No. 12 dated 06.02.2019).

The examination of patients was carried out in the polyclinics of the city of Karaganda and on the basis of the KGP "Multidisciplinary Hospital No. 1" of Karaganda.

Molecular genetic studies were conducted on the basis of the laboratory of collective use of the "Medical University of Karaganda" NC JSC (PON1 Q192R (rs662) and CYP2C19 (CYP2C19 \* 2-rs4244285, CYP2C19 \* 3-rs4986893 and CYP2C19\*17 - rs12248560), in the laboratory of genomic and personalized medicine, National Laboratory Astana, Nazarbayev University, Nur- Sultan (PON1 L54M (rs854560)).

Statistical processing of the results of the study was carried out using the programs STATISTICA 8 and IBM SPSS Statistics 20. The methods of descriptive statistics, quantile diagrams, histograms and the Kolmogorov-Smirnov criterion with the Lilliefors correction and the Shapiro-Wilk criterion were used to check the normality of the distribution. If the values of the statistical significance of the calculated criteria exceeded 0.01 (p>0.01), the actual distribution was formally considered to be no different from normal. Average trends were described by the mean and standard deviation. For data with a distribution different from normal, the statistical significance of differences in groups was determined using the Kraskel-Wallace criterion and the Mann- Whitney criterion. The differences were considered statistically significant at p <0.01. Qualitative data analysis was performed using Pearson's x2 with Yates correction. Spearman's rank correlation method was used for correlation analysis.

# Conclusions:

1. The level of vascular growth factor - PDGF-АА was increased in patients with coronary artery restenosis: in group I, 5188.6 [3676.1: 6701.1], group II - 6665.7 [4577.9:8753.5], group III - 3951.0 [2768.4: 5133.5]. A weak direct correlation was revealed between coronary artery restenosis and elevated levels of PDGF–AA after stent implantation (r=0.43, p=0.001).
2. Evaluation of gene polymorphism by gender showed that the following polymorphisms are mainly determined among women: PON1 Q192R (rs662) - 95%, PON1 L54M (rs854560) - 81%, CYP2C19\*3 - 81%. The frequency of gene polymorphism among men was more common than among women: PON1 l54M (rs854560) - 95%, PON1 Q192R (rs662) - 97%, CYP2C19\*3 - 88%, CYP2C19\*2 - 88%, CYP2C19\*17 - 69%.
3. Three models of inheritance (dominant, recessive and log-additive) of genetic polymorphism in the risk of coronary artery restenosis have been identified:

- dominant model by genotype T/C-C/C (rs12248560) OR 95% CI - 0.52 [0.24-1.12], p ≤ 0.09;

* log-additive model by genotype three genotype (rs12248560) OR 95% CI - 0.54 [0.29-0.99], p ≤ 0.03;

- recessive model by genotype A/A (rs4986893) OR 95% CI - 0.31 [0.08-1.14], p ≤ 0.05;

- recessive model by genotype G/G-A/G (rs4244285) OR 95% CI - 0.32 [0.09-1.15], p ≤ 0.05;

* dominant model by genotype C/T-C/C (rs662) OR 95% CI - 0.45 [0.17-1.2] p ≤ 0.09;
1. Correlation analysis revealed the following relationships between risk factors for coronary artery disease and genetic polymorphisms that increase the risk of coronary artery restenosis:
* direct correlation (r=1,153; r=0.973) with polymorphic genes CYP2C19\*2, PON1 (Q192R).
* weak inverse relationship with risk factors and CYP2C19\*17: smoking r=-0.2, alcohol r=-0.14, predisposition to coronary heart disease r=-0.14;
* direct strong association with coronary artery restenosis with polymorphic gene type CYP2C19\*3 (r=2.774);
* when evaluating laboratory parameters, weak feedback was revealed between the lipidogram, blood clotting and genetic polymorphism: TTL with PON1(L55M) r=0.13; platelets -r=0.11; fibrinogen - r=-0.12; PV - r=0.15; INR- r=0.11.

between risk factors with PON1(Q192R); weak feedback-R=-0.14; PTI - r=0.12; PV - r=-0.13;

* between CYP2C19\*17 and LDL - r=0.14; between CYP2C19\*3 and triglycerides - r=0.14; PTI
* r=0.17; CYP2C19 \* 2 and PTI-r=0.12 - weak positive correlation.