REPUBLIC OF AZERBAIJAN

On the rights of the manuscript

ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

DISTRIBUTION OF MUTATIONS LEADING TO THALASSEMIA AND OTHER HEMOGLOBINOPATHIES AND EFFICACY OF PRENATAL DIAGNOSIS IN AZERBAIJAN

Specialty:	2409.01 Genetics
Field of science:	Medicine
Applicant:	**Gunay Ziraddin Aliyeva**

Baku – 2024

The work was performed at National Hematology and Transfusion Center of the Ministry of Healthcare of Azerbaijan Republic

Scientific Supervisor: Doctor of philosophy in medicine, ass. prof. **Chingiz Dashdemir Asadov**

Scientific Advisor: Doctor of Biological Sciences, professor **Ellada Mirali Akhundova**

Official Opponents: Corresponding member of RAS, Doctor of medical sciences, professor **Rena Abulfaz Zinchenko**

Prof. dr. **Volkan Necati Baltaci**

Doctor of biological sciences, professor **Ramiz Taghi Aliyev**

Dissertation council BFD 4.27 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Azerbaijan Medical University

Chairman of the Dissertation council: Corresponding member of ANAS, Doctor of medical sciences, prof. **Elmar Mustafa Qasimov**

Scientific secretary of the Dissertation council: Doctor of philosophy in medicine, ass.prof. **Elnur Elman İbrahimov**

Chairman of the scientific seminar: Doctor of biological sciences, ass. prof. **Gunay Hafiz Akbarova-Ben-Tzvi**

İMZANI TƏSDİQ EDİRƏM
Azərbaycan Tibb Universitetinin
ELMİ KATİBİ
Tibb elmləri doktoru, professor
Nazim Adil oğlu Pənahov
01.07.24

GENERAL CHARACTERISTICS OF THE WORK

Relevance of the problem. Hemoglobinopathies are the most common autosomal recessive single gene disorders in the world.[1] They emerge as result of mutations on genes encoding for globin proteins, which are part of hemoglobin molecule.[2] These mutations can reduce the synthesis of the globin chain or lead to the production of an abnormal chain, depending on mechanisms and location on the relevant genes. Former scenario leads to thalassemia, while the latter is involved in the formation of structural variants of hemoglobin molecule. Because they are inherited in an autosomal recessive manner, a clinically significant phenotype occurs only in homozygous or compound-heterozygous conditions. Heterozygous carriers, on the other hand, are asymptomatic, expressing only minimal changes in hematological parameters which allows them to be identified through laboratory diagnosis.[3]

Thalassemia is a group of heterogeneous diseases that occur as a result of incomplete or reduced synthesis of the corresponding globin chain.[4] Quantitative changes in the synthesis of one chain leads to an imbalance of ratio between the two types of globin chains that make up hemoglobin molecule, which is the basis of the pathophysiological mechanism of thalassemia. Thalassemia is regionally specific, and each population has a characteristic mutation spectrum.[5] Investigation of the spectrum and frequencies of

---

[1] Modell, B., Darlison, M. Global epidemiology of haemoglobin disorders and derived service indicators. // Bulletin of the World Health Organization, – 2008. 86 (6), – p. 480–487.

[2] S Thein, S.L. Molecular basis of β thalassemia and potential therapeutic targets // Blood Cells, Molecules, and Diseases, – 2018. 70, – p. 54–65.

[3] Aliyeva, G., Asadov, C., Mammadova, T., et al. Thalassemia in the laboratory: pearls, pitfalls, and promises // Clinical Chemistry and Laboratory Medicine (CCLM), – 2018. 57 (2), – p. 165–174.

[4] Taher, A.T., Weatherall, D.J., Cappellini, M.D. Thalassaemia // The Lancet, – 2018. 391 (10116), – p. 155–167.

[5] Williams, T.N., Weatherall, D.J. World distribution, population genetics, and health burden of the hemoglobinopathies. // CSH perspectives in medicine, – 2012. 2 (9), – p. a011692.

mutations in populations with high prevalence of thalassemia has scientific and clinical-diagnostic value. Thalassemia has a high molecular heterogeneity, and today more than 500 mutations are known to cause thalassemia.[6] These mutations can have different phenotypic expressions and clinical manifestations depending on their localization, structure and transcription mechanism on the relevant gene.[7] Therefore, the study of mutations characteristic of the local population is very important in the organization of thalassemia control programs in these countries, in the diagnosis of the disease and in the selection of optimal treatment approaches.

Hemoglobin variants are mainly the result of single nucleotide polymorphisms, small insertions, deletions, or, in some cases, the 'fusion genes' that lead to changes in the amino acid sequence of the globin chain. Although the first discovered hemoglobin variants were named in alphabetical order, more than 700 variants are known today, and they are now named after the place where they were first discovered. Although numerous, not all hemoglobin variants are clinically significant, and only if the corresponding mutation affects the function, solubility, stability, or synthesis of the hemoglobin molecule can it cause hematological changes and disease. Hemoglobin variants can be co-inherited together with other variants or with different thalassemic mutations, which leads to high heterogeneity of their phenotypic manifestations and clinical picture. Therefore, the study of genotype-phenotype correlations of variants inherited in compound with heterozygous, homozygous and thalassemic mutations characteristic of the local population has a diagnostic and therapeutic value.

For prevention purposes, state-regulated population screenings are applied in areas with high prevalence of clinically significant

[6] Kountouris, P., Lederer, C.W., Fanis, P., et al. ithaGenes: An Interactive Database for Haemoglobin Variations and Epidemiology // PLoS ONE, – 2014. 9 (7), – p. e103020.

[7] Thein, S.L. The Molecular Basis of β-Thalassemia // Cold Spring Harbor Perspectives in Medicine, – 2013. 3 (5), – p. a011700.

hemoglobinopathies.[8] The target of these screenings are either newborns or potential childbearing couples. While neonatal screening is about early identification of children with thalassemia and providing them with appropriate treatment, screening of couples, on the other hand, is a more radical approach that addresses the problem by preventing the birth of a sick child. Thus, at-risk couples identified as carriers are sent to genetic counseling and prenatal diagnosis in the appropriate week of pregnancy, allowing to determine the genotype of the fetus in the second trimester and termination of pregnancy with the consent of both parents. Such preventive programs have been implemented in Cyprus, Greece, Turkey, Iran and other countries since the 1980s.[9] The number of sick children born in these populations had significantly decreased within 3-5 years of the initiation of the programs. In our country, within the framework of the "State Program on Prevention of Thalassemia for 2015-2020", a country-wide mandatory premarital screening identifies at-risk couples, directing them to appropriate genetic counselling and prenatal diagnosis since 2015. The analysis of the results of the program implemented for more than three years will reveal the efficacy of prenatal diagnostics in the prevention of thalassemia in Azerbaijan.

Object and subject of the study. The study involved 1719 individuals with various types of hemoglobinopathy who were referred the Scientific Research Institute of Hematology and Transfusiology. Participants were divided into control and research groups and further stratified into four subgroups: Group 1: β-thalassemia carriers (n=1413), Group 2: β-thalassemia patients (n=42), Group 3: α-thalassemia patients and carriers (n=96) and Group 4: Individuals with variant hemoglobin fractions detected (n=168). Molecular genetic methods were used to determine relevant

[8] Cao, A., Kan, Y.W. The prevention of thalassemia // Cold Spring Harbor perspectives in medicine, – 2013. 3 (2), – p. a011775.

[9] Angastiniotis, M.A., Hadjiminas, M.G. Pevention of Thalassemia in Cyprus // The Lancet, – 1981. 317 (8216), – p. 369–371.

globin gene mutations in each participant, and the phenotypic manifestations of these mutations were studied.

Aim of the study is to identify mutations leading to thalassemia and other hemoglobinopathies in Azerbaijan and determine their geographical association with different regions of the country, as well as to study the efficacy of prenatal diagnosis in prevention of thalassemia in the country.

Research objectives:

1. Identification of mutation spectrum of β-thalassemia in a group of carriers identified only through population screening in Azerbaijan;
2. Study of geographical and ethnic association of identified β-thalassemia mutations with all regions of Azerbaijan;
3. Identification of mutation spectrum and genotype-phenotype correlation of α-thalassemia in Azerbaijani population;
4. Identification of mutations leading to hemoglobin variants and their genotype-phenotype correlation in Azerbaijani population;
5. Retrospective analysis and evaluation of the efficacy of prenatal diagnosis in the prevention of thalassemia in Azerbaijan.

Research Methods: High-performance liquid chromatography (HPLC) and capillary electrophoresis biochemical methods were employed. DNA extraction was conducted using silica absorption, followed by polymerase chain reaction (PCR), reverse dot-blot hybridization, and sequencing molecular-genetic methods. The collected data were processed using Past3 statistical software. Geographic maps were designed using QGIS 3.2 Bonn cartography software, and illustrations were created using the Inkscape vector graphics editor.

Main findings presented for the defense:

1. Three previously unknown β-thalassemia mutations were identified in the Azerbaijani population (-92 [C>T], Codon 15 [TGG>TAG] and IVS-II-848 [C>A]) and phenotypical expression of Codon 14 [+T], which is unique to the local population, was described for the first time.

2. The mutation spectrum of β-thalassemia carriers, identified only through population screening, included more β^0 mutations with severe phenotypic manifestations comparing to previous groups.
3. The analysis of geographical distribution of β-thalassemia mutations across all regions of the country revealed molecular-genetic differences between the regions.
4. The study of the mutation spectrum of α-thalassemia in the Azerbaijani population revealed 9 mutations with 80% of the spectrum accounting for deletions with milder clinical manifestation.
5. 4 β-globin mutations leading to clinically significant variant hemoglobins were identified in the Azerbaijani population: Codon 6 [A> T] HbS, Codon 121 [G> C] HbD Punjab, Codon 26 [G> A] HbE and Codon 6 [G > A] HbC.
6. Prenatal diagnosis followed by selective abortion have been shown to be efficacious in preventing thalassemia in Azerbaijan.

Originality. To the best of our knowledge, the molecular basis of α-thalassemia, the distribution of β-thalassemic mutations across the country, and the genotype-phenotype correlation of hemoglobin variants of the local population have not been studied before, and the efficacy of prenatal diagnosis has not been analyzed to date. Therefore, the research work is original.

Theoretical and practical value. The results of the study enabled to identify hemoglobinopathy mutations characteristic of the local population and to propose population-specific molecular-genetic diagnostic algorithms. Furthermore, the analysis of geographical distribution of the mutations allowed the development of optimal diagnostic algorithms for each geographical region. The study of phenotypic manifestations of different hemoglobinopathy genotypes will be of great prognostic value in determining further treatment approaches and transfusion regimens for patients with certain genotypes. It was also found that prenatal diagnosis and selective abortion were efficacious preventing clinically significant hemoglobinopathies in Azerbaijan.

Approbation and presentation of the work. The main objectives and results of the dissertation were presented at the following scientific conferences and events:

- "14th International Conference on Thalassaemia and other Haemoglobinopathies" (*Thessaloniki, 2017*)
- "IX International Eurasian Hematology-Oncology Congress" (*Istanbul, 2018*)
- "World Immune Regulation Meeting XII" (*Davos, 2018*)
- "60th American Society of Hematology Annual Meeting" (*San Diego, 2018*)
- "Müasir Biologiyanın Aktual Problemləri elmi-praktik konfransı" (*Bakı, 2019*)
- "International Conference Erythropoiesis Control and Ineffective Erythropoiesis: from bench to bedside" (*Budapest, 2019*)
- "Human Genome and Health 2nd International Conference Translational Medicine in the Era of Omics" (*Tbilisi, 2019*)
- "1-ci Azərbaycan Beynəlxalq Hematologiya Mütəxəssisləri Konqresi" (*Bakı, 2019*)
- "ET Hematologiya və Transfuziologiya İnstitunun 75 İllik Yubileyinə Həsr Olunmuş Hematologiyanın Aktual Problemləri Beynəlxalq Konfransı" (*Bakı, 2019*)
- "24th Congress of European Hematology Association" (*Amsterdam, 2019*)
- "International Society of Laboratory Hematology 2019 Conference" (Vancouver, 2019)
- "8th Sardinian International Summer School - From Genomic discoveries to therapeutic targets" (*Sardinia, 2019*)
- "10th Eurasian Hematology-Oncology Congress" (*Istanbul, 2019*)

The work was presented as a panel speech in 2 of them, 2 oral presentations, and the rest were presented as poster presentations. The initial discussion of the work was held at seminar of the Scientific Council of National Hematology and Transfusion Center. The results and practical recommendations of the work are being implemented at National Hematology and Transfusion Center as well as National Perinatal Center.

Affiliation. The work was performed at the National Hematology and Transfusion Center (*prev.* Scientific-research Institute of Hematology and Transfusiology).

Publications. The results of the work have been summarized in 28 publications: 14 articles and 14 abstracts. 2 articles were published as single-authored, 5 article and 8 abstracts as first-author publications. 19 were published in international journals, of which 11 were in journals with Thomson Reuters impact factor. The author's scientific works in the Scopus and Web of Science indexing systems have been cited hundreds of times, and her current h-index is 5.

The structure and content of the dissertation. Dissertation was compiled on 178 A4 pages (199762 characters), including introduction (6 p., 11166 chac.), literature review (47 p., 75987 chac.) materials and methods (17 p., 23541 chac.), results of the study (42 p., 35093 chac.), conclusion (17 p., 34168 chac.), results and practical recommendations (4 p., 5972 chac.), and a list of abbreviations (2 p., 1245 chac.). It includes 390 literature sources (38 p.) and illustrated with 12 tables and 28 figures.

MATERIALS AND METHODS

The study participants were selected among samples directed to the Laboratory of Hereditary Pathology of Erythron and to the Department of Thalassemia Prevention of the Scientific Research Institute of Hematology and Transfusiology. The study consisted of control and research groups (Fig. 1). The control group consisted of healthy individuals with no hemoglobinopathy, and the study group was stratified into 4 groups (Fig. 1). The inclusion criteria were HbA_2 > 3.4% in the 1st study group of β-thalassemia carriers, HbF > 8% with cooccurring severe anemia in the 2nd study group of β-thalassemia patients, identification of HbH fraction or complex cases of potential α-thalassemia coinheritance were in 3rd study group, and the 4th group included samples in which variant hemoglobin fractions were detected at any level by chromatographic and electrophoretic methods (Fig. 1). Samples of the 1st research group consisted of individuals identified through premarital or premilitary screenings.

During the study period, a total of 1,819 samples were studied and either α or β-globin gene mutations were identified through molecular genetic methods.

Information on the geographical origins of the study participants was obtained and β-thalassemia carriers were stratified into 10 groups based on economic regions of the country - Absheron, Ganja-Gazakh, Sheki-Zagatala, Lankaran, Guba-Khachmaz, Aran, Mountainous Shirvan, Karabakh, Nakhchivan, and Western Azerbaijan (Fig. 2). Both parents of the participant included in the relevant group had to have settled in the region for at least the last two generations. Participants of mixed origin were not included in the geographic study and thus 1071 of the 1413 β-thalassemia carriers were allocated among the geographic association groups of mutations, as shown in Figure 2.

Blood samples containing EDTA (ethylenediaminetetraacetic acid) were taken for analysis, complete blood count and erythrocyte indices were determined using a fully automated hematology analyzer (XT-2000i. Sysmex Corporation, Japan). Hb fractions were evaluated mainly by HPLC (Variant II. Bio-Bio., USA) and in some cases by capillary electrophoresis (CAPILLARYS 2. Sebia, France) and acetate-cellulose electrophoresis (Hospitex, Italy).

DNA extraction was performed via *silica* absorption (ViennaLab Spin Micro DNA Extraction System: REF-2-020; QIAamp DNA Blood Mini Kit: Cat.No.51106), detection of β-globin gene mutations was performed by reverse dot-blot hybridization (RDB) method via β-Globin StripAssay AZE1 and β-Globin StripAssay AZE2 kits designed specifically for Azerbaijani population based on the results of previous studies (REF: 4-170, ViennaLab Diagnostics GmbH. A-globin mutations were detected using the universal α-Globin StripAssay kit (REF: 4-160, ViennaLab Diagnostics GmbH). In samples where it was not possible to detect a corresponding causative mutation with RDB, the complete sequence of the *HBB* gene was determined by the dye terminator cycle sequencing method.

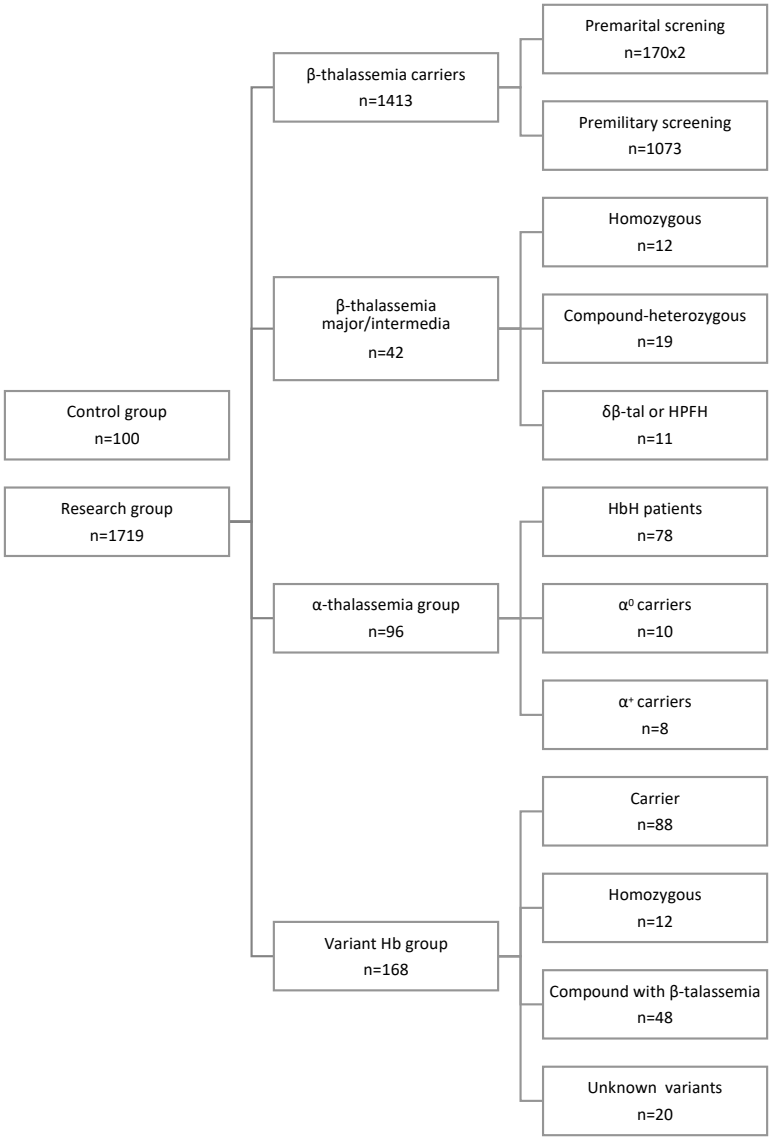


Figure 1. Design of the study

Region	Population mln	Sample size
Absheron	2.81	41
Ganja-Qazakh	1.27	92
Shaki-Zaqatala	0.61	198
Lankaran	0.92	112
Guba-Khachmaz	0.54	55
Aran	1.99	357
Mountanous Shirvan	0.31	84
Karabakh	0.92	73
Nakhchivan	0.45	24
Western Azerb	-	35

Figure 2. Research groups based on the geographical regions of Azerbaijan, corresponding population size and number of samples allocated to the relevant group

The data obtained was analyzed using the Past3 statistical program, geographical maps were generated via the QGIS 3.2 Bonn cartography software, and illustrations were prepared using the Inkscape vector graphics editor. Comparisons of the data from the two groups were analyzed using the Student's T-test, and the data on group phenotypes were described using box-and-whisker diagrams, a nonparametric descriptive statistical method. Data on the association of β-thalassemia mutations with geographical regions were analyzed using a contingency table and Pearson's residuals (ri) were calculated for each cell. Azerbaijan's geospatial vector data was obtained from the DIVA-GIS database in shp format. The citation and list of references was organized via the Mendeley Desktop (Elsevier) citation management program, and for the first time an electronic reference style developed in accordance with the requirements of the AAK was placed in Mendeley's style archive.

RESULTS AND DISCUSSION

Mutation spectrum of β-thalassemia in Azerbaijan. Although β-thalassemia mutations of Azerbaijani population have been examined in previous studies, substantial proportion of the study

groups consisted of β-thalassemia patients. It is possible that the lack of adequate blood supply and chelation therapy for more severe thalassemia genotypes could result resulting in a high mortality rate among young children with severe mutations, and a selective survival of patients with milder phenotypes, thus skewing their results. Therefore, we included only heterozygous carriers, detected through population screening, which is a true representation of the mutation spectrum of the population.

Total of 33 β-thalassemia mutations have been identified in the Azerbaijani population, including the results of 4 previous studies (Table 1). 32 of them were found in our study group, excluding the IVS-I-128 [T>G] β^+ mutation of Saudi origin, identified only by Çürük and colleagues, which was not found in our study group (and in any previous study). The identified mutations had more severe phenotypes, with 80% of the spectrum accounting for β^o mutations. Mutations were highly heterogeneous in terms of molecular mechanisms, including transcriptional mutations, RNA processing mutations, post-transcriptional and translational mutations.

As we considered when designing the study and the inclusion criteria of the research group, compared to previous studies, the fact that our research group consisted only of carriers identified through population screening was reflected in the ratio of mutations with mild and severe phenotypes. Thus, as we have seen in the example of IVS-I-6 [T>C] mutation with a mild β+ phenotype, the results of Çürük, Kuliev and Mammadova accounted for 7.1%, 5.9% and 8.5% of the spectrum, respectively. In our study group, on the other hand, much less, 2.76% was observed. In general, βo mutations accounted for 80.3% of the spectrum in our study group, while the results of Çürük, Tagiev, Kuliev and Mammadova were 63.4%, 75.5%, 67.3% and 53.7%, respectively. Thus, these results indicate that βo mutations with more severe phenotype were observed at a higher rate in our study group, consisted only of carriers detected by population screening. 3 mutations were detected for the first time in the Azerbaijani population: -92 [C> T], IVS-II-848 [C> A], Codon 15 [TGG> TAG].

Table 1. Mutation spectrum of β-thalassemia in Azerbaijan

№	Mutasiya	HGVS Nomenklaturası	Fenotip	Mənşəyi	Bizim Nəticə		Çürük 1992	Tagiev 1993	Kuliev 1994	Məmmədova 201
					N	%				
1	Codon 8 [-AA]	HBB:c.25_26delAA	β⁰	Mediterranian	494	34.96	21.2	32.7	32.6	25.1
2	IVS-II-1 [G>A]	HBB:c.315+1G>A	β⁰	Mediterranian, African-American	231	16.35	21.2	7.5	21.5	10.7
3	IVS-I-110 [G>A]	HBB:c.93-21G>A	**β⁺**	Mediterranian	143	10.12	20.2	4.7	12.6	6.7
4	Codon 8/9 [+G]	HBB:c.27_28insG	β⁰	Asian Indian	69	4.88	2	12.1	4.4	2.5
5	Codon 36/37 [-T]	HBB:c.112delT	β⁰	Kurdish	58	4.10	2	0.9	0.7	1
6	IVS-II-745 [C>G]	HBB:c.316-106C>G	**β⁺**	Mediterranian	50	3.54	3			1
7	Codon 82/83 [-G]	HBB:c.250delG	β⁰	**Azerbaijan**	47	3.33	3	2.8	3.7	
8	IVS-I-6 [T>C]	HBB:c.92+6T>C	**β⁺**	Mediterranian	39	2.76	7.1	0.9	5.9	8.5
9	Codon 44 [-C]	HBB:c.135delC	β⁰	Kurdish	35	2.48	3	2.8		1.5
10	Codon 39 [CAG>TAG]	HBB:c.118C>T	β⁰	Mediterranian	34	2.41	2	3.7		2.7
11	IVS-I-5 [G>C]	HBB:c.92+5G>C	β⁰	Asian Indian, SE Asian	34	2.41	1	2.8	1.5	1.7
12	Codon 5 [-CT]	HBB:c.17_18delCT	β⁰	Mediterranian	25	1.77			0.7	1.7
13	IVS-I-1 [G>A]	HBB:c.92+1G>A	β⁰	Mediterranian	24	1.70	2	4.7		1.7
14	Codon 16 [-C]	HBB:c.51delC	β⁰	Asian Indian	20	1.42	2			1.2
15	Codon 15 [TGG>TGA]	HBB:c.48G>A	β⁰	Asian Indian	16	1.42		2.8	2.2	0.5
16	-30 [T>A]	HBB:c.-80T>A	**β⁺**	Mediterranian, Bulgarian	14	0.99	2		1.5	1.2
17	Codon 41/42 [-TTCT]	HBB:c.124_127delTTCT	β⁰	Chinese, SE Asian, Indian	12	0.85				0.2

Table 1. continued

18	5'UTR +22 [G>A]	HBB:c.-29G>A	β^{++}	Mediterranian, Turkish	11	0.78	1		1.5	
19	-28 [A>C]	HBB:c.-78A>C	β^{+}	Kurdish	10	0.71	1		0.7	
20	IVS-I-5 [G>T]	HBB:c.92+5G>T	β^{+}	Mediterranian	10	0.71	1			
21	Codon 29 [C>T]	HBB:c.90C>T	β^{o}	Lebanese	8	0.57	3			
22	Codon 22 [-7bp del]	HBB:c.68_74delAAGTTGG	β^{o}	Turkish	6	0.42		0.9		0.5
23	IVS-I-130 [G>C]	HBB:c.93-1G>C	β^{o}	Italian, UAE, Japanese	5	0.35				1
24	Codon 30 [G>C]	HBB:c.92G>C	β^{o}	Mediterranian, Kurdish	5	0.35	1	0.9		0.5
25	Codon 37 [TGG>TGA]	HBB:c.114G>A	β^{o}	Saudi Arabian	2	0.14				0.7
26	-101 [C>T]	HBB:c.-151C>T	β^{++}	Mediterranian	2	0.14				0.5
27	IVS-I [25bp del]	HBB:c.93-21_96del	β^{o}	Asian Indian, UAE	2	0.14				0.5
28	Codon 14 [+T]	HBB:c.44_45insT	β^{o}	**Azerbaijan**	2	0.14		0.9		
29	Codon 15 [TGG>TAG]*	HBB:c.47G>A	β^{o}	Asian Indian	2	0.14				
30	IVS-II-848 [C>A]*	HBB:c.316-3C>A	β^{+}	Iran, Egypt	1	0.07				
31	-92 [C>T]*	HBB:c.-142C>T	β^{++}	Mediterranian	1	0.07				
32	-88 [C>A]	HBB:c.-138C>A	β^{+}	Kurdish	1	0.07			1.5	
33	IVS-I-128 [T>G]	HBB:c.93-3T>G	β^{+}	Saudi Arabian			1			
	Total xromosom (N)				**1413**		**99**	**107**	**135**	**402**
	Heteroziqotlar (N)				**1413**		**66**	**0**	**41**	**142**
	Total mutasiyalar (N)				**32**		**20**	**15**	**14**	**22**

In addition, the Codon 14 [+T] mutation, previously detected by Tagiev et al on only one chromosome and then not described in the literature, was detected in our research group in homozygous and heterozygous forms which was described together with phenotypic features.

Geographical distribution and ethnic association of β-thalassemia mutations in Azerbaijan. Although Codon 8 [-AA] was the most common mutation in the entire population, accounting for 35% of the spectrum, it is evenly distributed among other regions except Sheki-Zagatala, and its frequency does not exceed that of the general population (Fig. 3). The highest association of mutations was observed between Codon 8 [-AA] and Sheki-Zagatala, and between Codon 5 [-CT] and Mountainous Shirvan regions (ri>6.00). The largest difference from the expected results (*ie* the general population spectrum) was observed in the Lankaran region, with statistically significant association of IVS-II-1 [G>A], Codon 36/37 [-T], Codon 14 [+T], Codon 5 [-CT], -88 [C>A], -92 [C>T], and Codon 44 [-C] (ri > 2.00).

The identified mutations were grouped according to their well-known ethnic origins and the ethnic composition of the local population within the regions was studied. The mutations were distributed to 5 groups of Mediterranean and Turkish, Caucasian and European, Asian, Arab, and Kurdish origin, and their distribution was studied in 10 regions (Fig. 4). Although the vast majority of mutations found in the local population are of Mediterranean and Turkish origin, these mutations were observed more frequently in the northern regions of the country (Sheki-Zagatala - 0.924; Guba-Khachmaz - 0.872). The relatively low frequency of mutations of Mediterranean and Turkish origin was found in Karabakh (0.643) and Mountainous Shirvan (0.666) regions, and between 0.714–0.829 in Lankaran, Aran, Ganja-Gazakh, Nakhchivan and Absheron, respectively. The results revealed a high heterogeneity of the ethnic composition of the local population, reflecting the historical assimilation and interbreeding of a number of nations.

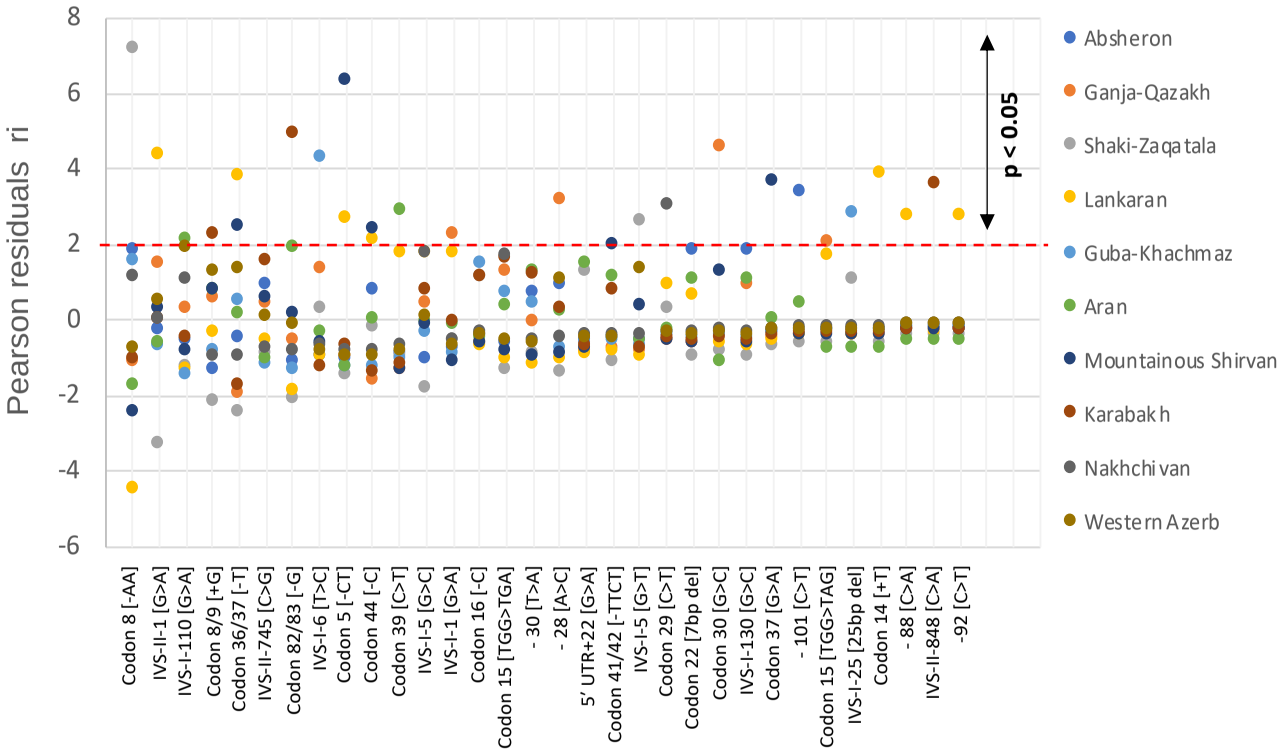
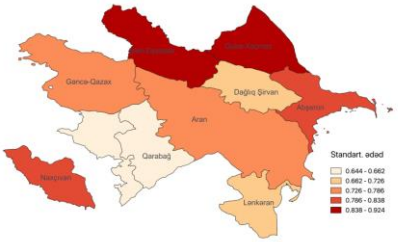
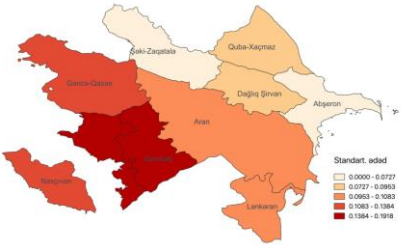


Figure 3. Association of β-thalassemia mutations with regions in Azerbaijan. Associations were analyzed through generation of a contingency table, and calculation of Pearson residuals (ri). Association of above 2.00 was considered as statistically significant ($p < 0.05$)



Mediterranean and turkish

European and Caucasus



Asian

Arab

Kurdish

Western Azerbaijanians	
Mediterranian	0.742
European	0.028
Asian	0.114
Arab	0
Kurdish	0.114

Figure 4. Choropleth maps illustrating distribution of mutations with different ethnic origins. Data of the maps represents standardized values scaled by population data.

Genotype-phenotype correlation of β-thalassemia mutations. There was a statistically significant difference from the control group in almost all phenotypic indicators of all other mutations except promoter mutations -30 [T>A], -28 [A>C], 5′ UTR + 22 [G>A], -88 [C>A], -92 [C>T], -101 [C>T] and consensus splays site mutations IVS-I-6 [T>C] and IVS-II-848 [C>A] ($p < 0.05$). It is known that these mutations are classified as β^{+} or β^{++} and usually have a very mild phenotypic manifestation. However, although the HbA_2 values of these mutations were lower than those of β^{o} mutations, they were significantly higher compared to the control group ($p < 0.05$).

Clinical manifestation in β-thalassemia patients. The study also identified the genotypes of a total of 31 β-thalassemia patients, including 22 thalassemia major and 9 thalassemia intermedia, and analyzed them in relation to their phenotypic characteristics. Total Hb levels were <7 g/dL in primary patients with $\beta^{IVS\text{-}I\text{-}6\ [T>C]}/\beta^{IVS\text{-}I\text{-}6\ [T>C]}$ and $\beta^{codon8\ [-AA]}/\beta^{IVS\text{-}II\text{-}1\ [G>A]}$ genotype, and was in the 7-9 g/dL range in patients with $\beta^{codon8\ [-AA]}/\beta^{IVS\text{-}I\text{-}110\ [G>C]}$ and $\beta^{codon\ 36/37\ [-T]}/\beta^{codon\ 36/37\ [-T]}$ genotypes.

Other cases associated with high HbF levels. The study also examined the *HBB* genes of 11 patients with high HbF levels (>8%) with absence of cooccurring profound anemia by the RDB method and found no mutations in the corresponding gene. In 2 of these patients, HbF ranged from 8 to 10%, and in 8 from 10 to 25%. All patients in the corresponding group had a total Hb >11 g/dL and no abnormal hemoglobin fraction was detected. Patients' total Hb levels not characteristic of anemia and normal HbA_2 values, ruled out the possibility that they were carriers or sick with thalassemia. The corresponding phenotype was found to be characteristic of δβ-thalassemia or hereditary persistence of fetal hemoglobin (HPFH).

Mutation spectrum and genotype-phenotype correlation of α-thalassemia in Azerbaijan. Examination of a total of 178 chromosomes included in the α-thalassemia group revealed 9 mutations (Table 2). The most common mutations were 20.5 kb double gene deletion of Mediterranean origin and 3.7 kb single gene deletion, observed worldwide.

Table 2. Mutation spectrum of α-talassemia in Azerbaijan

	Mutations	HGVS classification	Origin	n	%
1	**20.5 kb DEL**	NG_000006.1:g.15164_37864del22701	Mediterranian	70	39.5
2	**3.7 kb DEL**	NG_000006.1:g.34164_37967del3804	Worldwide	61	34.4
3	**α2 Poly A-1 [AATAAA>AATAAG]**	HBA2:c.*94A>G	Arab, Middle East, Mediterranean	12	6.7
4	**α2 IVS-I 5nt DEL**	HBA2:c.95+2_95+6delTGAGG	Mediterranean, Middle East	9	5.0
5	**MED DEL**	NG_000006.1:g.24664_41064del16401	Mediterranian	7	3.9
6	**α2 Poly A-2 [AATAAA>AATGAA]**	HBA2:c.*92A>G	Mediterranean, Turkish, Cypriot, Arab, Iranian	7	3.9
7	**α2 Codon 142 [T>C]**	HBA2:c.427T>C	Southeast Asian	6	3.3
8	**4.2 kb DEL**	N/A	Worldwide	4	2.2
9	**α2 Codon 19 [-G]**	HBA2:c.56delG	Iranian	1	0.5
	Total			177	

Together, these two mutations accounted for 74% of the spectrum. The mutations were mainly of Mediterranean, Middle Eastern, Arab, Iranian, Turkish and Southeast Asian origin. 9 different genotypes were observed among HbH patients. The most common genotype was $\alpha^{-3.7}/\!--^{20.5}$ and was found in 42 patients. In general, although point mutations (especially Hb Constant Spring, α2 Codon 142 [T>C]) associated with severe anemia and clinical course than deletions, such mutations are relatively rare in the local population, often coinherited with deletions. The most common point mutations – polyadenylation mutations (α2 Poly A-1, α2 Poly A-2) were associated with mild phenotypes due to their location on the gene. Moreover, coinheritance of *HBA* and *HBB* gene mutations was detected in 6 patients, indicating such inheritance patterns to complicate the diagnosis by changing the characteristic clinical course of the disease.

Variant hemoglobins and their genotypes in Azerbaijan. Examination of a total of 165 chromosomes included in the corresponding group revealed 4 variants of hemoglobin mutations -

Table 3. Variant hemoglobin mutations in Azerbaijan

Mutastions	HGVS classification	N
Codon 6 [A>T] *HbS*	HBB:c.20A>T	118
Codon 121 [G>C] *HbD*	HBB:c.364G>C	28
Codon 26 [G>A] *HbE*	HBB:c.79G>A	17
Codon 6 [G>A] *HbC*	HBB:c.19G>A	2
		165

Codon 6 [A>T] HbS, Codon 121 [G>C] HbD Punjab, Codon 26 [G>A] HbE and Codon 6 [G>A] HbC (Table 3). As expected, variant hemoglobins were commonly observed to be coinherited with β-thalassemia mutations than in homozygous form. In addition, the molecular-genetic methods of the study were not able to identify the causative mutation in 20 samples, where variant hemoglobin fraction detected through biochemical methods. According to their chromatographic patterns, these unknown variants were presumed to be Hb Lepore, D-Iran, J, Russ and O-Arab.

Thalassemia prevention program for 2015-2019

Analysis of the efficacy of prenatal diagnosis applied within the thalassemia prevention program in 2015-2019. From June 2015 to March 2019, 170 couples were sent to genetic counseling followed by identification of their genotypes. Molecular genetic analysis of 115 fetal samples obtained by transabdominal amniocentesis in these couples revealed the genotypes of the fetuses. 20 prenatal diagnoses were performed in 2016, 24 in 2017, 57 in 2018, and 14 by the end of March 2019. Of these, prenatal diagnosis was applied to two consecutive pregnancies in 9 couples and to 4 consecutive pregnancies in 1. There were 1 monozygotic twin (healthy), 1 dizygotic twin (1 affected fetus) and 1 triple pregnancy (2 affected fetuses). 29% of couples were in consanguineous marriage.

Most of the consanguineous marriages were observed in Aran (40.7%), Lankaran (14.8%), Sheki-Zagatala (14.8%) and Absheron (11.1%) regions. No consanguinity observed in Khachmaz and Nakhchivan. Of the 115 fetal samples examined, 61 were identified as heterozygous, 28 as homozygous or compound-heterozygous, and 26 fetuses were unaffected. With the exception of multiple pregnancies, all affected pregnancies were terminated by selective abortion in accordance with the legislation of the Republic of Azerbaijan. Premarital screening, prenatal diagnosis and termination of affected pregnancies under the State Program on Prevention of Thalassemia were observed to be efficacious, with cooccurring decline in incidence rate of thalassemia since 2014. The total number of patients with thalassemia major and intermediate per 100,000 population of Azerbaijan was 1.76 in 2014, 1.11 in 2017, and 0.89 in 2018. In general, the incidence rate decreased significantly since the initiation of the program (IRR = 0.6285, $p < 0.005$, 95% CI [0.4928, 0.7993]; PF = 18.92%).

CONCLUSION

1. For the first time in Azerbaijan, a mutation spectrum of β-thalassemia was determined in a group consisted of only carriers identified through population screening, and a total of 32 mutations were identified, 3 of which were novel in the local population (-92 [C>T], Codon 15 [TGG>TAG] and IVS-II-848 [C>A]) and the phenotypic manifestation of the Codon 14 [+T] mutation found only in Azerbaijan were described.
2. The geographical distribution of β-thalassemic mutations among the regions of the country was studied for the first time and the association of Codon 8 [-AA] mutation with Sheki-Zagatala and strong association between Codon 5 [-CT] mutation and mountainous Shirvan regions ($ri > 6.00$, $p < 0.05$) was determined. Lankaran region was observed to have the most unique mutation spectrum, (Codon 36/37 [-T], -88 [C>A], and Codon 44 [-C]; $ri > 2.00$, $p < 0.05$). There were statistically significant associations between Karabakh region and Codon 8/9 [+G] mutation, Guba-

Khachmaz with IVS-I-6 [T>C], Aran with Codon 39 [C>T], and the Mountainous Shirvan region with Codon 36/37 [-T] and Codon 44 [-C] mutations (ri>2.00, p <0.05).

3. The vast majority of the spectrum of β-thalassemic mutations is composed of mutations of Mediterranean and Turkish origin. The mutations of Asian origin were observed in the western regions of the country, Caucasian and European mutations in the western, northwestern and central regions, and mutations of Arab origin in central and southern regions. Mutations of Kurdish origin, on the other hand, were mainly observed in Nakhchivan and in the eastern regions of the country.
4. The mutation spectrum of α-thalassemia in Azerbaijan was studied for the first time and only 9 mutations were detected in the local population. The most common α-thalassemic mutations were the 20.5 kb dual gene deletion of Mediterranean origin and the 3.7 kb single gene deletion, which is observed worldwide, and together they accounted for 74% of the spectrum. The mutations were mainly of Mediterranean, Middle Eastern, Arab, Iranian, Turkish and Southeast Asian origin.
5. It was determined that 80% of the mutation spectrum of β-thalassemia in the Azerbaijani population consists of β^o mutations with severe phenotypes, 80% of the mutation spectrum of α-thalassemia, on the other hand, consists of deletions with milder phenotypes.
6. 4 variants of hemoglobin mutations were identified on a total of 165 chromosomes with a variant hemoglobin fraction: Codon 6 [A>T] HbS, Codon 121 [G>C] HbD Punjab, Codon 26 [G>A] HbE and Codon 6 [G>A] HbC. The last three mutations were detected for the first time, furthermore, we identified 6 unknown hemoglobin variants in the local population. The inheritance of variant hemoglobins was predominantly observed together with β and α-thalassemias rather than in homozygous form.
7. Retrospective analysis of the results of prenatal diagnosis conducted for the prevention of thalassemia determined that 29% of 170 couples who were directed to genetic counselling were in

consanguineous marriages. From total 115 examined fetal samples, 61 were identified as heterozygous, 28 as homozygous or compound-heterozygous, and 26 fetuses were unaffected. With the exception of multiple pregnancies, all affected pregnancies were terminated by selective abortion, leading to a significant reduction in the incidence rate of β-thalassemia in Azerbaijan (IRR = 0.6285, p <0.005, 95% CI [0.4928, 0.7993]; PF = 18.92%).

PRACTICAL RECCOMMENDATIONS

1. Since the mutation spectrum of β-thalassemia carriers detected through population screening is more reliable than previous studies of local population, it is recommended for laboratories conducting molecular genetic diagnosis of β-thalassemia in Azerbaijan to design an algorithm based on the results of current research.
2. Although the mutation spectrum of β-thalassemia in the local population is broad and consists of more than 30 mutations, the 5 most common mutations (Codon 8 [-AA], IVS-II-1 [G>A], IVS-I-110 [G>A], Codon 8/9 [+G], Codon 36/37 [-T]) together account for 70% of the spectrum. Therefore, multiplex screening of these mutations at the initial stage of molecular genetic analysis of β-thalassemia followed by sequence analysis of the *HBB* gene if no mutation is detected could save time and resources.
3. It is recommended to design first-line multiplex test composition in regional laboratories on the basis of the characteristic mutation spectrum of the corresponding region and to perform the sequence analysis of the *HBB* gene in the next stage in the central reference laboratory of the country.
4. Taking into account the mutation spectrum of β-thalassemia of the local population, the sequence analysis of the *HBB* gene is recommended to cover the -101 promoter region, untranslated region (5'UTR), first, second and third exons, and splice junctions including 110th position of intron 1 and 745th position of intron 2.

5. The most prevalent α-thalassemia mutations in the local population are deletions, with 4 of them together (20.5kb, 3.7kb, MED, 4.2kb) accounting for 80% of the spectrum. Therefore, in the initial stage of molecular genetic analysis of α-thalassemia, it is recommended to screen for these mutations by GAP-PCR method and, if none of these 4 mutations are detected, to sequence the *HBA1* and *HBA2* genes and/or examine the presence of large deletions by MLPA method.
6. It is recommended to perform a multiplex PCR of the most common variant mutations of the local population (Codon 6 [A>T] HbS, Codon 121 [G> C] HbD Punjab, Codon 26 [G>A] HbE and Codon 6 [G>A] HbC) as first-line clinically significant hemoglobin variants.
7. When determining the treatment approaches and transfusion regimens of relevant patients, clinical hematologists are recommended to take into account the fact that the vast majority of β-thalassemia mutations in the local population are severe and most of α-thalassemia mutations, on the other hand, are mild. In patients with unclear diagnostic criterias, molecular genetic analysis of the relevant genes should be performed to rule out the coinheritance of α and β-thalassemia.
8. Taking into account the efficacy of prenatal diagnosis applied within the prevention program in the country so far, it is recommended to continue the relevant practice in the following years.
9. Given the prevalence of double gene deletions of α-thalassemia in the local population, it is recommended to perform genetic counselling and molecular-genetic testing of α-thalassemia in couples with history of recurring hydrops fetalis as well as in those with one partner having HbH disease, followed by prenatal diagnosis and early termination of a fetus with Hb Bart's syndrome which could prevent related pregnancy complications.

LIST OF PUBLICATIONS ON THE SUBJECT OF THE DISSERTATION

1. **Aliyeva, G.,** Abdulalimov, E., Asadov, C., et al. First report of thalassemia intermedia in a patient compound-heterozygous for -92 [C>T] and codon 36/37 [-T] mutations // Hemoglobin, – 2020. 45(6), – p. 347-348.
   PubMed ID: 29893155; ***Impact Factor: 0.462***
2. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. Molecular and geographical heterogeneity of hemoglobinopathy mutations in Azerbaijanian populations // Annals of Human Genetics, – 2020. 84 (3), – p. 249–258.
   PubMed ID: 31755088; ***Impact Factor: 1.319***
3. **Əliyeva, G.Z.** Azərbaycanda Alfa Talassemiyanın Mutasiya Spektri və Genotip-Fenotip Korrelyasiyası // Azərbaycan Tibb Jurnalı, – 2019. 3, – s. 104–110.
4. **Əliyeva, G.Z.** Hemoqlobinopatiya və Onun Öyrənilməsinin Tarixi İnkişafı // Sağlamlıq, – 2019. 24 (5), – s. 44–50.
5. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. Spectrum and Geographical Distribution of β-Thalassemia Mutations in Azerbaijan // Leukemia Research, – 2019. 85 (Suppl 1), – p. S24.
   Impact Factor: 2.066
6. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. Geographical Distribution of β-Thalassemia Mutations in Azerbaijan // 8th Sardinian International Summer School 'From Genomic discoveries to therapeutic targets,' – Pula, Itally: – 2019. – p. 8.
7. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. Prenatal Diagnosis and Selective Abortion in Thalassaemia Prevention: Experience of Azerbaijan // International Journal of Laboratory Hematology, – 2019. 41 (Suppl 2), – p. 11.
   Impact Factor: 2.073
8. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. Large-Scale Genotyping of Hemoglobinopathies in Azerbaijan // International Journal of Laboratory Hematology, – 2019. 41 (Suppl 2), – p. 11.
   Impact Factor: 2.073

9. **Əliyeva, G.Z.,** Əsədov, Ç.D., Məmmədova, T.M., et al. Azərbaycanda Talassemiyanin Prenatal Diaqnostikası // Azərbaycan Pediatriya və Perinatologiya Jurnalı, – 2019. 5 (1), – s. 35–41.
10. Asadov, C., **Aliyeva, G.,** Mikayilzadeh, A., et al. Phenotypes and Genotypes of Hemoglobinopathies in Azerbaijan: PB2405 // Hemasphere, – 2019. 3 (S1), – p. 1069.
11. **Əliyeva, G.Z.,** Əsədov, Ç., Məmmədova, T., et al. Azərbaycanda Talassemiya və Digər Hemoqlobinopatiyalara Səbəb Olan Mutasiyaların Molekulyar, Etnik və Coğrafi Heterogenliyinin Tədqiqi // ET Hematologiya Transfuziologiya İnstitunun 75 İllik Yubileyinə Həsr Olunmuş "Hematologiyanın Aktual Problemləri" Beynəlxalq Konfransının Materialları, – Bakı, Azərbaycan: – 2019. – s. 215–231.
12. Əlimirzəyeva, Z.X., Əsədov, Ç.D., Məmmədova, T.M., **Əliyeva, G.Z.** Aralıq Talassemiya: Patofiziologiyadan Müalicəyə Qədər Panoram Baxış // ET Hematologiya Transfuziologiya İnstitunun 75 İllik Yubileyinə Həsr Olunmuş "Hematologiyanın Aktual Problemləri" Beynəlxalq Konfransının Materialları, – Bakı, Azərbaycan: – 2019. – s. 181–193.
13. Məmmədova, T.M., Əsədov, Ç.D., Abduləlimov, E., **Əliyeva, G.Z.,** et al. Azərbaycanda Hemoqlobinopatiyalar // ET Hematologiya Transfuziologiya İnstitunun 75 İllik Yubileyinə Həsr Olunmuş "Hematologiyanın Aktual Problemləri" Beynəlxalq Konfransının Materialları, – Bakı, Azərbaycan: – 2019. – s. 194–202.
14. Абдулалимов, Э., **Алиева, Г.** Спектр Наиболее Распространённых Мутаций в Азербайджане и их Фенотипическое Проявление // ET Hematologiya Transfuziologiya İnstitunun 75 İllik Yubileyinə Həsr Olunmuş "Hematologiyanın Aktual Problemləri" Beynəlxalq Konfransının Materialları, – Bakı, Azərbaycan: – 2019. – с. 235.
15. Farzaliyeva, G., **Aliyeva, G.,** Asadov, C., et al. Phenotypic manifestation of the coinheritance of α and β globin gene mutations // Human Genome and Health 2nd International

Conference Translational Medicine in the Era of Omics, – Tbilisi, Georgia: – 2019. – p. 25.

16. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. Genotype-Phenotype Correlation of S, D and E Variants Coinherited with Thalassemia Mutations // International Conference Erythropoiesis Control and Ineffective Erythropoiesis: from bench to bedside, – Budapest, Hungary: – 2019. – p. 27.
17. Fərzəliyeva, G., **Əliyeva, G.,** Rəsulova, M., et al. İnsan alfa (HBA1, HBA2) və beta (HBB) qlobin gen mutasiyalarının yanaşı ötürülməsi hallarında fenotipik təzahür // "Müasir Biologiyanın Aktual Problemləri" mövzusunda elmi-praktiki konfransın materialları, – Bakı, Azərbaycan: – 2019. – s. 26–27.
18. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. Thalassemia in the laboratory: pearls, pitfalls, and promises // Clinical Chemistry and Laboratory Medicine (CCLM), – 2018. 57 (2), – p. 165–174. *PubMed ID: 30138112;* ***Impact Factor: 3.556***
19. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. First Report of α-Thalassemia Genotypes from Transcaucasia // Blood, – 2018. 132 (Suppl 1), – p. 4899.
    Impact Factor: 15.132
20. Asadov, C., **Aliyeva, G.,** Mikayilzadeh, A., et al. Thalassemia prevention in Azerbaijan: what have we achieved so far? // Leukemia Research, – 2018. 73 (S1), – p. S70.
    Impact Factor: 2.319
21. **Aliyeva, G.,** Asadov, C., Mammadova, T., et al. Codon 14 (+T) (HBB: c.44_45insT): a Rare β-Thalassemia Mutation Reported Only in Azerbaijan // Hemoglobin, – 2018. 42 (4), – p. 276–277. *PubMed ID: 30422720;* ***Impact Factor: 0.462***
22. Asadov, C., Alimirzoeva, Z., Mammadova, T., **Aliyeva, G.,** et al. β-Thalassemia intermedia: a comprehensive overview and novel approaches // International Journal of Hematology, – 2018. 108 (1), – p. 5–21.
    PubMed ID: 29380178; ***Impact Factor: 1.942***
23. Nasrullayeva, G., Ibrahimova, S., **Aliyeva, G.,** et al. Identification of Louis-Bar syndrome in a family with beta-

thalassemia // Abstract Book of World Immune Regulation Meeting XII, – Davos, Switzerland: – 2018. – p. 85–86.
24. Asadov, C., Alimirzoeva, Z., Mammadova, T., **Aliyeva, G.,** et al. Thalassemia prevention program in Azerbaijan: preliminary report // 14th International Conference on Thalassemia and other Haemoglobinopathies, – Thessaloniki: – 2017. – p. 140.
25. Asadov, C., Gafarova, S., **Aliyeva, G.** Bone Abnormalities in β-Thalassemia Intermedia // Blood Research & Transfusion Journal, – 2017. OABTJ.MS.I, – p. 555551.
26. Asadov, C., Abdulalimov, E., Mammadova, T., **Aliyeva, G.,** et al. Genotype-Phenotype Correlations of β-Thalassemia Mutations in an Azerbaijani Population. // Turkish journal of haematology: official journal of Turkish Society of Haematology, – 2017. 34 (3), – p. 258–263.
PubMed ID: 28120779; ***Impact Factor: 0.650***
27. Асадов, Ч., Мамедова, Т., Абдулалимов, Э., **Алиева Г.,** et al. Фенотипические проявления талассемических мутаций в Азербайджане // Georgian medical news, – 2016. 12 (261), – с. 74–80.
PubMed ID: 28132047
28. **Aliyeva, G.,** Shahverdiyeva, I. Application of Genetic Methods in Prenatal Diagnosis of Thalassemias // Abstract Book of International Youth Forum on "Integration Processes of the World Science in the 21st Century," – Ganja, Azerbaijan: – 2016. – p. 191–192.

The defense will be held on "30" September 2024 at 16.00 at the meeting of the Dissertation Council BFD 4.27 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Azerbaijan Medical University.

Address: AZ1022, Baku city, A. Gasimzade str.14 (conference hall)

Dissertation is accessible at the Library of Azerbaijan operating at Azerbaijan Medical University.

Electronic versions of the dissertation and its abstract are available on the official website of Azerbaijan Medical University (www.amu.edu.az).

Abstract was sent to the required addresses on "01" July 2024.

Signed for print: 21.06.2024

Paper format: A5

Volume: 37760

Number of hard copies: 20