KARAGANDA MEDICAL UNIVERSITY
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ABSTRACT

of the thesis for the degree of Doctor of Philosophy on the topic:
«Association of gene polymorphism and molecular markers
in the risk of primary pulmonary hypertension development».

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Karaganda – 2025

Relevance of the study

Primary pulmonary hypertension (PPH), also known as idiopathic pulmonary arterial hypertension (IPAH), is a progressive and currently incurable rare disease of unknown etiology. PPH is characterized by the intense progression of right ventricular failure and leads to an early fatal outcome [1-3]. The incidence of PPH is 48-55 cases, and the prevalence is 6 cases per 1 million adults per year [4]. The mortality rate remains comparable to oncological diseases and reaches 55% within 3 years, even with specific therapy of pulmonary arterial hypertension (PAH) [5]. There is currently no registry for patients with IPAH in the Republic of Kazakhstan. Some highly specialized centers create databases, but there is no united and unified system for registering patients with pulmonary hypertension (PH), which makes it difficult to analyze the current situation in our country.

It is very difficult to diagnose IPAH, especially in the early stages, since the clinical symptoms are nonspecific. Initial symptoms include shortness of breath, fatigue, chest pain, pre-fainting condition/fainting, and swelling of the lower extremities. However, the clinical signs are hardly noticeable until the disease progresses. This leads to the average 2-year delay in diagnosis in most patients. Diagnosis of IPAH is difficult, mortality from this disease is high, and survival periods are only 2-5 years after diagnosis.

Genetic mutations, metabolic and molecular disorders play the role in the pathogenesis of IPAH [6].

IPAH correlates with the following genetic mutations: bone morphogenetic protein receptor type 2 (BMPR2), mothers against decapentaplegic homologs 1 and 9 (SMAD family member 1 and 9), potassium channel subfamily K member 3 (KCNK3) and caveolin 1 (CAV1). Genetic susceptibility (BMRP2 mutation and other genetic factors) leads to endothelial dysfunction, which causes the change in the synthesis of endothelial vasoactive substances that affect many intracellular pathways [7]. The main intracellular pathways are nitric oxide, endothelin, and the prostacyclin pathway, which leads to changes in the vascular system at PAH. Although there is the genetic basis in the development of IPAH, there are factors of the molecular mechanism that probably contribute to the pathogenesis. The primary lesion of the pulmonary arteries at IPAH is probably a combination of a genetic predisposition with the influence of biomarkers, which lead to a cascade of events, eventually causing vascular remodeling of the vessel wall [8]. Despite the multitude of biomarkers, no specific marker for IPAH has been found to date. Biomarkers that specifically indicate the pathophysiology, severity of the disease, and response to treatment would be ideal tools in diagnosing IPAH, assessing its progression, and evaluating the effectiveness of therapy.

Timely diagnosis, including instrumental, genetic, and molecular screening, will make it possible to prescribe early drug therapy, predict and prevent disease progression, provide secondary PAH prevention, and identify patients at risk. At

the same time, it is diagnostically important to assess the features of the association of genetic polymorphism and the specificity of molecular markers depending on ethnicity. These problems necessitate continued research into the complex instrumental, genetic, and molecular mechanisms that lead to the development of IPAH with the complex etiology and pathogenesis. Such areas of scientific research are still a very relevant scientific and medical problem.

Aim of the study: to evaluate the association of BMPR2 gene polymorphism with biomarkers of endothelial dysfunction and inflammation in the risk of idiopathic pulmonary arterial hypertension development in the ethnic group of Kazakhs.

Objectives of the study:

1. To conduct a systemic analysis of predictors of development and complexity of diagnostic search for idiopathic pulmonary arterial hypertension in the Republic of Kazakhstan.
2. To analyze the relationship of molecular markers of endothelial dysfunction and inflammation in patients with idiopathic pulmonary arterial hypertension.
3. To evaluate the polymorphism of the bone morphogenetic protein receptor type 2 gene (rs1061157, rs2228545, rs17199249, rs113305949) in patients with idiopathic pulmonary arterial hypertension in the ethnic group of Kazakhs.
4. To conduct the comparative analysis of clinical and hemodynamic parameters and patient survival depending on the association with the mutation of the BMPR2 gene.
5. To develop a diagnostic algorithm for the early diagnosis of idiopathic pulmonary arterial hypertension based on disease predictors.

Object of the study:

The study included 53 patients with idiopathic pulmonary arterial hypertension (IPAH) from the ethnic group of Kazakhs, classified as the main group. All patients underwent the comprehensive clinical and functional examination at the specialized pulmonary hypertension center. During the thorough clinical examination, all patients were given the differential diagnosis with the exclusion of other known causes of pulmonary arterial hypertension. The control group included 125 practically healthy individuals, comparable in age and gender, with no family history of IPAH or other cardiovascular diseases (congenital heart defects, coronary heart disease, chronic heart failure, arterial hypertension, etc.).

Methods of the study:

To achieve the objectives, demographic and clinical characteristics, as well as hemodynamic parameters from the data of the medical history and questionnaire were studied in patients with IPAH. Biomarkers (C-reactive protein, endothelin-1 (ET-1), and interleukin-6 (IL-6) were determined by enzyme-linked immunosorbent assay (ELISA), and genotyping of the BMPR2 polymorphism was performed by polymerase chain reaction (PCR) in Real Time. Flanking primers and destructible fluorescent probes were developed to analyze the single nucleotide polymorphism (SNP) of the BMPR2 gene loci. The choice of SNP was justified by the data from the literature review, allele frequencies, and the functional position of the genome. The study material was the venous blood of patients with IPAH (main group, n=53) and practically healthy individuals (control group, n=125).

The present scientific study was approved by the Ethics Committee of the Karaganda Medical University NC JSC (Protocol No. 62 dated 04/12/2021).

Scientific novelty:

- The results of the systemic analysis are presented, which determine the late diagnosis of idiopathic pulmonary arterial hypertension, their demographic characteristics and clinical features in the ethnic group of Kazakhs (Copyright certificate of entry of information in the State Register of Copyrighted Objects KZ No. 53552 dated January 17, 2025 «Questionnaire for patients with idiopathic pulmonary arterial hypertension»).

- For the first time, the association of genetic variants of the BMPR2 gene polymorphism with the risk of idiopathic pulmonary arterial hypertension development in patients belonging to the ethnic group of Kazakhs was determined (rs1061157, rs2228545, rs17199249, rs113305949).

- For the first time, the relationship of the genetic polymorphism BMPR2 with the risk of death in patients with idiopathic pulmonary arterial hypertension in the ethnic group of Kazakhs has been determined.

- For the first time, the complex of biomarkers was identified that make it possible to predict the risks of the progressive nature of the course and the unfavorable outcome of IPAH.

- Based on the materials of the thesis, the certificate of registration of the rights to the copyright object was obtained: Copyright certificate of entry of information into the State Register of Copyrighted Objects KZ No. 59143 dated June 3, 2025

«Diagnostic algorithm for IPAH», the authors are T. T. Nurpisova, D. Zh. Taizhanova

Practical significance:

- Determining of serum concentrations of endothelin-1 and interleukin-6 in the course of the disease will allow assessing the severity of the course and predicting the risk of the adverse outcome of the disease.

- Genetic testing will create conditions for the early detection of the group of patients at risk of IPAH developing among first-line relatives.

- Determining of the prerequisites for late diagnosis in association with the features of gene polymorphism will allow developing the algorithm for early IPAH diagnosis.

Implementation in practice:

The main findings and results of this dissertation were implemented in the clinical activities of the Laboratory of Personalized and Genomic Diagnostics of the Medical Center of the Presidential Administration of the Republic of Kazakhstan (Astana, Republic of Kazakhstan) (Act No. 1, Appendix G), Limited Liability Partnership Clinical Miras (Karaganda, Republic of Kazakhstan) (Act No. 2, Appendix D) and State Enterprise Multidisciplinary Hospital No. 1 of Karaganda (Karaganda, Republic of Kazakhstan) (Act No. 3, Appendix E)..

Personal contribution of the dissertator:

All the main parts of the study (analytical review of domestic and foreign literature on the problem under the study, collection and processing of primary material, database formation, questionnaires, molecular analysis by ELISA, manual DNA extraction, molecular genetic analysis, statistical processing, analysis and presentation of the results) were performed with the direct author's participation at the all stages.

Approbation of the thesis:

The main results of the study were presented and discussed at:

1. X Eurasian Congress of Cardiologists. The topic of the report: «Genetic aspects of pulmonary arterial hypertension», May 16-17, 2022.
2. XIV Congress of Cardiologists of the Republic of Kazakhstan, June 2-3, 2022. The report: «Idiopathic pulmonary arterial hypertension: How can we

not miss the time?» was presented at the Competition of Young Scientists within the framework of the Congress.

3. X anniversary All-Russian Congress «Pulmonary Hypertension – 2022», December 5-6, 2022. The topic of the oral report: «Genetic aspects of pulmonary arterial hypertension: diagnostic tasks and management tactics for patients with hereditary PAH».

4. Scientific and practical conference of young scientists, undergraduates and doctoral students «The World of Science and youth: traditions and innovations», dedicated to Science Day, April 12, 2023. The topic of the report: «Idiopathic pulmonary arterial hypertension».

5. XVI National Congress of Cardiology with International participation, dedicated to the 135th anniversary of S. D. Asfendiyarov, June 6-7, 2024, the report «Association of BMPR2 gene polymorphism with the risk of idiopathic pulmonary arterial hypertension in the ethnic group of Kazakhs» was presented at the Young Scientists Competition within the framework of the Congress.

6. 9th World Congress on Cancer and Heart, March 12-13, 2025, London, UK. Poster report: «Genetic polymorhphism in BMPR2 is associated with susceptibility to PAH in Kazakhstan».

Information about publications:

11 scientific papers were published, including 4 articles and 7 abstracts. Of these, 1 article is in the journal indexed in the international databases Scopus and Web of Science: Diagnostics, 2024, 14.2687, Scopus 62%, CiteScore 3.0, Web of Science Q2. 3 articles were published in journals 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. 7 abstracts were published in collections of international and domestic congresses and conferences.

As part of the study the following documents were received:

- Copyright certificate of entry of information in the State Register of Copyrighted Objects KZ No. 53552 dated January 17, 2025 «Questionnaire for patients with idiopathic pulmonary arterial hypertension».
- Copyright certificate of entry of information into the State Register of Copyrighted Objects KZ No. 59143 dated June 3, 2025 «Diagnostic algorithm for IPAH».

Main provisions submitted for defense:

- The duration of the diagnostic period from the time of manifestation of clinical symptoms to the establishment of clinical diagnosis of IPAH is on average 7.1 ± 6.0 years. Predictors of the early development of IPAH include young age and female sex. Patients with IPAH experience psychopathological disorders: anxiety (32.6%), depression (38.0%), stress (40%), fear of loss of income due to illness (65%).
- In the ethnic group of Kazakhs, the onset of the disease is recorded at the young age (35.0 – 51.0 years); the ratio of women to men is 6.5:1. Early clinical symptoms include shortness of breath during physical exertion (95%), weakness (85%) and fatigue (69%). At the same time, at the stage of verification of the clinical diagnosis of IPAH, patients had more expressed symptoms such as cough (OR = 4.67; 95% CI = 1.17 – 25.14) and palpitations (OR = 2.83; 95% CI = 1.29 – 11.60). The most significant triggers of the manifestation of the disease were acute respiratory illness and psychoemotional stress.
- At the stage of manifestation of the first clinical symptoms in real clinical practice, echocardiographic signs of pulmonary arterial hypertension were diagnosed only in 56% of patients, which indicates the need to determine targeted indications for examination.
- High ET-1 levels are associated with key hemodynamic parameters such as mean right atrial pressure (r = 0.728; p <0.001) and pulmonary vascular resistance (r = 0.360; p = 0.008), which affect the prognosis of the disease. The progressive increase in the IL-6 level correlates with the functional class (FC) of heart failure (H (3) = 16.12; p = 0.001). The IL-6 level is significantly higher in patients with heart failure: FC III (W = 11; p = .0415) and FC IV (W = 0; p = .036) than in IPAH patients with FC II and FC I (W = 128.5; p = 0.011).
- In the ethnic group of Kazakhs, the polymorphism of the BMPR2 gene (rs17199249) increases the risk of IPAH developing (T = 70.75%, G = 29.24%, MAF – 0.2925, χ^2 – 0.001, HWE p – .975). Polymorphism of the rs17199249 gene has the significant association with the risk of IPAH developing in the codominant (OR = 0.17, 95% CI (0.06 – 0.52); p = 0.001) and recessive models (OR = 0.14, 95% CI (0.05-0.40); p = 0.001).
- The carriage of the BMPR2 gene mutation at IPAH demonstrates the high degree of association with the risk of death in the next 5 years from the moment of diagnosis (OR = 2.896, 95% CI (1.165 – 7.065); p=0.022). At the same time, women with the BMPR2 gene mutation had the higher probability of death (OR = 3.142, 95% CI (1.212 – 8.143); p = 0.019) compared with men (OR = 1.414, 95% CI (0.088 – 22.64); p = 0.806).

Conclusions based on the results of the study:

- Predictors of the development of idiopathic pulmonary arterial hypertension include the combination of anamnestic and clinical genetic factors: young age (35.0 – 51.0), female sex and the presence of a mutation in the BMPR2 gene. The most common initial complaints are shortness of breath during physical exertion (95%), weakness (85%) and fatigue (69%), the trigger of the manifestation of the disease is the previous acute respiratory illness and psychoemotional stress. The duration of the diagnostic period of IPAH takes the average of 7.1±6.0 years. At the stage of manifestation of the first clinical symptoms, echocardiographic signs of pulmonary arterial hypertension were diagnosed only in 56% of patients.
- The increase in the ET-1 level is associated with key hemodynamic parameters: mean pressure in the right atrium (r = 0.728; $p<0.001$) and pulmonary vascular resistance (r = 0.360; $p = 0.008$), affecting the prognosis of the disease. The increase in the IL-6 level correlates with the progression of the FC of the heart insufficiency (HF) (H (3) = 16.12; $p = 0.001$) at IPAH. IL-6 at HF FC III ($W = 11$; $p = .0415$) and HF FC IV ($W = 0$; $p = .036$) is significantly higher than at HF FC I according to NYHA. IL-6 is significantly higher in patients with HF FC III ($W = 128.5$; $p = 0.01$) and HF FC IV ($W = 5$; $p = .004$) than with HF FC II. The comparative assessment of IL-6 depending on FC III and IV shows that IL-6 is lower in patients with IPAH FC III than in patients with FC IV ($W = 22$; $p = .017$).
- The prediction of the high risk of IPAH developing in the ethnic group of Kazakhs is associated with the identification of the polymorphism of the BMPR2 gene (rs17199249) (T = 70.75%, G = 29.24%, MAF – 0.2925, χ^2 – 0.001, HWE p – .975). Polymorphism of the rs17199249 gene has the significant association with the risk of IPAH developing in the codominant (OR = 0.17, 95% CI (0.06 – 0.52); $p = 0.001$) and recessive models (OR = 0.14, 95% CI (0.05 – 0.40); $p = 0.001$). At the same time, BMPR2 polymorphisms (rs1061157, rs113305949) did not show the association with the risk of IPAH developing in the ethnic group of Kazakhs.
- The analysis of the survival rate of patients with IPAH showed that carriers of the BMPR2 gene mutation demonstrate the high degree of mortality in the next 5 years from the moment of diagnosis (OR = 2.896, 95% CI (1.165 – 7.065); $p = 0.022$). Women with the BMPR2 gene mutation have the higher probability of death (OR = 3.142, 95% CI (1.212 – 8.143); $p = 0.019$) compared with men (OR = 1.414, 95% CI (0.088 – 22.64); $p=0.806$).

- A personalized algorithm has been developed for early diagnosis, reduction of the time required to establish a diagnosis of idiopathic pulmonary arterial hypertension, and timely prescription of pathogenetic therapy.

Practical recommendations:

1. Clinical symptoms in the form of shortness of breath during physical exertion, cough, rapid heartbeat, developing after an acute viral infection and psychoemotional stress, as well as young age and female sex, make it possible to predict the risk of IPAH developing, which determines indications for diagnostic echocardiography and genetic testing to detect polymorphism of the BMPR2 gene.
2. IL-6 and ET-1 levels in patients with IPAH can be included in the diagnostic algorithm for assessing the severity and predicting the disease. The high ET-1 level is associated with the prognosis of the disease, and the IL-6 level is associated with the outcome of the disease.
3. Identification of the rs17199249 gene polymorphism at IPAH demonstrates the higher association with death within the next 5 years from the moment of diagnosis.
4. The algorithm for the early diagnosis of IPAH was developed and proposed for use in the clinical practice of therapeutic physicians (cardiologist, internist, general practitioner, pulmonologist).

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