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ETHNO-TERRITORIAL DISTRIBUTION OF THE C174M AND C235T POLYMORPHISMS OF THE AGT GENE AND C677T OF MTGFR GENE IN THE POPULATION OF THE AZERBAIJAN REPUBLIC

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ЭТНО-ТЕРРИТОРИАЛЬНОЕ РАСПРЕДЕЛЕНИЕ МУТАЦИЙ C174M И C235T ГЕНА AGT И C677T ГЕНА MTGFR СРЕДИ НАСЕЛЕНИЯ АЗЕРБАЙДЖАНСКОЙ РЕСПУБЛИКИ

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Abstract. The prevention of hereditary diseases associated with gene and chromosomal disorders, in particular multifactorial-polygenic diseases is one of actual areas of medical genotyping. For the first time in the population of the Republic of Azerbaijan we have identified mutations C174T and C235T of the angiotensinogen gene and mutation C677T of the methylenetetrahydrofolate reductase gene both in the control group and among patients with diseases of the cardiovascular system. Reliable connections for the frequency of occurrence of polymorphism of the C174T and C235T alleles of the angiotensinogen gene and polymorphism of the C677T allele of the methylenetetrahydrofolate reductase gene were found with a statistical method. To identify the ethno-geographic relationship of the mutations C174T and C235T of the AGT gene for the population of the Azerbaijan Republic, we examined practically healthy individuals and patients with CVD. The composition of this group was multinational and corresponded to the main national and ethnic composition of the Republic. The distribution of the identified mutations C174T and C235T of the AGT gene, as well as the C677T polymorphism of the MTHFR gene among ethnic groups of the Azerbaijan Republic is identified as uneven.

Аннотация. Актуальные направления медицинского генотипирования заключается в профилактике наследственных заболеваний связанных с генными и хромосомными нарушениями, в частности, мультифактериальными-полигенными заболеваниями. Впервые нами у населения Азербайджанской Республики идентифицированы мутации C174T и C235T гена ангиотензиногена и мутация C677T гена метилентетрагидрофолатредуктазы как в контрольной группе, так и среди больных с заболеваниями сердечно-сосудистой системы. Обнаружена статистическим методом достоверная связь частоты встречаемости полиморфизма аллелей C174T и C235T гена ангиотензиногена и полиморфизма аллеля C677T гена метилентетрагидрофолатредуктазы. Для выявления этногеографической связи мутаций C174T и C235T гена AGT для населения Азербайджанской Республики нами обследованы практически здоровые лица и больные имеющие сердечно-сосудистые заболевания. Состав данной группы была многонациональной и соответствовало основному национальному и этническому составу Республики. Распределение идентифицированных мутаций C174T и C235T гена AGT, а также полиморфизма C677T гена MTHFR по этническим группам населения Азербайджанской Республики показал неравномерное их распространение.

Т. 7. №5. 2021
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Keywords: genetic polymorphism, angiotensinogen, methylenetetrahydrofolate reductase, polymerase chain reaction, cardiovascular diseases.

Ключевые слова: генетический полиморфизм, ангиотензиноген, метилентетрагидрофолатредуктаза, полимеразно-цепная реакция, сердечно-сосудистые заболевания.

Literature analysis shows that there is a difference in the frequency of C174T and C235T polymorphisms of the AGT gene not only racial (European ethnicity, Mongoloids and Negroids), but also ethnic, as well as the geographical difference. It was established not only the interethnic difference among the people living in the same regions and settlements, but also the difference among the same nationality living in different regions of the same country. Consequently, ethnogeographic features of their distribution in human populations were revealed for C174T and C235T mutations of the AGT gene [1].

We have examined practically healthy individuals and patients with CVD in order to identify ethnogeographic relationship of C174T and C235T mutations of the AGT gene for the population of the Republic of Azerbaijan.

Consequently, we organized two groups: the 1st group is practically healthy individuals of 20 to 52 years old. These individuals have been selected empirically and had no relationship with each other. The composition of this group was multinational and corresponded to the main national and ethnic composition of the Republic. They were Azerbaijanis — 58 people (53.7%), Lezghins — 21 people (19.4%), Russians — 9 people (8.3%), Talyshs — 12 people (11.1%) and other nationalities — 8 people (7.4 %). Totally, 108 people (60 men and 48 women).

Ethnogeographic reference. Lezghins is an ethnic group living mainly in the northern regions of the Republic, particularly in Gusar region. Talyshs live in the South-East part — Lankaran-Astara region of the Republic [2]. Examined Russians live mainly in Baku city. Due to the small number of Jews, Tatars, Georgians and Chechens, they were united into a group called others (Figure 1). Examined Azerbaijanis were originally from all regions of the Republic, including Baku city.

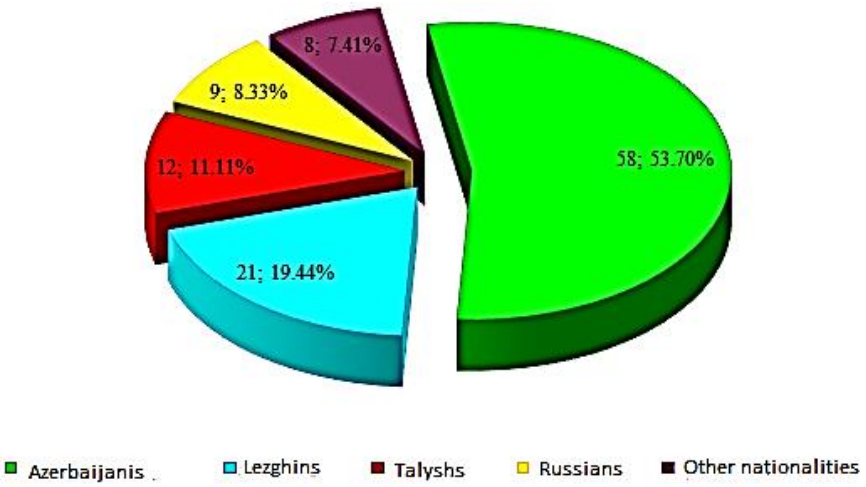


Figure.1. The percentage of national and ethnic groups included in the control group

As it could be seen from the figure, 53.7% of all people examined, included in the control group, are Azerbaijanis from various regions of the Republic, including Baku. Lezghins, Talyshs and Russians were 19.44%, 11.11% and 8.33%, respectively. 7.41% of the total number of

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66

examined is accounted for the share of other nationalities and ethnic groups living in the Republic (Georgians, Avars, Poles, etc.).

The 2nd group comprised the patients with CVD. 72 people (39 men, 33 women) at the age of up to 18 years old were examined in this group. The ethnic origin was as follows: Azerbaijanis — 43 people (59.7%), Lezghins — 8 people (11.1%), Russians — 6 people (8.3%), Talyshs — 9 people (12.5%) and other nationalities — 6 people (8.3%).

National and ethnic origin of the patients with CVD is given in the Figure 2.

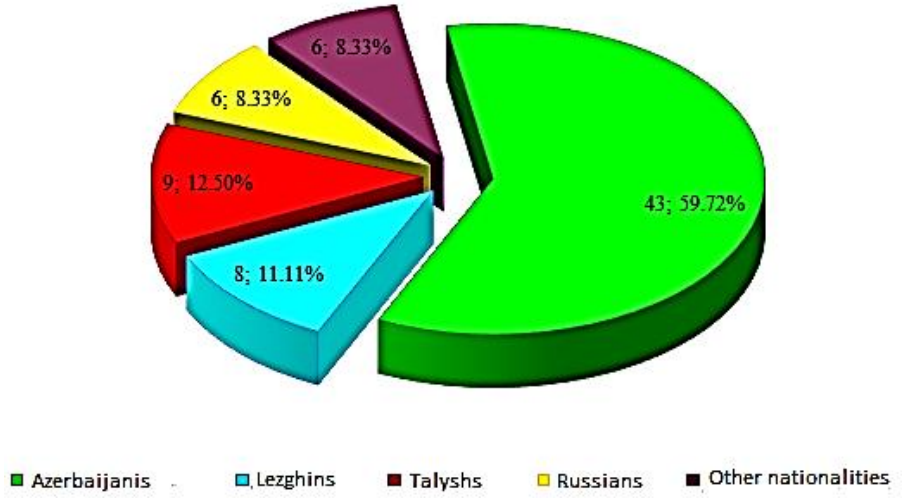


Figure 2. Percentage of national and ethnic affiliations of people with CVD

More than a half of examined (59.72%) in the group of patients with CVD as well as in the control group were Azerbaijanis from various regions of the Republic, including Baku city. Lezghins, Talyshs and Russians were 11.11%, 12.5% and 8.33%, respectively. 8.33% of those examined were of other nationalities and ethnic origins living in the Republic.

In our studies, we distributed the results of genetic analysis for C174T mutations in both groups: in the control group and in the group of patients with CVD. In the control group, 18 individuals were found to have the C174T mutation in a heterozygous state; among representatives of various ethnic groups, the following quantitative distribution was observed: in 9 cases out of 18 (50%) — among Azerbaijanis, in 3 cases (16.67%) among Lezghins and in 2 (11.11%) cases among Talyshs, Russians (11.11%), representatives of other nationalities and ethnicities (11.11%), in each group separately.

The results of distribution of phenotypical frequencies of this polymorphism are given in the Table 1.

C174T mutation was identified in the group of patients with CVD in both heterozygotic and homozygotic states. 10 Azerbaijani representatives had this mutation in heterozygotic state (23.25%), 2 representatives had it in homozygotic state (4.65%). 2 representative of the ethnic group of Lezghins had this mtutation in heteroygotic state (25%), and 1 representative — in homozygotic state (12.5%). C174T mutation among Talyshs, Russians and representatives of other nationalities and ethnic groups was found in 4 (44.44%), 3(50%) and 2(33.33%) cases, respectively, no homozygotic state of mutation was identified.

In the control group, differing phenotypic frequencies of C174T polymorphism were observed, ranging from 14.29% (Lezghins) to 22.22% (Russians) among ethnic groups living in Azerbaijan. Phenotypic frequency of polymorphism among Azerbaijanis was 15.52%.

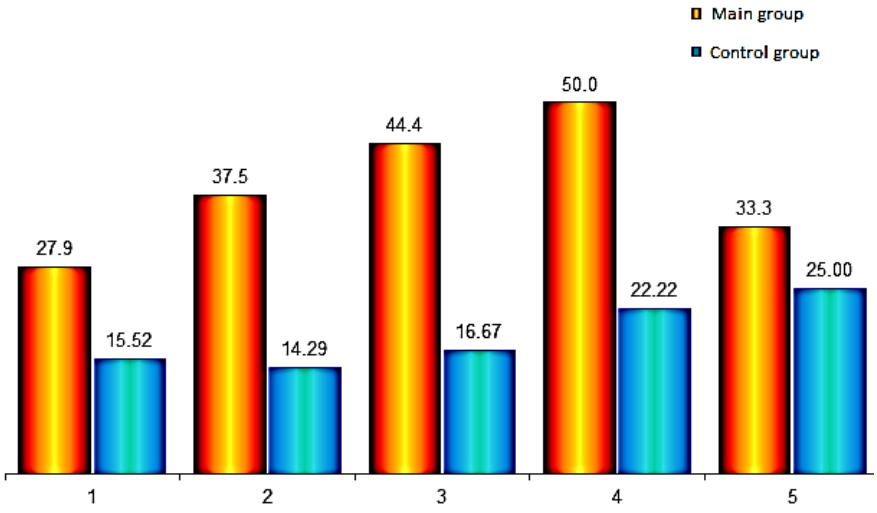
Table 1.

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66

DISTRIBUTIONS OF THE PHENOTYPE OF C174T IN THE CONTROL GROUP AND IN THE GROUP OF PATIENTS WITH CVD

Ethnic groups	*N*	*T/T*		*C/T*		*C/C*	
		abs.	*%*	*abs.*	*%*	*abs.*	*%*
Control group							
Azerbaijanis	58	0	–	9	15.52	49	84.48
Lezghins	21	0	–	3	14.29	18	85.71
Talyshs	12	0	–	2	16.67	10	83.33
Russians	9	0	–	2	22.22	7	77.78
Other nationalities	8	0	–	2	25.00	6	75.00
Patients with CVD							
Azerbaijanis	43	2	4.65	10	23.26	31	72.09
Lezghins	8	1	12.50	2	25.00	5	62.50
Talyshs	9	0	–	4	44.44	5	55.56
Russians	6	0	–	3	50.00	3	50.00
Other nationalities	6	0	–	2	33.33	4	66.67

In the group of patients with CVD, the phenotypic frequency of polymorphism was approximately two times higher than the results obtained in the control group and was distributed as follows according to the increase in phenotypic frequencies: Azerbaijanis — 27.9%, other nationalities — 33.33%, Lezghins — 37.5%, Talyshs — 44.44% and Russians — 50.0% (Figure 3).



1 – Azerbaijanis
2 – Lezghins
3 – Talyshs
4 – Russians
5 – other nationalities

Figure 3. The final phenotypic frequency of C174T in the control group and in the group of patients with CVD

Having combined these two groups separately based on the nationality of examined people, the following result was obtained according to the increase in phenotypic frequencies of C174T polymorphism: Azerbaijanis — 43.42%, Lezghins — 51.79%, other nationalities — 58.33%, Talyshs - 61.04% and Russians — 72.22%.

The results of distribution of phenotypic frequencies of this polymorphism are given in the following Table 2.

Table 2.

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66

DISTRIBUTION OF THE GENOTYPE OF C174T IN THE CONTROL AND CVD PATIENT GROUPS

Ethnic groups	*Control group*			*Patients with CVD*		
	T/T	*C/T*	*C/C*	*T/T*	*C/T*	*C/C*
Azerbaijanis	–	0.1552	0.8448	0.0465	0.2326	0.7209
Lezghins	–	0.1429	0.8571	0.125	0.25	0.625
Talyshs	–	0.1666	0.8334	–	0.4444	0.5556
Russians	–	0.2222	0.7778	–	0.5	0.5
Other nationalities	–	0.25	0.75	–	0.3333	0.6667

The results of distribution of frequencies of gene alleles of this polymorphism are given in the Figure 4.

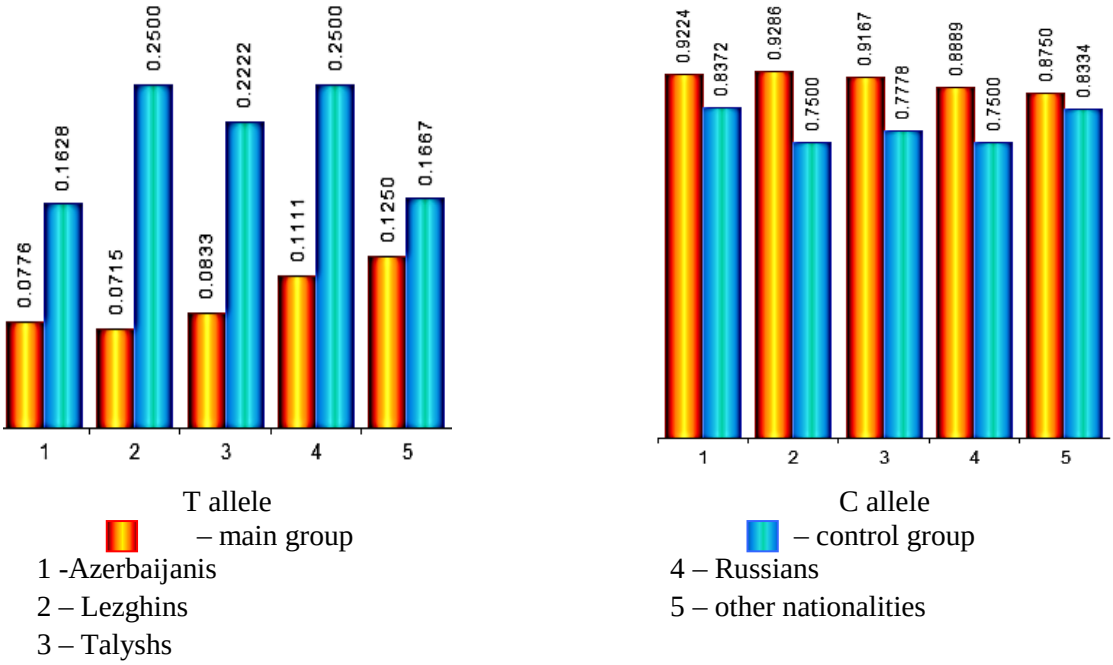


Figure 4. Distributions of gene alleles (in fractions of a unit) of C174T in the control and CVD patient groups

The next studied polymorphism of C235T angiotensinogen gene was identified in both experimental groups. In the control group of 108 people we identified 12 cases of this polymorphism in heterozygotic state. Among Azerbaijanis — 5 out of 58 cases (8.62%), among Lezghins — 2 out of 21 cases (9.52%), among Talyshs — one case (8.3%), among Russians — 2 cases (22.22%) and again in 2 cases among representatives of other nationalities (25%).

In the group of patients with CVD, consisting of 72 people, in 18 cases this mutation was idenified in a heterozygotic state and in 9 cases — in a homozygotic state. Among 43 representatives of Azerbaijani nationality, 7 cases were found to have this mutation in a heterozygotic state (16.27%), and in 6 cases in a homozygotic state (13.95%). Among Lezghins no heterozygotic form of mutation was identified, however in one case this mutation was observed in homozygotic state (12.5%). Among Talyshs heterozygotic state of mutation was observed in one case (11.11%), no homozygote was found out. Two cases of C235T were identified among the representatives of Russian nationality — in heterozygotic state in one case (16.67%) and in homozygotic state in another case (16.67%), respectively. Moreover, in one case C235T mutation in homozygotic state was identified among the representatives of other nationalities (16.67%).

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66

The results of distribution of phenotypic frequencies of polymorphism of C235T angiotensinogen gene in the control group and in the group of patients with CVD among the representatives of various nationalities are given in the Table 3.

As it can be seen from the table, differing phenotypic frequencies of C235T polymorphism were observed in the control group, ranging from 8.33% (Talyshs — 1 out of 12 people) to 25.0% (others — 2 out of 8 people) among ethnic groups living in Azerbaijan. The highest frequencies among the examined nationalities were observed among Russians — 2 out of 9 (22.22%). The frequency of polymorphism phenotype among Azerbaijanis was 5 out of 58 (8.62%).

Table 3.

DISTRIBUTIONS OF THE PHENOTYPE OF C235T
IN THE CONTROL AND CVD PATIENT GROUPS

Ethnic groups	*N*	*T/T*		*C/T*		*C/C*	
		abs.	*%*	*abs.*	*%*	*abs.*	*%*
Control group							
Azerbaijanis	58	0	–	5	8.62	53	91.38
Lezghins	21	0	–	2	9.52	19	90.48
Talyshs	12	0	–	1	8.33	11	91.67
Russians	9	0	–	2	22.22	7	77.78
Other nationalities	8	0	–	2	25.00	6	75.00
Patients with CVD							
Azerbaijanis	43	6	13.95	7	16.28	30	69.77
Lezghins	8	1	12.50	0	–	7	87.50
Talyshs	9	0	–	1	11.11	8	88.89
Russians	6	1	16.67	1	16.67	4	66.67
Other nationalities	6	0	0.00	1	16.67	5	83.33

Phenotypic frequency of C235T polymorphism in the group of patients with CVD was distributed as follows. 7 representatives of Azerbaijani nationality had this mutation in heterozygotic state (16.28%), and 6 representatives had it in homozygotic state (13.95%). No heterozygotic state of mutation among the representatives of Lezghin ethnic group was identified, and only one person had a mutation in homozygotic state (12.5%). C235T mutation among Talyshs and representatives of other nationalities was found out only in heterozygotic state (1 out of 9 — 11.11% and 1 out of 6 (16.67%). Among Russians this mutation was identified in heterozygotic state in one case out of 6 (16.67%) and in one case it was in homozygotic state (16.67%).

Phenotypic frequencies of C235T polymorphism were observed in the control group, ranging from 8.33% (Talyshs) to 22.22% (Russians) among ethnic groups living in Azerbaijan. Phenotypic frequency of polymorphism among Azerbaijanis was 8.62%.

In the group of patients with CVD, phenotypic frequency of polymorphism in accordance with the increase of phenotypic frequencies was distributed as follows: Talyshs — 11.11%, Lezghins — 12.5%, other nationalities — 16.67%, Azerbaijanis — 30.23% and Russians — 33.33% (Figure 5).

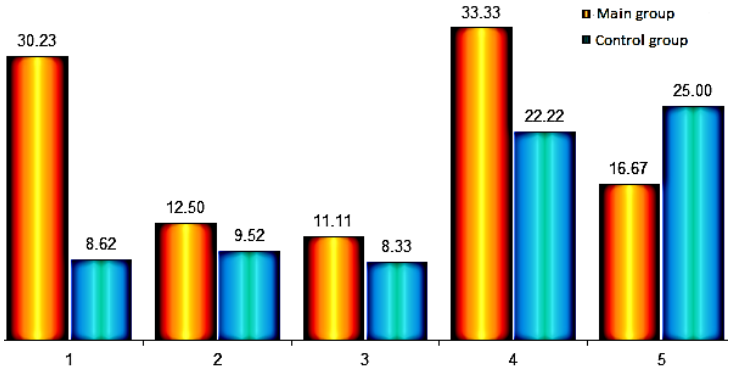
Having combined the control and experimental group separately by nationalities of those examined, we get the following image according to the increase of phenotypic frequency of C235T polymorphism: Talyshs — 19.44%, Lezghins — 22.02%, Azerbaijanis — 38.85%, other nationalities — 41.67%, Russians — 55.55%.

Based on the results of the study, it can be concluded that the lowest frequency of C235T

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66

polymorphism of the AGT gene was observed among Talyshs with a phenotypic frequency of 19.41%, and the highest among Russians — 55.56%.

The results of distribution of genotypic frequencies of C235T polymorphism of angiotensinogen gene in the control group and in the group of patients with CVD among the representatives of various nationalities are presented in the Table 4.



1 – Azerbaijanis
2 – Lezghins
3 – Talyshs
4 – Russians
5 – other nationalities

Figure 5. The final phenotypic frequency of C235T in the control and CVD patient groups

Table 4.

Genotype distribution of C235T in the control and CVD patient groups

Ethnic groups	Control group			Patients with CVD		
	T/T	C/T	C/C	T/T	C/T	C/C
Azerbaijanis	–	0.0862	0.9138	0.1395	0.1627	0.6978
Lezghins	–	0.0952	0.9048	0.125	–	0.875
Talyshs	–	0.0833	0.9167	–	0.1111	0.8889
Russians	–	0.2222	0.7778	0.1667	0.1667	0.6666
Other nationalities	–	0.25	0.75	–	0.1666	0.8334

The results of distribution of frequencies of gene alleles of this polymorphism are presented in the Figure 6.

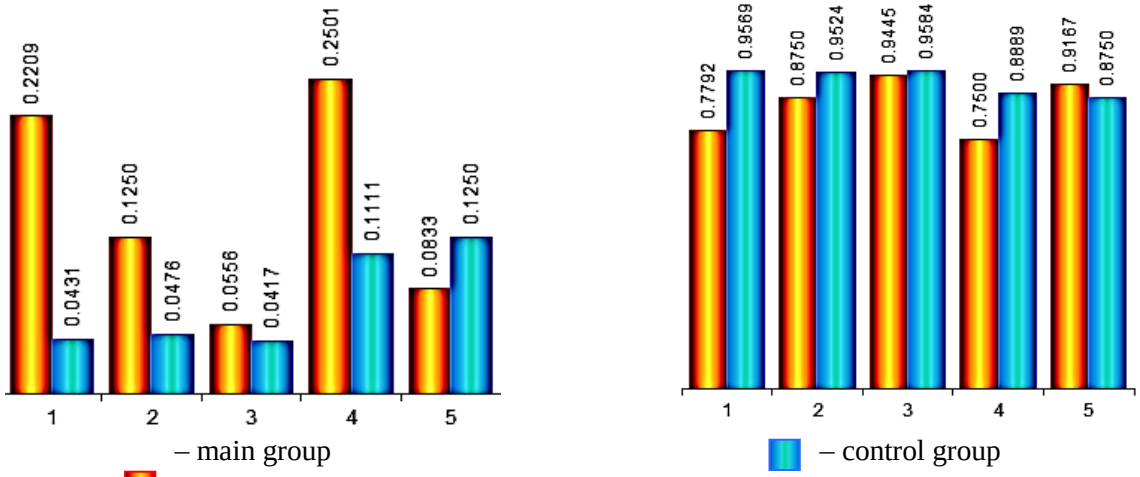
Analysis of results of C174T and C235T polymorphisms of AGT gene among the population of neighbouring countries suggests the following image.

Studies carried out by T. H. Nguyen and T. P. Shkurat (2011) showed the association of polymorphic variations of C174T and C235T of AGT with the ischaemic heart disease among Russians from Rostov-on-Don of the Rostov region, at the border with Krasnodar Krai of the Russian Federation [5].

Z. N. Kalakutok (2003), in her works, carried out in Adyghei Republic of the Russian Federation in 2000-2002, also identified association of C174T polymorphism of AGT gene with the risk of developing essential hypertension, mainly among Adyghes [4].

In the course of re-study in the Republic of Adyghei ten years later (2002-2010), the following results were obtained: 84% of craniological patients and 63.6% donors with hereditary burden on CVD had a heterozygotic state of C174T mutation. All patients with C235T had various forms of IHD from rhythm disturbance to myocardial infarction accompanied by hypertension.

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66



T allele
1 – Azerbaijanis
2 – Lezghins
3 – Talyshs

C allele
4 – Russians
5 – other nationalities

Figure 6. Distributions of gene alleles (in fractions of a unit) of C235T in the control and CVD patient groups

In the Republic of Adyghei, Z. N. Kalakutok (2009) divided all 115 examined patients (50 men and 65 women) according to ethnic origin: Russians, Adyghes, Chechens and others [4]. Of the 115 examined people 60 people (52.2%) were Adyghes (28 men, 32 women), 32 people were Russians and only 28.8% of the total number of examined were 10 men, 22 women, Chechens — 8 people (7%), 5 men and 3 women and 15 representatives of other nationalities (13%), 7 men and 8 women [3].

It's interesting that the frequency of occurrence of C235T polymorphism among Russians living in Rostov-on-Don is approximately three times higher than among Russians living in Maykop city and equals to 70%. The ratio of heterozygotic and homozygotic forms of the C235T mutation in two groups of Russians from RA and RR is 38%, 20% and 29%, 2%, respectively. The total frequency of heterozygotes and homozygotes for Russians in the Republic of Adygei is 67% (38% + 29%) and for Russians in the Rostov Region is 22% (20% + 2%).

According to the results of studies on C235T polymorphism among the control group of Russians living in the Republic of Azerbaijan, phenotypic frequency was similar (22.22%) to the frequencies, identified among Russians in the group of donors in Rostov Region — 22%, and differed from those among Russians living in the Republic of Adyghei — 67%.

M.G. Andreeva when studying the role of C174T polymorphism of the AGT gene in the formation of aptitude to the development of arterial hypertension, the characteristics of its course and the choice of a hypertensive drug depending on the genotype among the residents of Kazan, found out that the frequency of the C174T mutation is twice higher among Russians than among Tatars [1].

C677T polymorphism of the MTHFR gene in the control group was identified in heterozygotic state in 24 cases: 13 cases among Azerbaijanis (22.41%), 4 cases among Lezghins (19.05%), 3 cases among Talyshs (25%), 2 cases among Russians (22.22%) and 2 cases among the representatives of other nationalities (25%).

In the group of patients with CVD this polymorphism was identified in 27 cases in heterozygotic state: in 16 cases among Azerbaijanis (37.2%), in 3 cases among Lezghins (37.5%),

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66

in 4 cases among Talyshs (44.44%), in 2 cases among Russians (33.33%) and in 2 cases among the representatives of other nationalities (33.33%). This mutation wasn't identified in homozygotic state [6].

The results of distribution of phenotypic frequencies of C667T polymorphism of the MTHFR gene in the control group and in the group of patients with CVD among the representatives of various ethnic groups are presented in the Table 5.

Differing phenotypic frequencies of C667T polymorphism were observed in the control group, ranging from 19.05% (Lezghins — 4 out of 21 people) to 25.0% (Talyshs — 3 out of 12 people and others — 2 out of 8 people) among ethnic groups living in Azerbaijan (Figure 6). The frequencies among Talyshs and in the group of representatives of other nationalities are the same. The frequency among Russians was 22.22% (2 out of 9). The frequency of polymorphism phenotype among Azerbaijanis was 22.41% (13 out of 58).

Table 5.

DISTRIBUTIONS OF THE PHENOTYPE OF THE C677T OF THE MTHFR GENE IN THE CONTROL GROUP AND IN THE GROUP OF PATIENTS WITH CVD

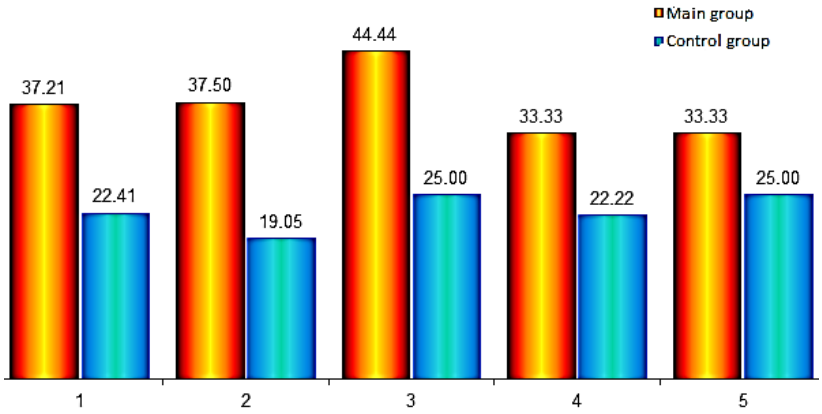
Ethnic groups	N	T/T		C/T		C/C	
		abs.	%	abs.	%	abs.	%
Control group							
Azerbaijanis	58	0	–	13	22.41	45	77.59
Lezghins	21	0	–	4	19.05	17	80.95
Talyshs	12	0	–	3	25.00	9	75.00
Russians	9	0	–	2	22.22	7	77.78
Other nationalities	8	0	–	2	25.00	6	75.00
Patients with CVD							
Azerbaijanis	43	0	–	16	37.21	27	62.79
Lezghins	8	0	–	3	37.50	5	62.50
Talyshs	9	0	–	4	44.44	5	55.56
Russians	6	0	–	2	33.33	4	66.67
Other nationalities	6	0	–	2	33.33	4	66.67

The phenotypic frequency of C667T polymorphism among the patients with CVD was distributed in accordance with the increase of phenotypic frequencies in the following manner: among Russians — 2 cases out of 6 (33.33%), among the representatives of other nationalities — 2 cases out of 6 (33.33%), among Azerbaijanis — 16 cases out of 43 (37.2%), among Lezghins — 3 cases out of 8 (37.5%), among Talyshs — 4 cases out of 9 (44.44%).

Having combined the control group and the experimental groups separately based on the nationality of examined people, we obtained the following result according to the increase in phenotypic frequencies of C667T polymorphism: Russians — 55.55%, Lezghins — 56.55%, other nationalities — 58.33%, Azerbaijanis — 59.62%, Talyshs — 69.44%.

Based on the results of the study, it can be concluded that the lowest frequency of C667T polymorphism of the AGT gene was identified among Russians with phenotypic frequency — 55.55%, and the highest frequency — in the group of Talyshs — 69.44%. Table 5.6 submits the results of distribution of phenotypic frequencies of C667T polymorphism of the MTHFR gene in the control group and in the group of patients with CVD among the representatives of various ethnic groups.

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66



1 – Azerbaijanis
2 – Lezghins
3 – Talyshs
4 – Russians
5 – other nationalities

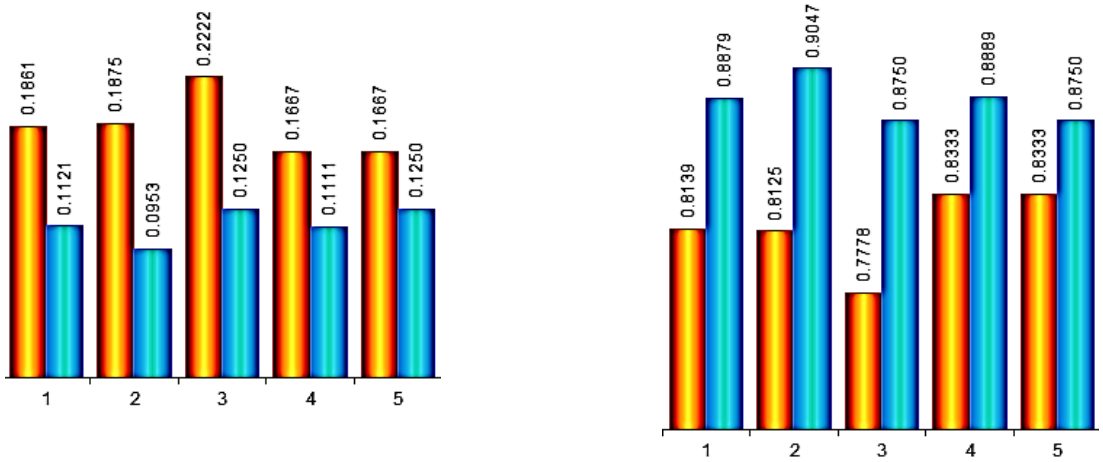
Figure 7. The final phenotypic frequency of C677T of the MTHFR gene in the control and CVD patient groups

Table 6.

DISTRIBUTION OF THE GENOTYPE OF C677T OF THE MTHFR GENE THE CONTROL AND CVD PATIENT GROUPS

Ethnic groups	Control group			Patients with CVD		
	T/T	C/T	C/C	T/T	C/T	C/C
Azerbaijanis	–	0.2241	0.7759	–	0.3721	0.6279
Lezghins	–	0.1905	0.8095	–	0.3750	0.6250
Talyshs	–	0.2500	0.7500	–	0.4444	0.5556
Russians	–	0.2222	0.7778	–	0.3333	0.6667
Other nationalities	–	0.2500	0.7500	–	0.3333	0.6667

The results of distribution of frequencies of gene alleles of this polymorphism are presented in the Figure 8.



T allele C allele

1 -Azerbaijanis
2 – Lezghins
3 – Talyshs
4 – Russians
5 – other nationalities

– main group – control group

Figure 8. Distributions of gene alleles of the C677T of the MTHFR gene in the control and CVD patient groups

Summing up all the above-mentioned, the distribution of identified C174T and C235T

Т. 7. №5. 2021
https://doi.org/10.33619/2414-2948/66

mutations of the AGT gene, as well as the C667T polymorphism of the MTHFR gene based on the ethnic groups of population of the Republic of Azerbaijan, showed their uneven distribution.

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