REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Science

GENETIC HETEROGENEITY AND BIOCHEMICAL POLYMORPHISM OF CERTAIN HEREDİTARY DISEASES (PHENYLKETONURIA, GALACTOSEMIA, MUCOVISSIDOSIS, LEUCINOSIS, FAMILIAL MEDITERRANEAN FEVER) IN THE POPULATION OF AZERBAIJAN

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GENERAL CHARACTERISTICS OF THE WORK

Relevance and degree of elaboration of the topic. Hereditary (or genetically transmitted) metabolic disorders are the result of genetic defects and are characterized by disturbances of biochemical functions at the cellular level. Defects arising in genes lead either to a reduction or to a complete loss of activity of enzymes involved in biochemical reaction chains. Biochemical metabolic disorders represent a distinct group of diseases that are individually rare but collectively expanding, characterized by new causative factors and clinical manifestations. Out of every 100 newborns, 5–6 is born with various genetically determined pathologies. In most cases, these are hereditary predisposed diseases. The observed 5–6% of pathologies include diseases that arise for the first time or are inherited from one of the parents. Relatively rare, but genetically completely distinct congenital metabolic diseases are also included in this group[1].

The majority of hereditary metabolic diseases present with similar clinical symptoms. Consequently, establishing an accurate diagnosis based solely on clinical and laboratory findings is often impractical.

In recent years, the application of methods such as whole-exome sequencing has significantly contributed to understanding the molecular and biochemical etiology of congenital metabolic diseases, leading to considerable advances in their treatment.

A precise diagnosis of hereditary metabolic diseases can be established only through the detection of reduced enzyme activity or the identification of a mutation in the corresponding gene.

Early diagnosis is crucial, as it enables timely treatment and can prevent both morbidity and mortality associated with these disorders.

One of the primary approaches widely employed in the study of human population diversity is the analysis of nuclear DNA polymorphisms. This method enables the assessment of balanced characteristics within the population gene pool and offers an objective description of genetic variation. A comprehensive evaluation of the population gene pool requires the integrated analysis of molecular-genetic, popu-

[1]Chiarelli, F. Early diagnosis of metabolic syndrome in children // The Lancet: Child & Adolescent Health, – 2020. Oct.; Vol. 1, No 2, – p.86-88.

lation-demographic, and population-statistical parameters.

The analysis of nuclear DNA polymorphisms in various human populations plays a crucial role in determining the molecular-genetic characteristics of gene pools and elucidating mutual genetic-functional relationships among different ethnic groups.

Investigating the genetic structure and degree of differentiation among populations of diverse nations and ethnic groups, using both classical and molecular approaches, enhances the applicability and relevance of research findings.

The study of the molecular-genetic basis of hereditary metabolic diseases that are prevalent in various regions of the Republic of Azerbaijan, along with the population-statistical analysis of these pathologies, represents one of the key challenges in the field of medical genetics.

The primary scientific focus of the present dissertation is the molecular-genetic analysis of hereditary metabolic diseases within the Azerbaijani population. However, considering the ethnic diversity of the country, the incorporation of population-demographic and population-statistical studies is also essential.

Hereditary metabolic diseases represent a significant group among human genetic disorders. Depending on the nature of the gene mutation, either the corresponding enzyme is not synthesized at all, or the enzymatic activity of the mutated protein is markedly reduced.

As a result of the absence or dysfunction of the enzyme, the conversion of the corresponding substrate into the subsequent chemical compound – either an intermediate or final product – is impaired or completely halted.

In most cases, toxic intermediate metabolites accumulate in tissues and organs, contributing to disease pathogenesis. Additionally, the synthesis of essential compounds required for normal development is disrupted during the progression of hereditary metabolic diseases.

More than 6,000 hereditary diseases have been identified in humans, with over 4,000 distinct variants of genetic disorders currently known. To date, approximately 500 hereditary metabolic diseases have been recorded.

The ongoing social and economic development in the Republic of Azerbaijan, along with the implementation of planned state programs,

may result in significant changes to the country's demographic landscape. Timely and accurate assessment of these changes requires up-to-date information on the current structure of the population.

Moreover, the Azerbaijani population is characterized by a relatively high rate of consanguineous marriages, which contributes to an increased incidence of hereditary disorders. The development of such disorders is directly linked to defects in genetic information or disruptions in genetic programming. Hereditary diseases encompass disorders resulting from gene, genome, and chromosomal mutations. While chromosomal and genomic mutations lead to chromosomal abnormalities, gene mutations are responsible for monogenic (single-gene) disorders – the specific category to which the diseases under investigation in this study belong.

The investigation of genetic polymorphisms associated with monogenic disorders forms an integral part of the comprehensive study of hereditary diseases that lead to clinical complications in the Azerbaijani population. Among the more than 4,000 known hereditary diseases, only 22 are gene-based disorders for which early molecular-genetic diagnosis allows for targeted phenotypic treatment. The disorders investigated in this research – phenylketonuria, galactosemia, cystic fibrosis (mucoviscidosis), leucinosis, and Familial Mediterranean Fever (FMF) – fall within this category.

Timely and accurate genetic diagnosis of these diseases enables the application of effective therapeutic interventions, such as dietary therapy and supportive treatments, significantly improving patients' phenotypic outcomes and quality of life. As hereditary diseases are unequivocally associated with gene mutations, one of the main objectives of this study is to investigate the specific mutations responsible for a range of such disorders.

Genetic screening studies in Azerbaijan remain limited. Research on the genetic heterogeneity and biochemical polymorphism of hereditary diseases in the Azerbaijani population is still in its early stages. Therefore, there is a pressing need for comprehensive investigations that include:

– the study of nuclear DNA polymorphisms – one of the most widely used approaches for assessing human population diversity – which enables the characterization of the gene pool and provides an

objective measure of genetic variability;

– a holistic evaluation of the Azerbaijani population's gene pool through molecular-genetic, population-demographic, and population-statistical analyses;

– the investigation of the prevalence and genetic basis of hereditary diseases such as phenylketonuria, galactosemia, cystic fibrosis, leucinosis, and FMF;

– a comparative analysis of the frequency and distribution of diagnostically significant mutations across subpopulations;

– and, based on these findings, an assessment of the genetic load in various regional and ethnic groups within the country.

The object of the research comprises patients diagnosed with phenylketonuria, galactosemia, mucoviscidosis (cystic fibrosis), leucinosis, and Familial Mediterranean Fever (FMF), representing various ethnic groups residing in the Sheki-Zagatala, Guba-Khachmaz, and Lankaran-Astara regions, as well as in Baku city, Republic of Azerbaijan.

The subject of this research is the investigation of gene mutations responsible for inherited diseases – phenylketonuria, galactosemia, mucoviscidosis (cystic fibrosis), leucinosis, and Familial Mediterranean Fever – among various ethnic groups residing in the Sheki-Zagatala (Balakan, Gakh, Gabala, Oguz, Zagatala, Sheki), Guba-Khachmaz (Shabran, Khachmaz, Guba, Gusar, Siyazan), and Lankaran-Astara (Jalilabad, Astara, Lerik, Masalli, Yardimli, Lankaran) economic regions, as well as in Baku city of the Republic of Azerbaijan. The research also aims to explore the potential and methodologies for assessing the genetic heterogeneity and biochemical polymorphism of these hereditary disorders within the target populations.

The aim of the research. The primary objective of this study is to investigate, for the first time, the genetic heterogeneity, biochemical polymorphism, and prevalence of inherited metabolic diseases – specifically phenylketonuria, galactosemia, cystic fibrosis (mucoviscidosis), maple syrup urine disease (leucinosis), and Familial Mediterranean Fever – among various ethnic groups residing in the Sheki-Zaqatala, Guba-Khachmaz, Lankaran-Astara regions and Baku city of Azerbaijan. Additionally, the study aims to identify the underlying mutations responsible for these disorders.

To achieve this goal, the following research tasks have been set:

a) To assess the role of key genetic-demographic indicators in shaping the genetic structure of populations in selected regions of Azerbaijan;

b) To conduct a comparative analysis of reproductive behavior and the structure of consanguineous marriages within the studied populations;

c) To evaluate genotypes, allele frequencies, and genetic differentiation across populations based on nuclear genome polymorphism;

d) To perform a comparative analysis of alleles and haplotypes at polymorphic DNA loci on both normal and mutant chromosomes;

e) To elucidate the molecular-genetic mechanisms of mutations that cause inherited metabolic diseases;

f) To conduct population-genetic and statistical analyses of genetic heterogeneity, biochemical polymorphism, and disease-associated mutations;

g) To determine the prevalence of congenital metabolic disorders in Azerbaijan through prospective analyses and large-scale neonatal screening;

h) To perform a comparative analysis of common and diagnostically significant mutations associated with congenital metabolic diseases across different ethnic groups;

i) To construct pedigrees and calculate inbreeding coefficients, taking into account family structure, age, sex, and disease prevalence of affected individuals;

j) To implement genetic screening of newborns;

k) To evaluate the effectiveness of preventive strategies aimed at reducing the likelihood of affected births;

l) To assess the applicability and potential clinical outcomes of dietary therapy in newborns diagnosed with inherited metabolic diseases;

m) To develop evidence-based diagnostic and preventive algorithms grounded in the population frequency of disease-causing alleles and haplotypes.

Research tasks:

1. To identify gene complex mutations at the DNA level associated with phenylketonuria, galactosemia, cystic fibrosis (mucoviscidosis), maple syrup urine disease (leucinosis), and Familial Mediterranean Fever.

2. Study of the effects of specific mutations detected in patients with phenylketonuria, galactosemia, mucovissidosis (cystic fibrosis), leucinosis, and Familial Mediterranean Fever on biochemical polymorphisms in various ethnic groups living in the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara regions, and Baku city of Azerbaijan, including determination of amino acid levels in blood and urine, blood sugar, liver enzymes, chloride levels in the sweat of cystic fibrosis patients, and leukocytes, fibrinogen, and C-reactive protein (CRP) in the blood of Mediterranean fever patients.

3. To analyze the biochemical, phenotypic-clinical, and symptomatic manifestations associated with specific mutations identified in patients with the aforementioned inherited metabolic disorders.

4. To examine the structure of consanguineous marriages and to calculate kinship and inbreeding coefficients within families of patients diagnosed with these disorders, across various ethnic groups living in the specified regions of Azerbaijan.

5. To calculate gene frequencies for phenylketonuria, galactosemia, cystic fibrosis, leucinosis, and FMF in families identified as being within genetic risk groups.

6. To assess the prevalence of phenylketonuria, galactosemia, cystic fibrosis, leucinosis, and FMF among different ethnic populations residing in the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara regions, and Baku.

7. To evaluate the incidence and territorial distribution of these hereditary disorders throughout the population of Azerbaijan, with an emphasis on regional specificity.

8. To determine the influence of sex on the prevalence and manifestation of phenylketonuria, galactosemia, cystic fibrosis, leucinosis, and FMF.

Research methods. In this study, a combination of modern molecular-genetic, biochemical, and statistical analysis methods was employed. Molecular techniques included polymerase chain reaction (PCR) – based methods and nucleotide sequencing of DNA fragments, the latter performed using an automated DNA analyzer.

The research focused on identifying the genetic heterogeneity, biochemical polymorphisms, and pathogenic mutations associated with phenylketonuria, galactosemia, cystic fibrosis (mucovissidosis), maple

syrup urine disease (leucinosis), and Familial Mediterranean Fever (FMF) among various ethnic groups residing in the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara regions, and Baku city of Azerbaijan.

Population-genetic and statistical analyses were conducted to assess the distribution and frequency of these inherited disorders. Additionally, clinical examinations were performed on neurologically symptomatic and suspected newborns.

Blood samples were collected from patients examined and treated in maternity and pediatric hospitals located within the Sheki-Zagatala, Guba-Khachmaz, and Lankaran-Astara economic zones. Genomic DNA was isolated from blood lymphocytes using standard extraction protocols and subsequently analyzed to detect gene mutations. The data obtained were subjected to statistical analysis to evaluate genetic variation, mutation frequency, and disease prevalence across the studied populations.

Screening programs targeting neurologically affected patients and newborns were also implemented as part of the research, contributing to the early identification of genetic disorders and enhancing the understanding of their epidemiology in the Azerbaijani population.

The methodological basis of the research is grounded in a comprehensive set of principles, methods, and theoretical frameworks applied to conduct genetic screening for phenylketonuria, galactosemia, cystic fibrosis (mucovissidosis), maple syrup urine disease (leucinosis), and Familial Mediterranean Fever (FMF). This framework supports the identification and characterization of mutations responsible for these inherited disorders, as well as the investigation of their genetic heterogeneity and biochemical polymorphism.

Statements submitted for defense:

1. Among the Azerbaijani population, the following mutations and polymorphisms have been identified: in the PAH gene – R261Q, V245V, P281L, R241C, V399V, E280K, R261X, A434D, R176X, Ex6-96A>G, R241C, R243Q, R252Q, Y356X and polymorphisms Q232Q, L385L, V245V; in the GALT gene – P325L, H132Q, Q334K, N314D; in the MEFV gene – R761H, M694I, M694V, V726A, M680I, E148Q and polymorphisms D102D, G138G, A165A, R202Q, R314R, G474G, G476G, D510D.

2. Among mucovissidosis (cystic fibrosis) patients from Baku city,

Sheki-Zagatala, Guba-Khachmaz, and Lankaran-Astara regions of Azerbaijan, the disease is associated with mutations in the *CFTR* gene – R117H, R334W, delF508, IVS8-5T, as well as a novel mutation in exon 7 – L322P(965T>C); among leucinose patients, novel mutations have been identified in the *DBT* gene exon 10 – 1199A>G, *BCKDHB* gene exon 2 – 508C>T, *BCKDHB* gene exon 9 – 972C>T, and *BCKDHB* gene exon 10 – 1221A>G.

3. Out of 33 mutations causing phenylketonuria, galactosemia, mucovissidosis (cystic fibrosis), leucinosis, and Familial Mediterranean Fever and leucinosis in the population of Baku city, Sheki-Zagatala, Guba-Khachmaz and Lankaran-Astara zones of Azerbaijan, 20 (61%) are transitions, 11 (33%) are transversions, 1 (3%) are duplications, 1 (3%) are deletions, 27 are missense, 1 is silent and 5 are nonsense mutations.

4. Mutations in the *PAH* gene lead to an increase in the levels of phenylalanine in blood and urine. Mutations in the GALT gene result in decreased blood sugar levels and increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alpha-fetoprotein (AFP), and bilirubin. The activity of the G6PD enzyme (glucose-6-phosphate dehydrogenase) is partially reduced in heterozygotes, while in homozygotes, there is a complete deficiency of the enzyme. Mutations in the *CFTR* gene increase the concentration of sodium chloride (NaCl) in sweat. Mutations in the *BCKDHB* and *DBT* genes cause elevated levels of leucine, isoleucine, and valine amino acids in blood and urine. Mutations in the *MEFV* gene increase the levels of leukocytes, fibrinogen, and C-reactive protein (CRP) in blood.

5. The phenotypic-clinical, symptomatic, and biochemical characteristics of the individual mutations studied influence the severity of the diseases.

6. In the Azerbaijani population, the incidence of phenylketonuria, galactosemia, mucovissidosis (cystic fibrosis), leucinosis, and Familial Mediterranean Fever depends on consanguineous marriages, including the coefficients of kinship and inbreeding.

7. The territorial heterogeneity of patients with phenylketonuria, galactosemia, mucovissidosis (cystic fibrosis), leucinosis, and Familial Mediterranean Fever as well as the territorial and ethnic distribution of

mutant alleles and the ethnic specificity in the distribution of mutant genotypes among various ethnic groups living in the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara zones, and Baku city, directly affects the prevalence of these diseases.

The relevance and originality of the research. The relevance and originality of this research are supported by extensive literature reviews in international journals and conference proceedings. To date, there have been no comprehensive studies aimed at determining the spectrum and molecular mechanisms of mutations causing phenylketonuria, galactosemia, cystic fibrosis (mucovissidosis), leucinosis, and Familial Mediterranean Fever, nor at analyzing the genetic heterogeneity and biochemical polymorphism of these diseases among various ethnic groups residing in the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara regions, and Baku city. This dissertation represents the first investigation into the prevalence of these inherited metabolic disorders within these populations, utilizing study results to assess genetic load across diverse groups and conducting a comparative analysis of the frequency of common and diagnostically significant mutations. These aspects emphasize the significance and timely importance of the research.

Novelty of the research. For the first time in the Azerbaijani population, five previously unreported mutations in the *DBT, BCKDHB, and CFTR* genes have been identified in patients diagnosed with leucinosis and cystic fibrosis (mucovissidosis). Notably, in an Azerbaijani patient newly diagnosed with cystic fibrosis, a novel mutation L322P (965T>C) was detected in exon 7 of the *CFTR* gene. Among patients with leucinosis, previously undescribed mutations were identified: a homozygous 1199A>G mutation in exon 10 of the *DBT* gene in one patient; a homozygous 508C>T mutation in exon 2 of the *BCKDHB* gene in a second patient; and compound heterozygous mutations 972C>T (exon 9) and 1221A>G (exon 10) in the *BCKDHB* gene in a third patient.

In addition to these novel findings, 28 known mutations in the *PAH, GALT, CFTR,* and *MEFV* genes were identified and their structural and molecular characteristics were comprehensively analyzed. The study established geographic and ethnic differences in mutation carriage, revealing ethnic specificity and regional heterogeneity in the distribution of these genetic variants.

Furthermore, the prevalence of the investigated diseases and their distribution among genetic risk groups were determined. The frequency of consanguineous marriages and inbreeding coefficients were calculated, demonstrating a significant association between these factors and the incidence of genetic diseases. The phenotypic and biochemical consequences of the mutations were studied, and their impact on the clinical severity of the diseases was statistically evaluated.

Theoretical significance of the research. The identification of mutation mechanisms within gene complexes associated with phenylketonuria, cystic fibrosis (mucovissidosis), leucinosis, galactosemia, and Familial Mediterranean Fever, along with elucidating the functional consequences of these mutations, holds substantial theoretical importance. In Azerbaijan, where consanguineous marriages are prevalent, genetic screening for these inherited metabolic disorders has been largely unexplored. This research contributes theoretically by analyzing the structure of consanguineous marriages within affected families, calculating inbreeding coefficients, assessing phenotypic frequencies, and constructing a gene map specific to the Azerbaijani population.

For the first time, the prevalence of phenylketonuria, galactosemia, cystic fibrosis, leucinosis, and Familial Mediterranean Fever was evaluated among various ethnic groups residing in the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara regions, and Baku city. Additionally, the comparative frequency of widely distributed and diagnostically significant mutations was assessed, providing fundamental theoretical insights. These findings enable estimation of the genetic load across different populations.

Finally, the development of diagnostic and preventive algorithms based on the frequency of pathogenic genes, alleles, and haplotypes offers not only practical applications but also enriches the theoretical framework underlying genetic epidemiology and molecular pathology of these diseases.

Practical significance of the research. The implementation of early molecular-genetic diagnostics and the application of the obtained results in clinical treatment bear considerable practical significance. The use of advanced molecular-genetic techniques facilitates mass genetic screening of newborns and other patients, enabling early identification of mutant genes during the neonatal period. This early detection allows for

prompt diagnosis, continuous monitoring, and timely medical intervention, which are essential for effective disease management.

Furthermore, the study supports prospective medical-genetic counseling for families at genetic risk, neonatal genetic screening programs, and prenatal diagnostics during pregnancy in at-risk families. The development of diagnostic and preventive algorithms based on the frequency of pathological alleles and haplotypes at polymorphic DNA loci significantly enhances prenatal and neonatal diagnostic capabilities.

Preventive measures aimed at minimizing the birth of affected children, coupled with the timely initiation of dietary therapy when indicated, hold critical practical importance. Additionally, the creation of genetic maps detailing the distribution of these pathologies within the Azerbaijani population, alongside the formulation of preventive programs targeted at newborns and healthcare professionals, contributes substantially to improving public health outcomes.

Approval and application. The materials and findings of this research have been presented and discussed at both international and local scientific conferences, as well as reviewed by the Scientific Council of the Institute of Genetic Resources of the Azerbaijan National Academy of Sciences. To date, the results have been disseminated through 65 scientific publications. These findings have broad applicability across various disciplines, including molecular biology, medical genetics, pediatrics, neurology, and other related branches of medical biology.

Within the scope of this study, a diagnostic and preventive program targeting five inherited diseases – phenylketonuria, galactosemia, cystic fibrosis (mucovissidosis), leucinosis, and Familial Mediterranean Fever – has been developed for newborn screening and is intended for implementation by healthcare professionals, underscoring its practical significance. Implementation of specific preventive strategies based on these findings can reduce the incidence of affected births.

Furthermore, genetic maps detailing the distribution of these diseases within the Azerbaijani population have been constructed. By incorporating variables such as patients' sex, age, marital structures, and local customs, population-statistical and demographic analyses enable accurate estimation of disease prevalence across the general population and among various ethnic groups.

The fundamental nature of the research, grounded in contemporary molecular-genetic technologies, establishes a robust foundation for its academic and practical application. Additionally, the results serve as valuable resources for the development of new educational materials and the preparation of lectures for students at Azerbaijan Medical University.

Discussion of the dissertation. The results of the dissertation work were presented at the conference "Current Problems of Modern Natural Sciences" held in Ganja in 2017, the conference "Modern Achievements in Health" dedicated to the 95th anniversary of Zarifa Aliyeva in 2018, the scientific-practical conference "Current Problems of Medicine" (2019) dedicated to the 100th anniversary of the establishment of the Faculty of Medicine at Baku State University, the II International Conference on "Human Genome and Health" in Tbilisi, Georgia in 2019, the conference on "II International Health Problems" of Khazar University (2019), the I International Scientific-Practical Conference "Education, Science, Technology" of Universities of Azerbaijan and Turkey (2019), the II International Scientific-Practical Conference on "Problems of Modern Medicine" in Almaty, Kazakhstan in 2020, the conference "Human Genetics and Genetic Diseases: Problems and Development" held at Azerbaijan Medical University in 2020 perspectives", the I International Scientific-Practical Virtual Conference on "Modern Problems of Science, Education, Innovation" held in Tallinn, Estonia in 2020, the International Scientific-Practical Congress on "Actual Problems of Medicine-2020" dedicated to the 90th anniversary of Azerbaijan Medical University, the International Conference on "Ponto-Caspian and Caucasus Region: Changes in the Connection and Isolation of Ecosystems, Phylogeny, Geology, Ecology and Geography" held at Khazar University in 2020, the International Conference on "Problems of Psychology" held at Western University in 2020, the III International Scientific-Practical Conference on "Scientific Society: Interdisciplinary Research" held in Hamburg, Germany in 2021, the I International Scientific-Practical Conference on "Theoretical and Practical Aspects of Science" held in Rome, Italy in 2021, At the II International Scientific and Practical Conference on "Global and Regional Aspects of Development" held in Copenhagen, Denmark in 2021, at the VIII International Scientific and Practical

Conference on “Scientific Research of the XXI Century” held in Ottawa, Canada in 2021, at the VIII International Scientific and Practical Conference on “Development of Science in Conditions of Social Crisis” held in Tokyo, Japan in 2021, at the “IV Health and Innovation Sciences” conference held in Baku in 2021, at the X International Scientific Conference on “Scientific Achievements and Challenges in Biology” held at the Baku State University of the Ministry of Education of the Republic of Azerbaijan, at the XI International Scientific and Practical Conference on “Science and Experience: Application to Modern Society” held in Manchester, Great Britain in 2021, at the “Global Approach to Scientific Research” held in Salvador, Brazil in 2021. Reports and discussions were made at the II International Scientific-Practical Conference on the topic, the "IX International Istanbul Scientific Research" Congress held in Istanbul, Turkey in 2022, the International Forum on the topic "Problems and their scientific solutions" held in Melbourne, Australia in 2022, the XI International Conference on the topic "Achievements and Problems in Biology" held at Baku State University in 2022, and other international scientific-practical conferences.

Organization where the dissertation work was conducted. The research was conducted at the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan.

Structure of the dissertation. The dissertation comprises an introduction, seven chapters, conclusions, recommendations, and a comprehensive list of references.

The **introduction** (29 414 characters) outlines the relevance of the research topic, the objectives and tasks of the study, applied methods, scientific novelty, and the theoretical and practical significance of the work.

The **first chapter** (136 667 characters) presents a detailed literature review on the subject matter.

The **second chapter** (10 574 characters) describes the materials and methods used for laboratory analyses, including molecular-genetic and biochemical techniques.

The **third chapter** (50 265 characters) focuses on phenylketonuria, detailing the organization of the experiment, biochemical and molecular-genetic investigations, genetic heterogeneity, biochemical poly-

morphism, and the identification of mutations in the PAH (phenylalanine-4-hydroxylase) gene. It also includes population-statistical analyses, calculation of phenotypic-clinical and gene frequencies, examination of territorial heterogeneity in mutation distribution, analysis of ethnic specificity, assessment of disease prevalence, construction of family pedigrees, calculation of kinship and inbreeding coefficients, and statistical comparisons by region, settlement, and gender.

The **fourth chapter** (33 822 characters) addresses galactosemia, covering biochemical and molecular-genetic studies, genetic heterogeneity, biochemical polymorphism, and mutation identification in the GALT (galactose-1-phosphate-uridyltransferase) gene. It includes population-statistical analyses, determination of phenotypic-clinical and gene frequencies, evaluation of territorial and ethnic mutation distribution, family pedigree construction, kinship and inbreeding coefficient calculations, and statistical comparisons by region, settlement, and gender.

The **fifth chapter** (43 583 characters) investigates mucoviscidosis (cystic fibrosis), with biochemical and molecular-genetic studies focusing on the CFTR (cystic fibrosis transmembrane conductance regulator) gene mutations. It includes biochemical polymorphism analysis, phenotypic-clinical and gene frequency calculations, territorial heterogeneity studies, ethnic specificity assessment, prevalence evaluation, family pedigree construction, kinship and inbreeding coefficients determination, and statistical analysis across regions, settlements, and gender.

The **sixth chapter** (24051 characters) presents biochemical and molecular-genetic studies on leucinosis. It examines genetic heterogeneity and biochemical polymorphism, focusing on mutations in the BCKDHA (branched-chain keto acid dehydrogenase E1 alpha polypeptide), BCKDHB (branched-chain keto acid dehydrogenase E1 beta polypeptide), DLD (dihydrolipoamide dehydrogenase), and DBT (dihydrolipoyl branched-chain transacylase) genes. Population-statistical analyses, phenotypic-clinical and gene frequency calculations, territorial mutation heterogeneity, ethnic distribution, prevalence, family pedigree construction, kinship and inbreeding coefficients, and statistical comparisons by region, settlement, and gender are also included.

The **seventh chapter** (58 516 characters) covers Familial Mediterranean Fever, including biochemical and molecular-genetic studies,

mu-tation identification in the MEFV (innate immunity regulatory pyrin) gene, biochemical polymorphism, phenotypic-clinical and gene frequency calculations, territorial and ethnic heterogeneity, prevalence assessment, family pedigree construction, kinship and inbreeding coefficient determination, and statistical analyses by territory and gender.

The dissertation concludes (11 679 characters) with a summary of the obtained results (5 603 characters), followed by conclusions and recommendations (1 765 characters).

The dissertation is presented in 397 pages, supported by 110 tables and 180 figures. The reference list contains 396 scientific sources, including 367 in Russian and English. Additionally, a list of abbreviations and symbols is provided after the references.

Chapter I. Literature review

The literature review of this dissertation systematically summarizes and critically analyzes the findings of numerous studies, drawing upon authoritative sources published in leading international scientific journals relevant to the research topic. For the first time in the Azerbaijani language, comprehensive, clearly structured, and logically coherent information is presented regarding mutations in the *PAH*, *GALT*, *BCKDHA*, *BCKDHB*, *DLD*, *DBT*, *CFTR*, and *MEFV* genes. These discussions are supported by references to priority journals and leading authors, ensuring the scientific validity and reliability of the presented data. Moreover, the review provides detailed information on the molecular-genetic diagnostic technologies employed for hereditary diseases. It systematically examines and critically compares the advantages and limitations of these methods, emphasizing the methodological diversity within current diagnostic approaches. In recent years, molecular-genetic research on hereditary disorders such as phenylketonuria, galactosemia, leucinosis, cystic fibrosis, and Familial Mediterranean Fever has significantly expanded. This chapter presents a comprehensive overview of such studies, highlighting both the theoretical foundations and practical implications of their findings.

Each section of the literature review integrates references to international scientific research, ensuring that the analysis transcends local perspectives and incorporates a comparative evaluation of global

knowledge. This approach provides a holistic understanding of the current state of the field, identifying both key achievements and existing research gaps.

Chapter II. Research material and methods

Between 2015 and 2020, a total of 4,530 individuals from the Azerbaijani population were enrolled in the study across the Baku city, Sheki-Zagatala, Guba-Khachmaz, and Lankaran-Astara economic regions. The research focused on hereditary diseases, including phenylketonuria, galactosemia, leucinosis, cystic fibrosis, and Familial Mediterranean Fever. Among the participants, 700 served as control subjects – healthy individuals with no observable clinical symptoms – while 3,830 were patients presenting with clinically diagnosed symptoms.

For the separation and quantification of amino acids, the High-Performance Liquid Chromatography (HPLC) method was utilized. Serum parameters – including glucose, bilirubin, and liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alpha-fetoprotein (AFP), and glucose-6-phosphate dehydrogenase (G6PD) enzyme activity – were measured using the enzyme-linked immunosorbent assay (ELISA). Leukocyte counts were determined using a counting chamber. C-reactive protein (CRP) levels were assessed via the particle-enhanced turbidimetric immunoassay (PETIA) method, while fibrinogen concentrations were measured using a chronometric assay. Additional diagnostic methods included the ferric chloride test and the sweat chloride test.For molecular-genetic analyses, DNA extraction from venous blood was performed using the QIAamp Genomic DNA and RNA Kit (QIAGEN, Germany). DNA fragments were amplified using the polymerase chain reaction (PCR) technique, carried out on the T100™ Thermal Cycler (Bio-Rad, Germany), with forward (F) and reverse (R) primers employed[2,3].

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[2]Амелина С.С. Молекулярно-генетическое типированис больных с фенилкетонурией в Ростовской области // Медицинская генетика, – 2004. - том 3, №3, – с. 139.

[3]Chong, J.X. A population-based study of autosomal-recessive disease-causing mutations in a founder population // Am. J. Hum. Genet. – 2012. v.91, – p.608.

Electrophoresis of DNA fragments was performed on a 1.7% agarose gel using the PowerPac Basic Gel Doc™ EZ system (Bio-Rad, USA). The Lambda DNA Mixed Digest marker served as a reference, while a 100 bp DNA ladder was employed to determine the molecular weights of the DNA fragments.

In the first stage, PCR-amplified DNA fragments were purified using magnetic bead-based systems, specifically the Agencourt AMPure XP PCR Purification Kit and the SPRIPlate 96 Super Magnet Plate. Following purification, the DNA fragments underwent nucleotide sequencing with the Applied Biosystems (Hitachi) 3130xl Genetic Analyzer. The resulting nucleotide sequences obtained via Sanger sequencing were analyzed and compared using the SeqScape™ software. Reference sequences were retrieved from GenBank (www.ncbi.nlm.nih.gov/genbank), the PAH database (http://www.pahdb.mcgill.ca), and the NCBI SNP database for variant identification and validation.

Mutations in the CFTR gene, associated with cystic fibrosis, were partially detected using a targeted genetic panel method performed on the Rotor-Gene Q platform (QIAGEN) [4,5].

For the statistical analysis of research outcomes, Pearson's chi-square test (χ^2) was applied when the sample size n≥5n \geq 5n≥5, while Fisher's exact test was used for n<5n < 5n<5. Segregation analysis and calculation of the inbreeding coefficient were conducted according to the methodology proposed by N.P. Bochkova. The coefficient of kinship (Q) and the inbreeding coefficient (Fi) for various degrees of consanguinity were determined using the standard method developed by S. Wright.

Bioinformatic analysis was conducted using the Swiss in silico modeling program (www.swiss-prot.org).

Chapter II. Research on phenylketonuria

3.1. Molecular analysis of the *PAH* gene. A genetic study of phenylketonuria (PKU) was carried out among different ethnic groups

[4]www.ncbi.nlm.nih.gov/snp

[5]Feuchtbaum L. Birth prevalence of disorders detectable through newborn screening by race ethnicity. Genet. Med. 14: 2012. 937-945.

within the Azerbaijani population. A total of 670 individuals were analyzed, including 110 controls and 560 patients. The study identified multiple mutations in the PAH gene, namely: V399V, R261Q, P281L, R241C, E280K, R261X, A434D, R176X, Ex6-96A>G, R241C, R243Q, R252Q, and Y356X. Additionally, the polymorphisms Q232Q, V245V, and L385L were detected.

Table 3.1.1. Number of different mutations and polymorphisms of the *PAH* gene in experimental and control groups

Group	Total number of samples involved in the study	Number of patients with detected mutations	Number of patients with polymorphism detected	Total
Experimental	560	25	-	25
Control	110	1	4	5
Total	670	26	4	30

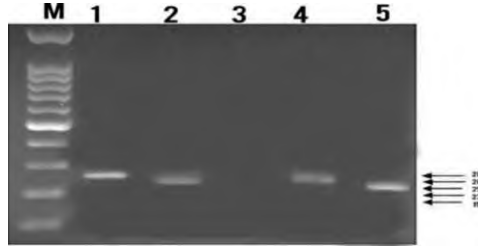


Figure 3.1.1. Image of 0.7% agarose gel electrophoresis of exons 6, 7, 8, 11, and 12 of the *PAH* gene: M – Marker; 1 – exon 6 (283 b.p.); 2 – exon 7 (256 b.p.); 3 – exon 8 (normal); 4 – exon 11 (260 b.p.); 5 – exon 12 (232 b.p.).

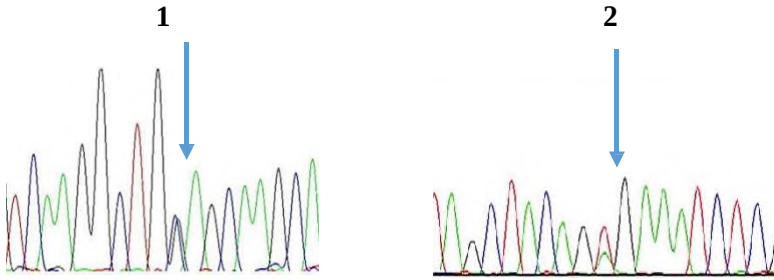


Figure 3.1.2. Electropherogram of changes in the nucleotide sequence of the *PAH* gene: Heterozygous form of 1-L385L(1155G>C) polymorphism; 2-Heterozygous form of the V399V(1197A>T) polymorphism

3.2. Comparison of various mutations detected in the *PAH* gene in the studied Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara zones and Baku city. Among the 670 individuals studied, 140 were from Guba-Khachmaz, 160 from Sheki-Zagatala, 190 from Lankaran-Astara, and 180 from Baku city, including both newborns and patients. Statistical analysis showed no significant differences in the number of patients between these economic regions and Baku city ($p > 0.05$).

However, ethnic specificity was observed in both the frequency and spectrum of PAH gene mutations. These findings are detailed in Figure 3.2.

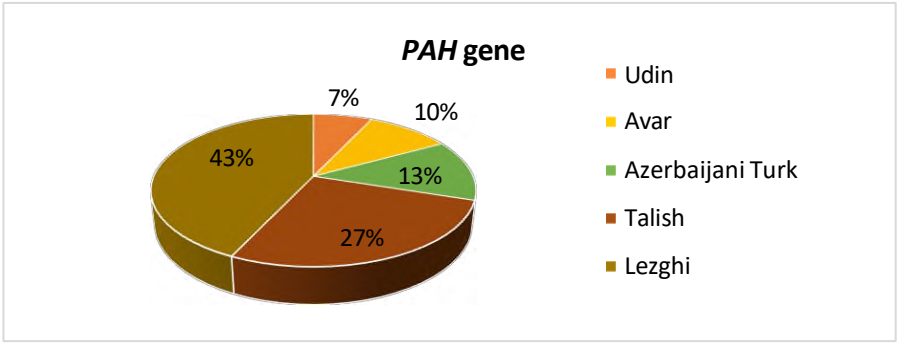


Figure 3.2. Prevalence of *PAH* gene mutations in the Azerbaijani population by different ethnic groups (in %)

3.3. Biochemical polymorphism of phenylketonuria. Phenylalanine levels in the blood were measured in 30 patients with phenylketonuria. Among them, 15 patients exhibited phenylalanine concentrations ranging from 120 to 350 µmol/L, 12 patients had levels between 1180 and 1470 µmol/L, and 3 patients showed elevated levels from 2290 to 2310 µmol/L. These measurements were used to evaluate the severity of the disease in the affected individuals.

In the control group, one individual with the V399V mutation and four individuals carrying the Q232Q, V245V, and L385L polymorphisms displayed phenylalanine levels within the 120–350 µmol/L range. Given the relatively low phenylalanine concentrations and the absence of clinical symptoms, the disease severity in these control subjects was classified as mild.

Among the 670 individuals studied for phenylketonuria, mutations and polymorphisms in the PAH gene were identified in 4 of the 110 control

samples and 26 of the 560 experimental samples. Statistical analysis revealed no significant difference in the number of affected individuals between the control and experimental groups (p>0.05) (Table 3.3.2).

Table 3.3.1. Comparison of phenylketonuria patients studied in the Azerbaijani population by severity

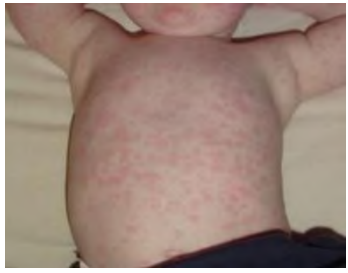
Name of the disease	N	Severity level	Number of patients		P_1	P_2
			Absolute	%		
Phenylketonuria	26	Severe	3	11		
		Moderate-severe	12	46,7	0,007	
		Mild	11	42,3	0,007	>0,05

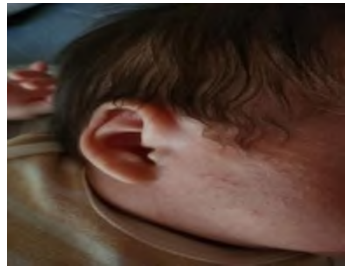
Note: P1 is the accuracy of the difference compared to severe, P2 is the average severity.

Table 3.3.2. Comparison of patients from control and experimental groups on the *PAH* gene

№	Group	Total	Number of patients		P
			Absolute	%	
1	Control	110	4	3,6	
2	Experimental	560	26	4,97	>0,05

a)





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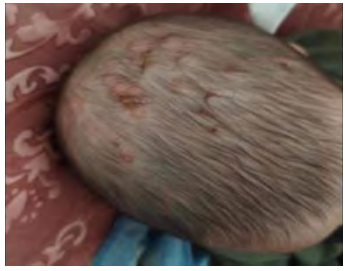

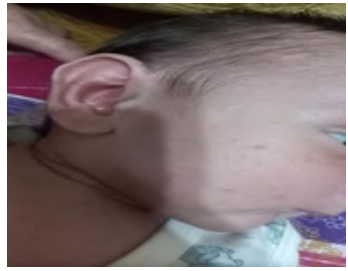

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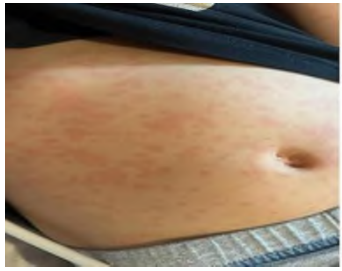


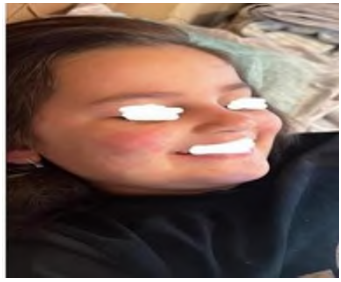


Figure 3.3.1. a) A 1-year-old girl with a heterozygous form of the A434D mutation, which causes high residual activity of the PAH enzyme (experimental group); b) A 1-year-old girl with a heterozygous form of the R243Q mutation, which causes high residual activity of the PAH enzyme (experimental group); c) A 4-year-old girl with a heterozygous form of the P281L mutation, which causes high residual activity of the PAH enzyme (experimental group)

3.4. Genealogy construction of families diagnosed with phenylketonuria, calculation of kinship and inbreeding coefficients. The coefficient of kinship and the inbreeding coefficient were calculated based on the pedigree analysis of each patient, with the results presented in Table 3.4.1.

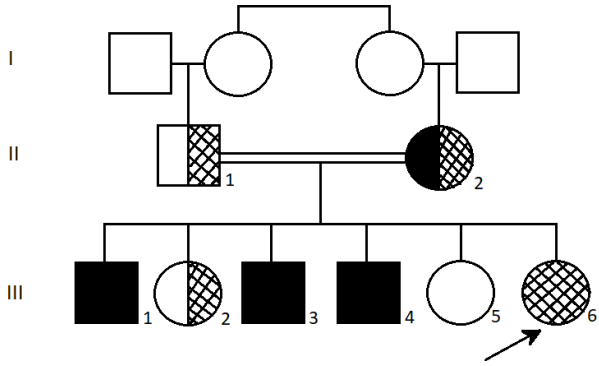


Figure 3.4.1. Family tree of G.M.: father (II-1) – heterozygous carrier of the R261G mutation, mother (II-2) – heterozygous carrier of the R261G mutation and G6PD enzyme, proband (III-6) – homozygous for R261G-fermentation (III-6) – R261G-fermentation (III-P3) (III-43) and brothers (III-43rd variant) – hemizygous variants, brother (III-2) – heterozygous carrier of the R261G mutation, brother (III-5) – healthy.

Table 3.4.1. Kinship and inbreeding coefficients

№	Number of patients	Coefficient of kinship (Q)	Coefficient of inbreeding (F)
1	7	0,32	0,16
2	2	0,25	0,125
3	11	0,75	0,375
4	3	1	0,5
5	3(mutation)+3(polimorphizm)	0,625	0,31
6	1	0,5	0,25

3.5. Comparison of genotypes of phenylketonuria patients. Among the 26 patients, 21 (80.7%) carried PAH gene mutations in a heterozygous form, while 5 (19.3%) were homozygous for the mutations ($p > 0.05$). No cases of compound heterozygosity or compound homozygosity were observed in the studied cohort ($p < 0.001$). Of these 26 patients, 15 (57.7%) were female and 11 (42.3%) were male.

3.6. Discussion of PAH gene mutations across neighboring countries. Taking into account the most common PAH gene mutations in neighboring countries, the following mutations were found in the Azerbaijani population: Ex6–96A > G, R176X, W187C, L348V mutations and Q232Q polymorphism in exon 6 of this gene; R261Q, P281L, R252Q, R243X, R241C, R243Q, E280K, R261X, R241C, G272X, S273F, L255S, V245A mutations and V245V polymorphism in exon 7; R408W, İ283N, L287V, H290Q mutations in exon 8; Y356X, V399V, E390G, S359X, V388M mutations in exon 11; The R413P, A434D, Y414C and D415N mutations in exon 12 were studied. In the Azerbaijani population, as in Iran, the most common mutation in the PAH gene was R261Q in exon 7, and the most common polymorphism was Q232Q in exon 6. Unlike the Iranian population, the R261Q (20%) polymorphism was recorded at a higher frequency, and the Q232Q (6.7%) polymorphism was recorded at a lower frequency. Unlike the results of studies conducted on PAH gene mutations in Turkey, the R408W mutation was not detected in the Azerbaijani population. Our results are similar to the results of the study conducted in the Russian and Georgian populations. However, unlike the Georgian population, the frequency of the P281L (6.7%) mutation in the PAH gene was recorded at a lower frequency in the Azerbaijani population.

Chapter IV. Research on galactosemia disease

A total of 944 individuals participated in the galactosemia study, comprising 130 control subjects and 814 experimental subjects.

4.1. Molecular-genetic analysis of *GALT* gene. In the Azerbaijani population, the following mutations in the GALT gene were investigated: H132Q, M142K, and R148W mutations located in exon 5; R258G and R231H mutations in exon 8; K285N mutation in exon 9; and Q334K, P325L, and N314D mutations in exon 10.

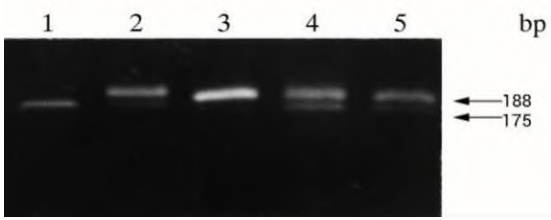


Figure 4.1.1. Intactness of DNA fragments in 1.7% agarose gel: 1 – Marker sample; 2 – Heterozygous form of the N314D (190(A>G)) mutation; 3 – Heterozygous form of the Q334K (1030(C>A)) mutation; 4 – Homozygous form of the N314D mutation; 5 – Compound form of the Q334K and N314D mutations

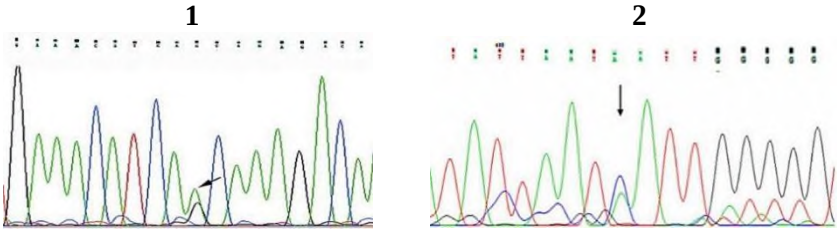


Figure 4.1.2. Electropherogram of changes in the nucleotide sequence of the *GALT* gene: 1-Heterozygous form of the N314D (190(A>G)) mutation; 2-Heterozygous form of the Q334K ((1030C>A)) mutation

Ethnic specificity was identified in both the frequency and spectrum of the GALT gene mutations studied in the Azerbaijani population, and the results are presented in Figure 4.1.3.

No mutations in the GALT gene were detected in the control group samples (Table 4.1.2). Among 523 experimental samples, 9 individuals were identified as carriers of four different GALT gene mutations

– P325L, H132Q, N314D, and Q334K – present in homozygous, heterozygous, and compound heterozygous forms.

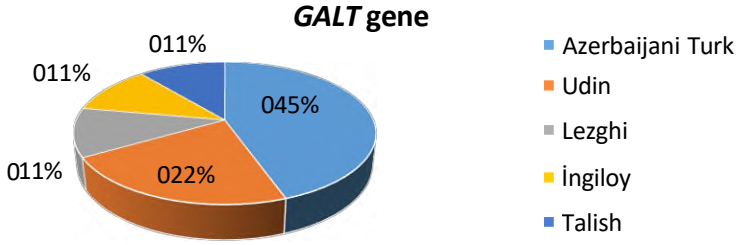


Figure 4.1.3. Distribution of *GALT* gene mutations among different ethnic groups in the Azerbaijani population (in %).

Table 4.1.1. Detected mutations of the GALT gene among the studied patients

№	Patient	Mutation	Genotype	Region	Ethnic group	Gender
1	Newborn Ə.A.	N314D	Heterozygote	Sheki, Cheshmali village	Udin	male
2	Newborn Ə.Ə.	Q334K	Heterozygote	Gabala, Zargarli village	Azerb.	male
3	Newborn R.R.	N314D	Homozygote	Lerik, Guneshli village	Talysh	female
4	M.A. (1 year 6 months)	N314D	Homozygote	Baku	Azerb.	male
5	F.N. (1 year 9 months)	Q334K/ N314D	Compound	Baku	Azerb.	male
6	Newborn B.E.	P325L	Heterozygote	Sheki, Cheshmali village	Udin	male
7	Newborn C.S.	H132Q	Heterozygote	Balakan, Gerakli village	Ingiloy	male
8	Newborn Ə.N.	N314D	Heterozygote	Gabala, Vandam village	Azerb.	male
9	Newborn C.N.	N314D	Heterozygote	Gusar	Lezgi	female

Table 4.1.2. Comparison of patients from the control and experimental groups on the *GALT* gene

№	Group	Total	Number of patients		P
			Absolute	%	
1	Control	130	-	1,3	
2	Experimental	814	9	1,1	>0,05

4.2. Comparison of the various mutations detected in the *GALT* gene in the studied Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara zones and Baku city. Of the 944 people involved in the study, 232 were newborns and patients from Guba-Khachmaz, 286 from Sheki-Zagatala, 170 from Lankaran-Astara zones, and 256 from Baku city.

Table 4.2.1. Comparison of various detected mutations of the *GALT* gene across the studied zones and Baku city

№	Regions	N		Number of patients		P_1	P_2	P_3
				Abs.	%			
1	Sheki-Zagatala	286	30 control 256 exp.	5	2,16			
2	Baku	256	40 control 216 exp.	2	0,78	>0,05		
3	Lankaran-Astara	170	30 control 140 exp.	1	0,59	>0,05	>0,05	
4	Guba-Khachmaz	232	30 control 202 exp.	1	0,35	>0,05	>0,05	>0,05
	Total:	944	130 control 814 exp.	9	0,95			

There was no significant difference in the number of newborns and patients from hospitals and maternity homes located in different regions of Azerbaijan and Baku city involved in the study of galactosemia ($p>0.05$).

4.3. Biochemical polymorphism of galactosemia. Mutations in the GALT gene led to reduced enzyme activity, resulting in the accumulation of galactose in the blood. This was accompanied by increased levels of alpha-fetoprotein (AFP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and elevated synthesis of liver enzymes, all of which influenced the severity of the disease. Among the

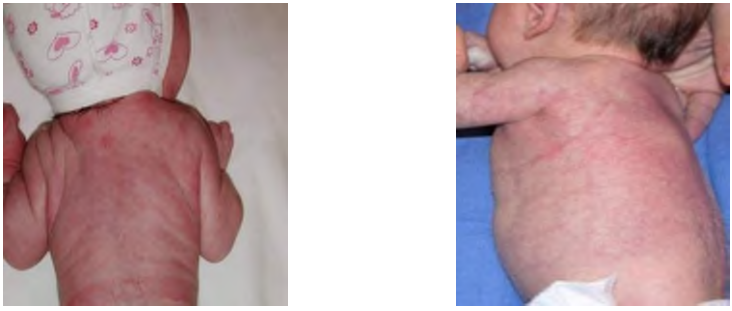
9 galactosemia patients, 4 exhibited blood galactose levels of 10 mg or higher, alongside a twofold or greater increase in AFP, ALT, and AST. These cases were classified as severe. The remaining 5 patients had galactose levels below 10 mg, corresponding to a mild disease severity. No cases of moderate severity were observed in the study. Statistical analysis showed no significant difference in the frequency of severe versus mild forms of galactosemia ($p > 0.05$).

Table 4.3.1. Comparison of galactosemia severity in patients studied in the Azerbaijani population

Name of disease	N	Severity level	Number of patients		P_1	P_2
			Absolute.	%		
Galactosemia	9	Severe	4	44,4		
		Moderate-severe	0	0,0	>0,05	
		Mild	5	55,6	>0,05	0,026

Note: P_1 is the accuracy of the difference compared to severe, P_2 is the average severity.

Of the 9 patients, 7 were female (77.7%) and 2 were male (22.2%).



a) b)

Figure 4.3.1. A newborn with high residual activity of the *GALT* enzyme: a) heterozygous form of the P325L mutation; b) heterozygous form of the Q334K mutation.

The homozygous form of the N314D mutation (M.A; R.R.) was detected in 2 patients with severe symptoms, and the heterozygous form of this mutation (A.A; A.N.) was detected in the other (Figure 4.3.2, 4.3.3).

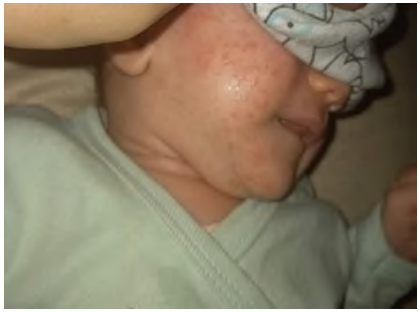


Figure 4.3.2. Galactosemia. A newborn (N.N.) with a heterozygous form of the N314D mutation in the *GALT* gene is diagnosed.

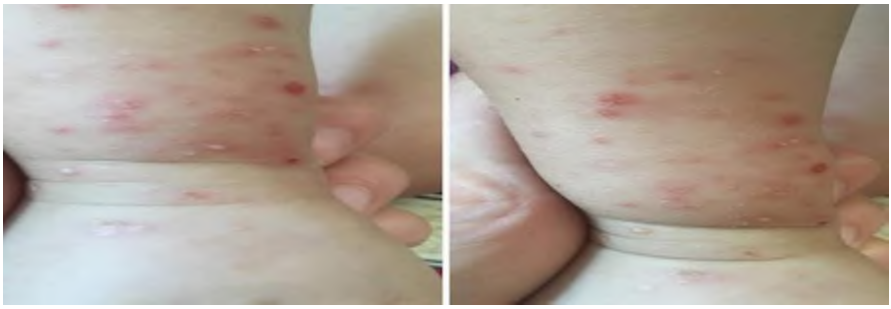


Figure 4.3.3. Patient (F.N.) with a compound form of the Q334K/N314D mutation in the *GALT* gene

4.4. Genealogy of families with diagnosed galactosemia, calculation of kinship and inbreeding coefficients. The kinship and inbreeding coefficients of the patients were calculated by constructing a pedigree. In 1 of 9 patients, the kinship coefficient was 0.32, the inbreeding coefficient was 0.16, in 4, the kinship coefficient was 0.625, the inbreeding coefficient was 0.31, in 2, the kinship coefficient was 0.75, the inbreeding coefficient was 0.375, and in 2, the kinship coefficient was 1, the inbreeding coefficient was 0.5.

4.5. Comparison of genotypes of galactosemia patients. Out of the 9 patients, 6 (66.7%) were heterozygous, 2 (22.2%) were homozygous, and 1 (11.1%) was compound heterozygous. No cases of compound homozygosity were detected among the studied patients.

4.6. Discussion of GALT Gene Mutations in Neighboring Countries. Taking into account the most prevalent GALT gene mutations in

neighboring countries, a comparative study was conducted in the Azerbaijani population. Unlike the Turkish population, mutations such as M142K, K285N, R231H, and R258G, which were investigated in Azerbaijan, were not detected. Among the mutations identified in the Iranian population – K285N, R148W, N314D, Q334K, H132Q, and P325L – the following were also observed in Azerbaijan: N314D (66.6%), Q334K (22%), H132Q (11.1%), and P325L (11.1%), whereas K285N and R148W were absent. In the Georgian population, the N314D mutation was reported at a frequency of 5.9%. In Azerbaijan, this mutation was found to be the most prevalent among the detected GALT gene mutations, occurring at a frequency of 66.6%.

Chapter V. Research on cystic fibrosis disease

5.1. Molecular-genetic analysis of the *CFTR* gene. The CFTR gene was analyzed in a total of 1,344 individuals, comprising 1,144 experimental subjects and 200 controls, representing various ethnic groups from different regions of the Republic of Azerbaijan.

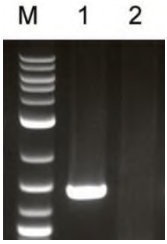


Figure 5.1.1. Gel electrophoresis image of the IVS8-5T mutation in intron 8 of the *CFTR* gene: M – DNA marker; 1 – mutation (134 b.p.); 2 – control sample (250 b.p).

In a subset of the studied patients, comprising 138 affected children and 548 newborns, CFTR gene mutations were identified using a genetic panel on the Rotor-Gene instrument. This panel screened for six specific CFTR mutations: delF508, W1282X, N1303K, delT2143, 3849+10kb C→T, and del2,3 (21kb). The homozygous form of the delF508 mutation was found in four patients. However, the mutations W1282X, N1303K, delT2143, 3849+10kb C→T, and del2,3 (21kb) were not detected in any of the studied individuals.

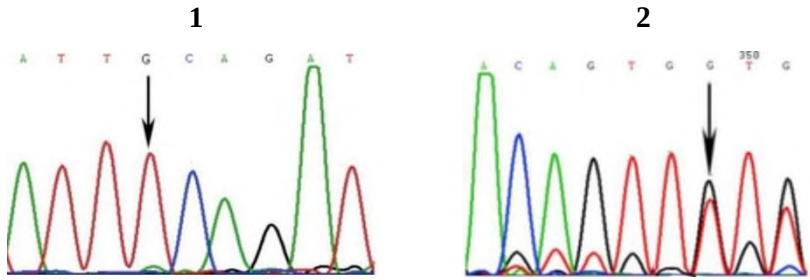


Figure 5.1.2. Electropherogram of changes in the nucleotide sequence of the *CFTR* gene: compound form of the R117H/R334W mutation: 1 – R117H (1000G>T) mutation; 2 – Electropherogram of changes in the nucleotide sequence of the R334W (328 (G>A)) mutation.

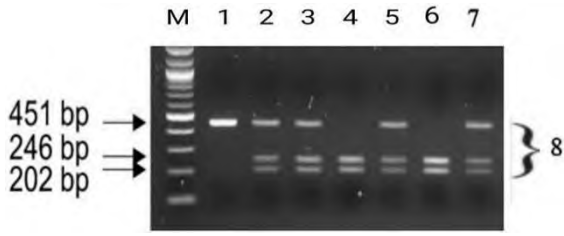


Figure 5.1.3. Gel electrophoresis image of the delF508 mutation detected in the *CFTR* gene: M – DNA marker; 1 – healthy; 2 – delF508 mutation in heterozygous form (451, 246, 202 b.p.; 3 – heterozygous form (451, 246, 202 b.p.); 4 – homozygous form (246, 202 b.p.); 5 – heterozygous form (451, 246, 202 b.p.); 6 – homozygous form (246, 202 b.p.); 7 – heterozygous form (451, 246, 202 b.p.); 8 – mutant region (covers the area where the 202-451 b.p. fragments are located).

Among the experimental group, 18 patients carried various CFTR gene mutations. Specifically, the following mutations were identified: R117H in exon 4, R334W in exon 7, a novel mutation L322P in exon 8 (reported for the first time in this study), delF508 in exon 10, and R553X in exon 11.

The mutations detected in the Azerbaijani population – including Phe508del/IVS8-5T, R117H/R334W, R553X, and the novel L322P (965(T>C)) mutation not previously reported in the literature – were observed at a frequency of 0.0007, while the delF508 mutation showed

a higher frequency of 0.01.

The prevalence of various CFTR gene mutations was 0.0052 in Baku and 0.0082 in the northern regions of Azerbaijan. No cystic fibrosis cases were detected in the southern regions.

Ethnic specificity was noted in both the frequency and spectrum of CFTR mutations. The delF508 mutation (present in 8 of the 18 patients) was found among individuals of the Lezgi ethnic group, whereas the other 10 patients were Azerbaijani Turks residing in Baku and the Khachmaz district. Consequently, the prevalence of cystic fibrosis was estimated at 0.0074 among Azerbaijani Turks and 0.0059 among the Lezgi ethnic group.

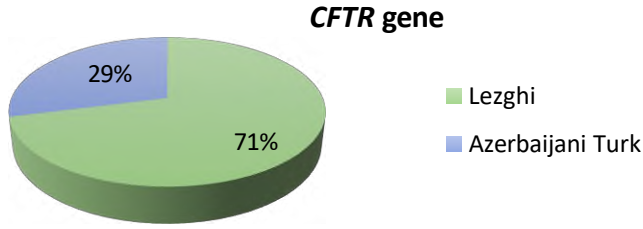


Figure 5.1.4. Prevalence of *CFTR* gene mutations in the Azerbaijani community by ethnic group (in %)

Of the 18 patients with *CFTR* gene mutations, 7 were male (38.9%) and 11 were female (61.9%). There was no significant difference between the number of male and female samples among cystic fibrosis patients ($p>0.05$).

There was no significant difference in the number of patients in the control and experimental groups ($p>0.05$).

Table 5.1.1. Comparison of patients from control and experimental groups on the *CFTR* gene

№	Group	Total	Number of patients		P
			Absolute	%	
1	Control	200	-	0,5	
2	Experimental	1144	18	1,24	>0,05

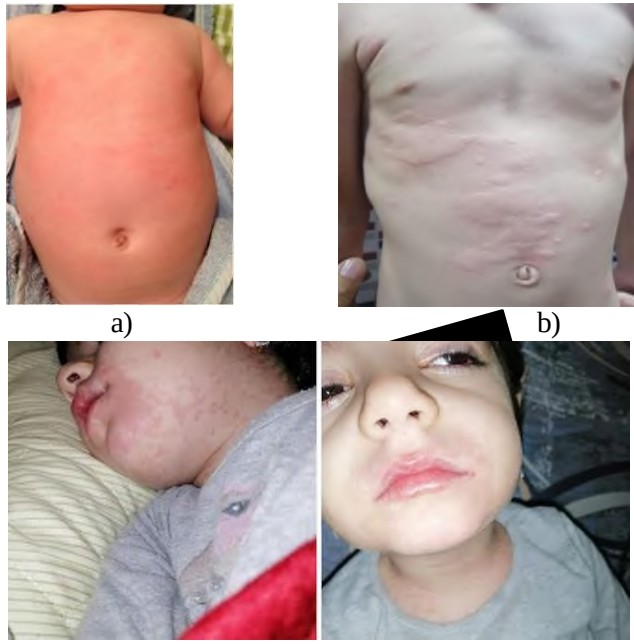


Figure 5.1.5. Cystic fibrosis: a) A newborn with a heterozygous form of the L322P (965T>C) mutation in exon 8 of the CFTR gene (B.D.); b) Patient with a homozygous form of the delF508 mutation (A.N.); c) Patient with a compound form of the delF508/IVS8-5T mutation (D.X.).

5.2. Biochemical polymorphism of cystic fibrosis. By correlating the results of sweat tests with molecular genetic analyses, the clinical severity of cystic fibrosis was classified in 18 patients carrying various CFTR gene mutations. Among them, 10 patients exhibited a severe form of the disease, characterized by sweat chloride concentrations exceeding 100 mmol/L and the presence of the delF508 mutation. The remaining 8 patients displayed a mild clinical form, with sweat chloride levels ranging from 60 to 100 mmol/L. These patients carried mutations such as R553X, R117H/R334W, 5T/delF508, and the novel L322P (965T>C) mutation, which has not been previously reported in the literature.

No cases of the moderate-severe form of cystic fibrosis were observed among the patients. Furthermore, there was no statistically significant difference in the number of patients presenting with severe

versus mild clinical forms ($p > 0.05$).

Table 5.2.1. Comparison of cystic fibrosis patients studied in the Azerbaijani population by severity

Name of disease	N	Severity level	Number of patients		P_1	P_2
			Absolute	%		
Cystic fibrosis	18	Severe	10	55,6		
		Moderate-severe	0	0,0	<0,001	
		Mild	8	44,4	>0,05	0,001

5.3. Comparison of the identified *CFTR* gene mutations across the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara regions, and Baku city. Among the 1,344 individuals included in the cystic fibrosis study, 322 were from the Guba-Khachmaz, 227 from Sheki-Zagatala, 183 from Lankaran-Astara economic regions, and 612 from Baku city. CFTR gene mutations were detected only in individuals from the Guba-Khachmaz region and Baku city. No statistically significant difference was observed in the number of cystic fibrosis patients between these two regions ($p > 0.05$). Notably, no CFTR gene mutations were identified in patients from the Sheki-Zagatala and Lankaran-Astara regions ($p < 0.001$) (Table 5.3.1).

Table 5.3.1. Comparison of the identified *CFTR* gene mutations across the studied regions.

№	Regions	N		Number of patients		P_1	P_2
				Absolute	%		
1	Guba-Khachmaz	322	50 control 272 exp.	11	3,4		
2	Baku	612	50 control 562 exp.	7	1,1	>0,05	
3	Shaki-Zagatala	227	50 control 177 exp.	0	0,0	<0,001	<0,05
4	Lankaran-Astara	183	50 control 133 exp.	0	0,0	<0,001	<0,05
	Total:	1344	200 control 1144 exp.	18	1,3		

5.4. Pedigree construction, calculation of kinship and inbreeding coefficients of families with cystic fibrosis. Based on the pedigree of each proband with *CFTR* gene mutations, inbreeding and kinship coefficients were determined. Out of 18 patients, 5 had a kinship coefficient of 0.32, an inbreeding coefficient of 0.16, 1 had a kinship coefficient of 0.25, an inbreeding coefficient of 0.125, 3 had a kinship coefficient of 0.75, an inbreeding coefficient of 0.375, 2 had a kinship coefficient of 1, an inbreeding coefficient of 0.5, 3 had a kinship coefficient of 0.625, an inbreeding coefficient of 0.31.

5.5. Comparison of genotypes of cystic fibrosis patients. Among the 18 patients, 12 (66.7%) were homozygous, 4 (22.2%) were heterozygous, and 2 (11.1%) were compound heterozygous.

5.6. Discussion of *CFTR* gene mutations in neighboring countries. The prevalence, gene frequency, and specificity of *CFTR* gene mutations in the populations of neighboring countries were investigated and the most characteristic ones for this population were identified, and the D110H, R117H mutations in exon 4 of the *CFTR* gene, R334W, R352Q mutations in exon 7, delF508, S492F mutations in exon 10, and G542X and R553X mutations in exon 11 were studied. The most frequently detected *CFTR* gene mutations in the Iranian population were delF508, IVS85T, R334W, G542X, D110H, R352Q and R117H mutations, which were also studied in the Azerbaijani population. Of these mutations, delF508 (83.3%), R117H (5.5%), R334W (5.5%), IVS85T (5.5%) were detected in the Azerbaijani population, while G542X, D110H and R352Q mutations were not detected. Of the mutations IVS8-5T, DelF508, G542X, R553X, R117H, R334W and S492F detected in the *CFTR* gene in the Turkish population, IVS8-5T, DelF508, R553X, R117H, R334W and S492F mutations were detected in the Azerbaijani population. The frequency of other mutations detected in the Azerbaijani population, except for the delF508 mutation, was found to be lower than in the Turkish population. Unlike the Turkish population, the G542X and S492F mutations were not detected in the Azerbaijani population. The DelF508, R553X, R117H, R334W mutations detected in the *CFTR* gene in various ethnic groups of Kazakhstan were also detected in the Azerbaijani population. The frequency of the DelF508 mutation in the Kazakh population was determined to be 51.7%, and in the Azerbaijani population it was relatively

high – 66.6%. Of the delF508, R117H, G542X mutations detected in the *CFTR* gene in the Russian population, the delF508 and R117H mutations were also detected in the Azerbaijani population. The frequency of the detected delF508 mutation was observed more frequently in the Azerbaijani population than in the Russian population.

Chapter VI. Molecular-genetic study of leucinosis disease

6.1. Molecular genetic analysis of *BCKDHA, BCKDHB, DLD* and *DBT* genes. A total of 940 individuals were included in the genetic study of leucinosis within the Azerbaijani population, comprising 140 control samples and 800 experimental samples**.** Molecular diagnostics were conducted targeting four key genes associated with the disease *(BCKDHA, BCKDHB, DLD and DBT).*

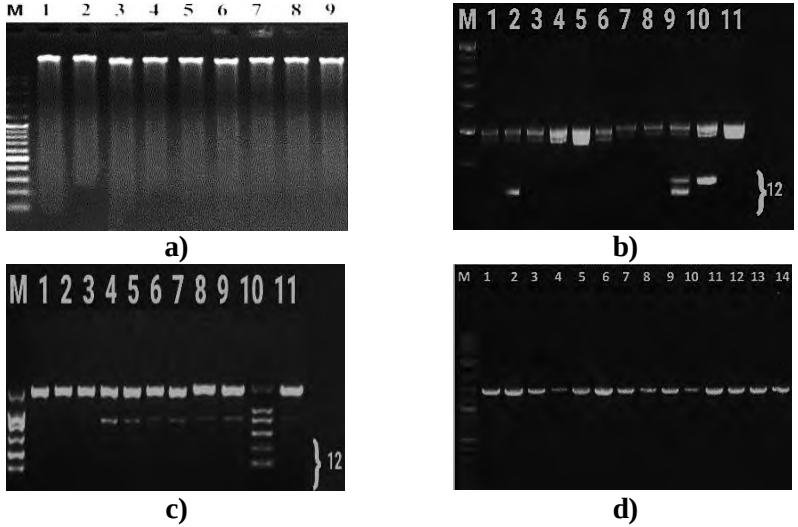


Figure 6.1.1. Image of electrophoresis of *BCKDHA, BCKDHB, DBT, DLD* genes on 1.7% agarose gel: Image of electrophoresis of a-*BCKDHA* gene: M-DNA marker, exons 1-9 – no mutation detected; Image of electrophoresis of b-*BCKDHA* gene: M-DNA marker, exons 1, 3-8, 11 – no mutation detected 1, 3-8 and 11, 2-9-10 – mutation in exons 2, 9, 10 in *BCKDHB* gene, 12 – mutant region; Image of electrophoresis of c-*DBT* gene: M-DNA marker, exons 1-9, 11 – no mutation detected, 10-mutation in exon 10 of *DBT* gene, 12 – mutant region; Electrophoresis image of the d-*DLD* gene: M – DNA marker, exons 1-14-1-14 where the mutation was not detected.

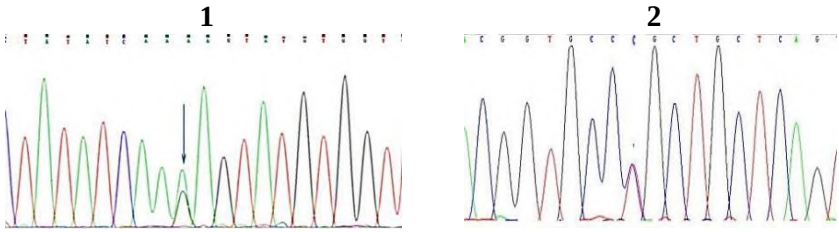


Figure. 6.1.2. Electropherogram of nucleotide sequence changes in the *BCKDHB* gene: Electropherogram of nucleotide sequence changes in the heterozygous compound form of the *BCKDHB* gene: novel 1221(A>G) mutation found in exons 1-10; novel 972(C>T) mutation found in exons 2-9.

Three of the 800 experimental samples included in the study were found to have mutations that cause leusinosis. Each of these three identified mutations is a novel mutation not reported in the literature. In one patient, the 1199A>G mutation in exon 10 of the DBT gene was homozygous, in one patient, the 508C>T mutation in exon 2 of the BCKDHB gene was homozygous, and in the third patient, the 972C>T (exon 9) and 1221A>G (exon 10) mutations of the BCKDHB gene were found in compound heterozygous form. No mutations were recorded in any of the 140 control samples included in the study. No mutations were found in the BCKDHA and DLD genes.

Data on the territorial and ethnic distribution of mutant alleles of the BCKDHB and DBT genes in Azerbaijan were obtained. The disease was detected in patients living in the northern region of Azerbaijan. Thus, 2 out of 3 patients were found in patients from the Khinalıq village of Guba, and 1 in the Khachmaz region.

Ethnic specificity was determined in the frequency and spectrum of the studied mutations. 1 of these patients is an Azerbaijani Turk, and 2 are from the Lezgi ethnic group. The incidence of leucinosis disease was recorded to be very low among Azerbaijani Turks living in Azerbaijan and was equal to 0.001, and among the population belonging to the Lezgi ethnic group it was 0.0021.

6.2. Biochemical polymorphism of leucinosis disease. The amount of valine, leucine and isoleucine amino acids was determined in the blood and urine of patients with general symptoms of leucinosis disease, the amount of valine amino acid in the urine of patients was

1.5-2 times higher than normal, isoleucine 1.5-2 times higher than normal, and leucine 3-4 times higher than normal. The amount of the amino acid valine in the patients' blood serum was recorded as 2-3 times higher than normal, isoleucine 7-8 times higher, and leucine 25-30 times higher.

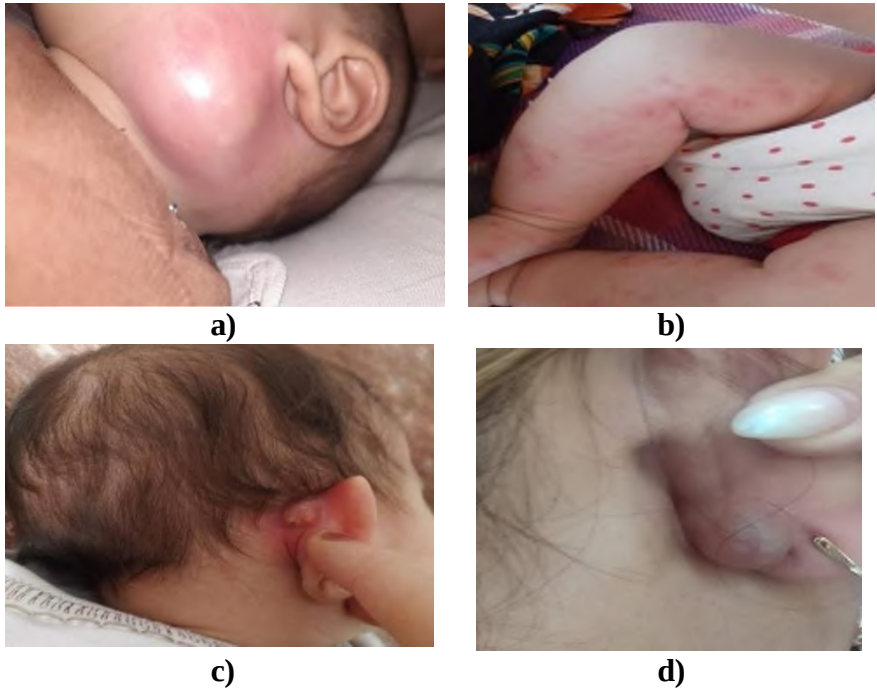


Figure 6.2.1. Newborn with leucosis disease: a) Homozygous form of the 1199A>G mutation in exon 10 of the *DBT* gene (T.E.); b) Newborn boy from the same family with a compound heterozygous form of the novel (972(C>T)/1221(A>G) mutation of the *BCKDHB* gene (A.T.); c) 4-year-old girl from the same family with a homozygous form of the novel (508(C>T)) mutation of the BCKDHB gene (A.M.).

Among the patients identified with mutations in the *BCKDHB* gene, the clinical presentation of leucinosis was generally mild. No cases of leucinosis were detected in any of the control group samples. As detailed in Table 6.2.3**,** a statistical comparison between the number of patients exhibiting mild versus severe clinical forms of the disease showed no significant difference ($p > 0.05$).

Table 6.2.1. Results of molecular genetic research on leucinosis disease

№	Pa-tient	Mutation	Genotype	Region	Ethnic group	Gen-der	Age
1	T.E.	DBT gene (1199A-G)	Homozygote	Xachmaz	Azerb.	female	4
2	A.M.	BCKDHB gene (508C-T)	Homozygote	Guba (Khinaliq village)	Lezghi	female	3
2	A.T.	BCKDHB gene (972C-T/ 1221A-G)	Compaund	Guba (Khinaliq village)	Lezghi	male	new-born

Table 6.2.2. Comparison of the severity of leucinosis in patients studied in the Azerbaijani population

Name of disease	N	Severity level	Number of patients		P_1	P_2
			Abs.	%		
Leucinosis	3	Severe	1	33,3		
		Moderate-severe	0	0,0	>0,05	
		Mild	2	66,7	>0,05	>0,05

Table 6.2.3. Comparison of patients from the control and experimental groups on the *BCKDHB, DBT* genes

№	Group	Total	Number of patients		P
			Absolute	%	
1	Control	140	-	1,4	
2	Experimental	800	3	2,6	>0,05

These findings suggest that the identified *BCKDHB* gene mutations may be associated with a milder phenotypic expression of the disorder in the studied Azerbaijani population. However, the limited number of patients necessitates further research to confirm genotype-phenotype correlations.

Various mutations of the *BCKDHB* and *DBT* genes were detected in 3 out of 800 experimental samples, the number of patients was statistically calculated, and based on the results of the calculations, it was determined that there was no significant difference in the number of

patients from the control and experimental groups (p>0.05).

Among the three patients diagnosed with leucinosis, one was male (33.3%) and two were female (66.7%)**.** Statistical analysis **revealed** no significant difference in the distribution of the disease between male and female patients **(**p > 0.05**),** indicating the absence of a gender-based predisposition to leucinosis in the studied sample.

6.3. Diseases in the Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara zones and Baku city, where the study of certain mutations of the *BCKDHB* and *DBT* genes was conducted. The table of certain mutations of the *BCKDHB* and *DBT* genes in the studied zones is given in 6.3.1.

Table 6.3.1. Comparison of *BCKDHB* and *DBT* genes by zones

№	Regions	Total number involved in screening		Total number of patients	Gene	Number of patients
1	Sheki-Zagatala	245	30 control	-		
			215 experimental			
2	Guba-Khachmaz	240	30 control	3	BCKDHB gene	2
			210 experimental		DBT gene	1
3	Lankaran-Astara	265	40 control	-		
			225 experimental			
4	Baku	290	40 control	-		
			250 experimental			
	Total:	940	140 control	3		
			800 experimental			

6.4. Genealogy of families with diagnosed leusinosis, calculation of kinship and inbreeding coefficients. According to the genealogy, it was recorded that 2 of 3 leusinosis patients had a kinship coefficient of 1 and an inbreeding coefficient of 0.5, and 1 had a kinship coefficient of 0.25 and an inbreeding coefficient of 0.125.

6.5. Comparison of genotypes of leusinosis patients. It was recorded that 2 out of 3 patients were homozygous (66.7%), and 1 was a compound heterozygous (33.3%) (Table 6.5.1).

Table 6.5.1. Comparison of genotypes of leucinosis patients

Genotype	Number of patients		P
	Absolute	%	
Homozygote	2	66,7%	
Compaunt homozygote	1	33,3%	>0,05
Heterozygote	-	-	<0,001
Compaunt heterozygote	-		<0,001
Total	3	100%	

Three distinct missense mutations were identified in the *BCKDHB* gene during molecular genetic analysis. In the control sample, the nucleotide sequence in the region corresponding to the 508th nucleotide within exon 2 was determined to be GTGACAAT. However, in individuals carrying the mutant allele, the nucleotide sequence in the same region was observed to be GTGATAAT, indicating a single-nucleotide variation. Furthermore, in exon 9, the nucleotide sequence at the site of the 972nd nucleotide was CGGTGCCCGCTGCTCAG in the control sample, whereas it was found to be CGGTGCCTGCTGCTCAG in the mutant gene carriers. Likewise, in exon 10, the control sequence at the position of the 1221st nucleotide was TATATCAAAAGTATGTGGT, while in individuals harboring the mutation, the sequence was altered to TATATCAAGAGTATGTGGT. These findings suggest point mutations occurring at specific loci within the *BCKDHB* gene, potentially altering gene function.

As a result of bioinformatic analysis performed on the *BCKDHB* and *DBT* genes in three selected patients, multiple amino acid substitutions with likely functional consequences were identified. In the first patient, a substitution of cytosine (C) with thymine (T) at nucleotide position 508 in exon 2 of the *BCKDHB* gene resulted in an amino acid change from lysine (K) to glutamine (Q) at position 332 of the encoded polypeptide. This specific mutation is expected to affect the protein's charge properties and may alter its structural stability or interaction with other subunits in the BCKDH complex.

In the second patient, a compound heterozygous missense mutation was detected. A C-to-T substitution at nucleotide position 972 in exon 9 of the *BCKDHB* gene led to an arginine (R) to serine (S) amino acid

change at position 673. Additionally, an A-to-G substitution at position 1221 in exon 10 of the same gene resulted in a phenylalanine (F) to glutamine (Q) substitution at position 947. Both changes may significantly disrupt the enzymatic function of the resulting branched-chain alpha-keto acid dehydrogenase subunit by affecting its active site or tertiary structure.

In the third patient, an A-to-G substitution at nucleotide position 1199 in exon 10 of the *DBT* gene was identified, leading to an amino acid substitution of lysine (K) with glutamine (Q) at position 907 of the polypeptide. Given the critical role of the DBT gene in encoding the E2 subunit of the BCKDH complex, such a mutation could impair the multi-enzyme complex's activity and contribute to disease pathogenesis.

Another novel mutation, designated L322P, was observed in exon 8 of the *CFTR* gene in a newborn diagnosed with cystic fibrosis. While the wild-type nucleotide sequence at position 965 in this region was expected to be ACTATAGAG, sequencing revealed a mutated sequence of ACTACAGAG, indicating a C-to-T substitution. As a result, the leucine (L) at position 322 in the CFTR protein was replaced by proline (P). This substitution is of particular importance due to proline's structural rigidity, which may introduce kinks into alpha-helices or disrupt local secondary structures, potentially compromising CFTR protein folding or chloride channel function.

All mutations identified in the present study are classified as pathogenic, based on their predicted impact on protein structure and function, as well as their absence in healthy control populations. These findings provide further insight into the molecular mechanisms underlying disorders such as maple syrup urine disease and cystic fibrosis, and highlight the importance of genetic screening for early diagnosis and clinical management.

Chapter VII. Molecular-genetic study of Familial Mediterranean Fever (FMF) disease

7.1. Molecular-genetic analysis of the *MEFV* gene. A genetic study on Familial Mediterranean Fever (FMF) was conducted among individuals from various ethnic groups within the Azerbaijani popular-

tion**.** A total of 632 individuals were included in the study, comprising 120 controls and 512 experimental subjects. E148Q, R202Q mutations and D102D, G138G, A165A polymorphisms in exon 2 of the *MEFV* gene, R314R polymorphism in exon 3, G474G, G476G, D510D polymorphisms in exon 5, and M694V, M694I, M680I, V726A, R761H, A744S, K695R mutations in exon 10 were studied. All seven detected mutations were previously identified in peoples living in the Mediterranean region, mainly in Turkish populations.

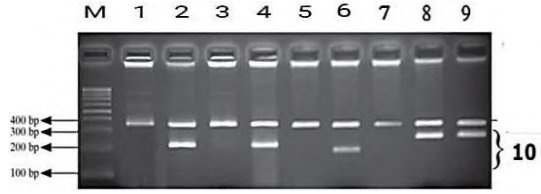


Figure 7.1.1. Gel electrophoresis image of mutations detected in the *MEFV* gene: M-DNA marker; 1 – healthy; 2 – heterozygous form of the M694V mutation (212 b.p.); 3 – healthy; 4 – heterozygous form of the M680I mutation (220 b.p.); 5 – healthy; 6 – heterozygous form of the M694I mutation (184 b.p.); 7 – healthy; 8 – heterozygous form of the R761H mutation (247 b.p.); 9 – homozygous form of the R761H mutation (247 b.p.); 10 – mutant region (covers the area where the fragments 184-247 b.p. are located)

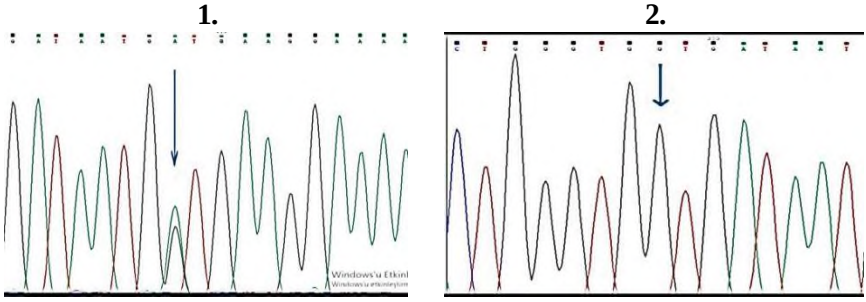


Figure 7.1.2. Electropherogram of changes in the nucleotide sequence of the *MEFV* gene: 1 – heterozygous form of the M694V (2080(A>G)) mutation; 2 – homozygous form of the V726A (2177(T>C)) mutation

The nucleotide sequence of exons 2, 3, 5 and 10 of the *MEFV* gene was studied, and 6 different mutations (R761H, M694I, M694V, V726A, M680I, E148Q,) and 8 different polymorphisms (R202Q, D102D, G138G, A165A, R314R, G474G, G476G, D510D) were detected.

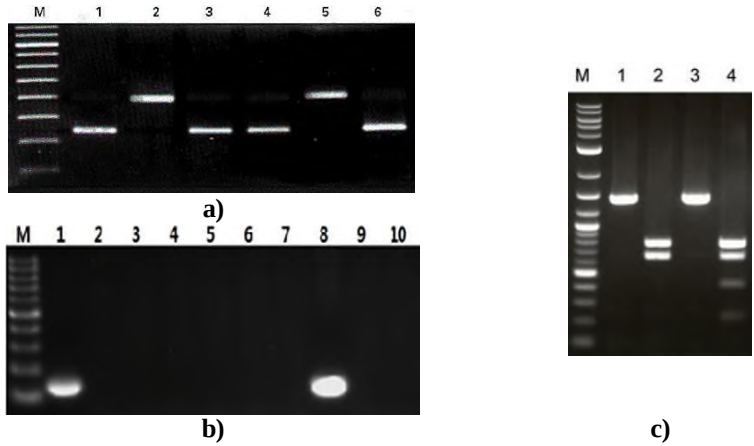


Figure 7.1.3. Gel electrophoresis image of polymorphisms detected in the *MEFV* gene in the control group**:** a – M-DNA marker; 1 – Heterozygous form of the D510D polymorphism; 2 – Healthy; 3 – Heterozygous form of the D102D polymorphism; 4 – Heterozygous form of the R324 polymorphism; 5 – Healthy; 6 – Heterozygous form of the G474G polymorphism; b – M-DNA marker: 1 – Homozygous form of the G138G polymorphism; 2-7 – Healthy, 8 – Homozygous form of the G474G polymorphism; 9, 10 – Healthy; c – M-DNA marker; 1 – Healthy; 2 – Homozygous form of the A165A polymorphism; 3 – Healthy; 4 – Homozygous form of the G474G polymorphism.

Ethnic specificity was determined in the frequency and spectrum of polymorphisms studied in the *MEFV* gene in the territory of Azerbaijan. The detected polymorphisms were recorded in representatives of four different ethnic groups (Figure 7.1.4).

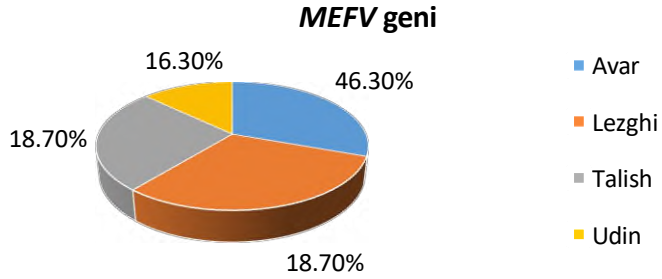


Figure 7.1.4. Prevalence of *MEFV* gene mutations in the Azerbaijani population by different ethnic groups (in %)

Various mutations and polymorphisms in the MEFV gene were detected in both the control and experimental groups. Specifically, 84 out of 120 control samples (70%) and 42 out of 512 experimental samples (8.2%) were found to carry one or more of the investigated variants.

Statistical analysis revealed no significant difference in the frequency of *MEFV* gene mutations and polymorphisms between the control and experimental groups **(**$p > 0.05$**).** This suggests that the presence of these variants alone may not be directly correlated with the clinical manifestation of Familial Mediterranean Fever within the studied population.

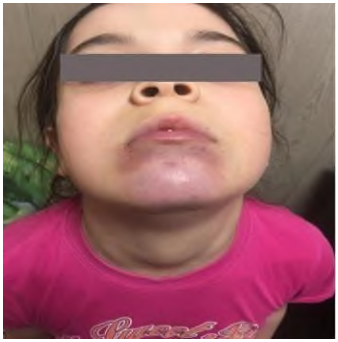
Table 7.1.1. Comparison of patients from control and experimental groups on the *MEFV* gene

№	Group	Total	Number of patient		P
			Absolute	%	
1	Control	120	84	36,6	
2	Experimental	512	42	8,2	>0,05

The mutations that we detected had the highest frequency of occurrence. The frequency of the M694V mutation was 0.0283, the frequency of the R761H mutation was 0.0236, the frequency of the M694I mutation was 0.0078, the frequency of the E148Q and V726A mutations was 0.0047, and the frequency of the M680I mutation was 0.0031.

7.2. Biochemical polymorphism of the Familial Mediterranean Fever disease. It was determined that in newborns with MEFV gene mutations, the number of leukocytes was more than 35,000 per 1 μl of blood, in patients aged 1-15 years, more than 20,000 per 1 μl of blood, and in patients over 15 years old, more than 12,000 per 1 μl of blood, the amount of fibrinogen in plasma increased to 28-32 μmol/l, and the concentration of C-reactive protein (CRZ) increased to 40-100 mg/l. Out of 42 patients, 17 had severe disease, 17 had mild disease, and 8 had moderate disease.

As shown in Table 7.2.1, the disease was observed in both severe, moderate and mild forms. The number of patients with moderate severe form was lower than those with severe and mild forms of the disease ($p<0.05$).



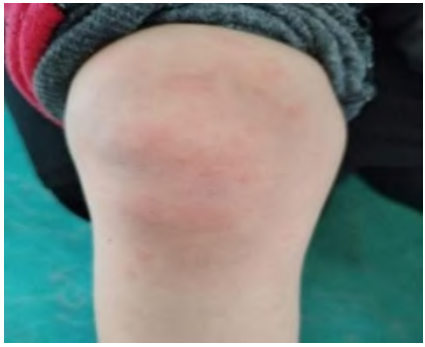


Figure 7.2.1. 4-year-old girl (M.X.) with FMF (heterozygous mutation R761H of the MEFV gene)

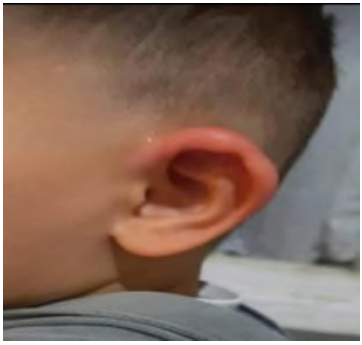
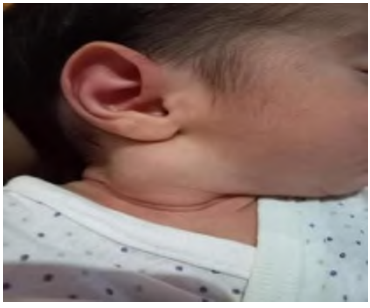


Figure 7.2.2. 8-year-old boy (N.R.) with FMF (heterozygous form of the M694V mutation in the MEFV gene)



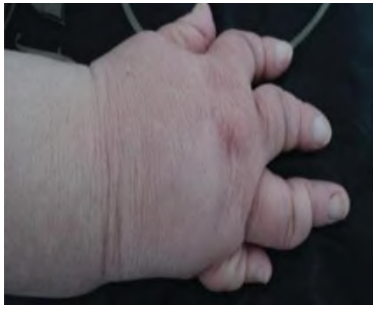


Figure 7.2.3. Newborn with FMF (F.A.) (MEFV gene R761H mutation in homozygous form)

Table 7.2.1. Comparison of the severity of FMF patients studied in the Azerbaijani population

Name of disease	N	Severity level	Number of patients		P_1	P_2
			Absolute	%		
Familial Mediterranean Fever (FMF)	42	Severe	17	40,5		
		Moderate-severe	8	19,0	<0,05	
		Mild	17	40,5	>0,05	<0,05

Among the 42 patients, 25 were male (59.5%) and 17 were female (40.5%). No statistically significant difference was observed between the number of male and female patients with Aghdeniz fever ($p > 0.05$).

7.3. Comparison of the detected different mutations of the MEFV gene in the studied Sheki-Zagatala, Guba-Khachmaz, Lankaran-Astara zones and Baku city. Of the 632 patients involved in the study, 93 were from Guba-Khachmaz, 132 from Sheki-Zagatala, 160 from Lankaran-Astara zones, and 247 from Baku city (Table 7.3.1).

Table 7.3.1. Comparison of different mutations detected in the *MEFV* gene across the studied zones

№	Regions	N		Number of patients		P_1	P_2	P_3
				absolute	%			
1	Sheki-Zagatala	132	30 control 102 exp.	16	12,1			
2	Guba-Khachmaz	93	30 control 63 exp.	10	10,8	>0,05		
3	Lankaran-Astara	160	30 control 130 exp.	10	6,3	>0,05	>0,05	
4	Baku	247	30 control 217 exp.	6	2,4	<0,001	<0,005	>0,05
	Total	632	120 control 512 exp.	42	6,6			

Compared to individual regions, the number of patients from Baku was relatively low ($p < 0.001$), while no significant difference was observed between the numbers of patients from the other zones ($p > 0.05$).

7.4. Genealogy of families diagnosed with FMF calculation of kinship and inbreeding coefficients. Inbreeding and kinship coefficients were determined based on the genealogy of each of the probands in whom mutations of the *MEFV* gene were detected. It was determined that 12 out of 42 patients had a kinship coefficient of 0.32 and an inbreeding coefficient of 0.16, 14 had a kinship coefficient of 0.25 and an inbreeding coefficient of 0.125, 3 had a kinship coefficient of 0.75 and an inbreeding coefficient of 0.375, 9 had a kinship coefficient of 0.625 and an inbreeding coefficient of 0.31, 2 had a kinship coefficient of 0.5 and an inbreeding coefficient of 0.25.

7.5. Comparison of genotypes of patients with FMF. Among the 42 patients, 13 (31%) were homozygous, 22 (51.3%) were heterozygous, and 7 (16.7%) were compound heterozygous. No cases of compound homozygosity were observed in the studied group.

7.6. Discussion of *MEFV* gene mutations in neighboring countries. The prevalence, gene frequency, and specificity of *MEFV* gene mutations in the population of neighboring countries were investigated and the most characteristic ones for this population were determined, and the E148Q mutation and R202Q, D102D, G138G, A165A polymorphisms in exon 2 of the MEFV gene, R314R polymorphism in exon 3, G474G, G476G, D510D polymorphisms in exon 5, and M694V, M694I, M680I, V726A, R761H, A744S, K695R mutations in exon 10 were studied. Of the M694V, M680I, M694I, V726A, E148Q, R761H, K695R and A744S mutations found in the *MEFV* gene with the highest frequency in the Turkish population, the M694V, M680I, M694I, V726A, E148Q, R761H mutations were detected in the Azerbaijani population, while the K695R and A744S mutations were not detected. The M694V (2.83%) and R761H (2.36%) mutations were observed with a higher frequency in the Azerbaijani population. The D102D, G138G, A165A, G474G, G476G, R314R and D510D polymorphisms observed with a relatively high frequency in the Turkish population were also studied and detected in the Azerbaijani population. The R202R polymorphism was observed with the highest frequency in the Azerbaijani population. As in the Georgian population, the E148Q, M680I and M694V mutations were observed with a high frequency in the Azerbaijani population. Unlike the Georgian population, the R671H mutation was detected with a high frequency in the

Azerbaijani population. The M694V, M694I, M680I, V726A, R761H, A744S, K695R mutations detected in the Iranian population were also studied and detected in the Azerbaijani population, while the A744S and K695R mutations were not detected. The M694V, M694I, M680I, V726A mutations were detected with a higher frequency in the Iranian population, and the M694V and R671H mutations were detected with a higher frequency in the Azerbaijani population.

RESULTS

1. For the first time, novel mutations not previously reported in the literature were identified in the *DBT* and *BCKDHB* genes in Azerbaijani patients diagnosed with leucinosis. Among 800 leusinosis patients, novel mutations in the *DBT* and *BCKDHB* genes were found in 3 individuals – in one patient, the 1199A>G mutation in exon 10 of the *DBT* gene was found in a homozygous state, in the second patient, the 508C>T mutation in exon 2 of the *BCKDHB* gene was homozygous, and in the third patient, the 972C>T (exon 9) and 1221A>G (exon 10) mutations in the *BCKDHB* gene were identified in a compound heterozygous form. No mutations were detected in the *BCKDHA* and *DLD* genes in the patients, and in the control group, no mutations were found in the *DBT, BCKDHB, BCKDHA,* and *DLD* genes.

2. For the first time, a novel mutation not previously reported in the literature – L322P (965T>C) in exon 7 of the CFTR gene – was identified in an Azerbaijani patient diagnosed with cystic fibrosis. Additionally, among 1,144 cystic fibrosis patients, single mutations in the CFTR gene were detected in 17 individuals in exons 4, 7, and 10, and intron 8, corresponding to R117H (350G>A), R334W (1000G>T), delF508 (1521-1523del), and IVS8-5T (1210-1211T>G), respectively. In one patient, a compound form of R117H (350G>A) and R334W (1000G>T) mutations was identified. No mutations were detected in exon 11 of this gene in the patients, nor were any of the studied mutations found in any member of the control group.

3. Among 560 patients with phenylketonuria, 25 individuals were found to carry one of the following mutations in the *PAH* gene: R261Q, V245V, P281L, R241C, V399V, E280K, R261X, A434D,

R176X, Ex6-96A>G, R241C, R243Q, R252Q, and Y356X, and 4 patients carried one of the polymorphisms: Q232Q, L385L, or V245V. In the control group, a single individual was found to carry one mutation (V399V) in this gene.

4. Among 814 patients with galactosemia, 8 individuals were found to carry one of the mutations in the *GALT* gene: P325L, H132Q, Q334K, or N314D, and one patient carried a compound form of the Q334K and N314D mutations. No mutations were detected in the control group.

5. Among 512 patients with Familial Mediterranean Fever (FMF), 42 individuals carried one of the *MEFV* gene mutations: R761H, M694I, M694V, V726A, M680I, or E148Q, and 7 patients carried compound forms of these mutations. In the control group of 84 individuals, the polymorphisms D102D, G138G, A165A, R202Q, R314R, G474G, G476G, and D510D were detected, but no mutations were found.

6. In a total of 3,830 individuals from Baku city and the Shaki-Zagatala, Guba-Khachmaz, and Lankaran-Astara regions of Azerbaijan, diagnosed with phenylketonuria, galactosemia, mucoviscidosis, familial Mediterranean fever, and leucinosis, 28 known and 5 novel mutations across 8 genes were identified; in the control groups, a single known mutation was detected, making a total of 34 mutations. Among these, 21 (61%) were transitions, 11 (33%) were transversions, 1 (3%) was a duplication, 1 (3%) was a deletion, 27 were missense, 1 was silent, and 5 were nonsense mutations.

7. In various ethnic groups living in the Shaki-Zagatala, Guba-Khachmaz, Lankaran-Astara zones of Azerbaijan and in Baku city, the need for amino acids in the blood and urine of phenylketonuria is 120-2310 μmol/l, the useful blood of galactosemia production is 1.2-1.9 mmol/l, the additional sugar is 1.2-1.9 mmol/l, the I02 enzyme/ the need for amino acids in the blood and urine is 498.6-3782 μmol/ mucoviscidosis (cystic fibrosis) supply is 62.8-102 mmol/l, the additional active leukocytes in the blood for FMF are more than 12000, and the concentration of fibrinogen is more than 20-32. It was concluded that it is in the range of 40-120 mg/l.

8. The phenotypic, clinical, symptomatic, and biochemical effects

of specific mutations detected in phenylketonuria, galactosemia, leucinosis, mucoviscidosis (cystic fibrosis), and familial Mediterranean fever (FMF) were studied to assess disease severity. Among phenylketonuria patients, no significant difference was found between the number of patients with moderate and mild forms of the disease ($p = 0.007$). Similarly, no significant differences were observed between the numbers of patients with severe and mild forms among those with galactosemia, mucoviscidosis, and FMF ($p > 0.05$). However, the number of patients with moderate and severe forms of FMF was significantly lower than those with severe and mild forms ($p < 0.05$).

9. The structure of consanguineous marriages among families of patients diagnosed with phenylketonuria, galactosemia, mucoviscidosis, leucinosis, and FMF in the Azerbaijani population was analyzed, and consanguinity and inbreeding coefficients were calculated. Among the 98 patients with various mutations studied, the inbreeding coefficient was found to be 0.125 in 18 patients (18.2%), 0.16 in 22 patients (23.23%), 0.25 in 7 patients (7.1%), 0.5 in 9 patients (9.1%), 0.31 in 19 patients (19.2%), and 0.375 in 23 patients (23.23%).

10. The incidence rates of mucoviscidosis (cystic fibrosis), leucinosis, phenylketonuria, galactosemia, and FMF were calculated for families within genetic risk groups. The incidence rates in the Azerbaijani population were as follows: phenylketonuria at 0.045, galactosemia at 0.009, mucoviscidosis (cystic fibrosis) at 0.013, leucinosis at 0.003, and FMF at 0.066.

11. Territorial heterogeneity in the frequency and spectrum of mutations studied in the territory of Azerbaijan was determined, information was obtained on the territorial and ethnic distribution of mutant alleles, and the ethnic specificity and prevalence of diseases in the distribution of mutant genotypes were assessed. As a result of comparing patients from the Avar, Udin, Sakhur, Tat, Tatar and Kurdish ethnic groups with patients from the Azerbaijani Turks and Lezgi ethnic groups, the statistical difference was estimated as $p<0.001$. There was no statistically significant difference between these ethnic groups (Avar, Udin, Sakhur, Tat, Tatar and Kurd) ($p>0.05$).

12. A regional comparison of patients with phenylketonuria, galactosemia, leucinosis, mucoviscidosis (cystic fibrosis), and FMF in the Azerbaijani population showed that 17.3% of patients with mutations

were registered in Baku city, 26.5% in the Sheki-Zagatala zone, 40.9% in the Guba-Khachmaz zone, and 15.3% in the Lankaran-Astara zone. When comparing patients by gender, no significant difference was observed ($p > 0.05$).

RECOMMENDATIONS

1. When performing molecular genetic diagnostics of phenylketonuria, galactosemia, leucinosis, mucoviscidosis a(cystic fibrosis) and FMF in the Azerbaijani population of various ethnic groups, the sequence of exons 6, 7, 11 and 12 of the *PAH* gene related to phenylketonuria, exons 5, 6 and 10 of the *GALT* gene related to galactosemia, exons 4, 7, 8, 10, 11 and intron 8 of the *CFTR* gene related to mucoviscidosis, exons 10 of the *DBT* gene related to leucinosis, exons 2, 9 and 10 of the *BCKDHB* gene, exons 2 and 10 of the *MEFV* gene related to FMF is recommended for the search for pathogenic variants. If the results of the sequence-related genetic analysis are unsatisfactory, a panel of genes or an extended molecular genetic analysis to determine all exon sequences can be used.

2. It is recommended to define specific mutation panels for ethnic groups as a result of generalizing the information obtained on the diversity and characteristics of mutation spectra in different regions and ethnic groups.

3. When developing DNA diagnostic protocols, it is recommended to take into account the significant variation in the frequencies and spectra of both high-frequency and rare mutations identified in different regions of Azerbaijan during the study.

4. New information obtained on the characteristics of phenylketonuria, galactosemia, leucinosis, (cystic fibrosis) and FMF and the results of the study will allow healthcare professionals to predict metabolic gene diseases in patients and determine the likelihood of manifestation of metabolic syndrome.

8. The described mechanisms of the formation of phenylketonuria, galactosemia, leucinosis (cystic fibrosis) and FMF will allow for a differential approach to the correction of metabolic disorders, treatment, and prevention of diseases.

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