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Molecular Genetic Features of Congenital Angiodysplasias of the Brain in Patients with Cephalgic Syndrome

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Abstract
The lack of awareness of many specialists in a narrow circle complicates the problem, since erroneous diagnosis and incorrect management of patients with cephalgic syndrome can lead to aggravation of their condition, on the one hand, and an increase in cases of serious complications. Aim of the study is evaluation of molecular genetic aspects of cerebral vascular abnormalities, we conducted a molecular genetic analysis of the VEGFA and MMP 3 genes. Research methods were study the role of these genes in the development of vascular anomalies, we first carefully assessed the prognostic effectiveness of the studied markers. The results of the work are based on data from a DNA study of 178 patients, who were divided into three subgroups: 92 (51.7%) patients had MSA; 41 (23.0%) were MBA, 45 (25.3%) were with DESA. A group of 172 healthy volunteers of Uzbek nationality was examined as a control. Study results have shown that P polymorphism C 634 G of the VEGF gene has 3 genotypic variants: C / C, C / G and G / G. Genotype 634 C / C is wild - major, G / G minor, and is relatively rare in the population. We can conclude that the polymorphic marker 5a/6a of the MMP3 gene plays a significant role in the formation of cerebral vascular abnormalities with the development of migraine and dyscirculatory encephalopathy.

Keywords
Congenital angiodysplasias, Cephalgic syndrome, VEGFA, MMP 3 genes, Molecular genetic aspects

1. Introduction
The problem of molecular and genetic features of congenital angiodysplasia of the brain in patients with cephalgic syndrome is due to the most common occurrence of various cerebrovascular diseases, including acute cerebrovascular accidents. Because they have a number of distinctive specific features that distinguish them from those without precerebral angiodysplasia [1, 3]. Aim of the study is evaluation of molecular genetic aspects of cerebral vascular abnormalities, we conducted a molecular genetic analysis of the VEGFA and MMP 3 genes.

2. Material and Methods
To study the role of these genes in the development of vascular anomalies, we first carefully assessed the prognostic effectiveness of the studied markers. The results of the work are based on data from a DNA study of 178 patients, who were divided into three subgroups: 92 (51.7%) patients had MSA; 41 (23.0%) were MBA, 45 (25.3%) were with DESA. A group of 172 healthy volunteers of Uzbek nationality was examined as a control.

3. Results of the Study

P polymorphism C 634 G of the VEGF gene has 3 genotypic variants: C / C , C / G And G / G . Genotype 634 C / C is wild - major, G / G minor, and is relatively rare in the population. Distribution of actual and theoretical frequencies of genotypes of the C 634 G polymorphism of the VEGF gene and their Compliance with the population balance of RCV was carried out separately in groups of migraine patients with cerebral vascular abnormalities and healthy persons of Uzbek nationality.

For the C 634 G polymorphism of the VEGF gene, the distribution of actual genotypes in the patient group and the control sample corresponded to that expected at RCV equilibrium (p>0.05) [1,3]. Thus, the expected and observed frequencies of the C / C genotype in the patient group were almost similar, that is, 0.57 and 0.58, respectively. The expected frequency of the heterozygous genotype C / G was 0.37 versus 0.35 observed, the expected frequency of the unfavorable homozygous genotype G / G was 0.06 versus 0.07 observed [1, 3, 4].

The expected and observed frequencies of occurrence of C / C, C / G and G / G genotypes in the control group were: 0.75/0.76, 0.23/0.21 and 0.02/0.02, respectively (Table 1.).

Table 1 Frequency distribution of genotypes of the C 634 G polymorphism of the VEGFA gene in accordance With RHV in the group of patients

Gene	Polymorphism	Genotype	Genotype distribution		χ^2	R
			Expected	Observable		
VEGFA	r s 2010963	C/C	0.57	0.58	0.045	**0.4**
		C/G	0.37	0.35	0.272	
		G/G	**0.06**	**0.07**	**0.415**	

Table 2 Frequency distribution of genotypes of the C 634 G polymorphism of the VEGFA gene in accordance With the Hardy-Weinberg equilibrium in the control group

Gene	Polymorphism	Genotype	Genotype distribution		χ^2	R
			Expected	Observable		
VEGFA	r s 2010963	C/C	0.75	0.76	0.009	**0.5**
		C/G	0.23	0.21	0.114	
		G/G	**0.02**	**0.02**	**0.379**	

Below are the results of the analysis of gene diversity (Table 2.) by polymorphism C 634 G of the VEGFA gene according to the following criteria: calculated expected heterozygosity (h_{exp}), and the relative deviation of the observed heterozygosity (h_{obs}) from the expected one (h_{exp}), in the main and population samples.

The relative deviation *(D)* of the expected heterozygosity from the observed one was calculated using the formula: $D = (h_{obs} - h_{exp})/h_{exp}$, where h_{obs} and h_{exp} *are* the observed and expected heterozygosity, respectively.

In the main group, relatively high heterozygosity for the studied polymorphism was revealed, $h_{exp} = 0.37$ and $h_{obs} = 0.35$ ($0.37 < H_{exp} < 0.4$), which indicates moderate gene diversity of this polymorphism.

In a population sample h_{exp} and h_{obs} corresponded to values of 0.23 and 0.21, which corresponds to low genetic diversity of the population for this polymorphism [1, 3].

In the main and control samples, the coefficient of relative deviation of the observed heterozygosity from the expected polymorphism C 634 G of the VEGFA gene turned out to be negative, i.e. D< 0 (D = -0.05 and -0.04, respectively), which indicates a lack of heterozygotes, the level of which corresponds to moderate (Table 3).

Table 3 Difference between expected and observed frequencies heterozygosity

Groups	hexp	hobs	D *
Main group	0.37	0.35	-0.05
Control group	0.23	0.21	-0.04

Note: D = (0.35-0.37)/0.37 = -0.05 for the main group; D = (0.21-0.22)/0.22 = -0.04 for the control group. The functionally unfavorable allele 634 G statistically significantly prevailed in patients compared to the control group (24.7% versus 13.1%, respectively) [1, 3].

Thus, the presence in the genotype of the unfavorable allele 634 G of the VEGF gene contributes to the progression of angiodysplasia and increases the risk of developing both migraine and dyscirculatory encephalopathy, frequent TIAs, strokes [1].

Table 4 Difference between alleles and genotypes of the polymorphic marker VEGF C 634 G in the main and control groups

No.	Groups	VEGF gene allele frequency				Frequency of VEGF gene genotypes					
		C		G		C / C		C / G		G / G	
		N	%	n	%	N	%	n	%	N	%
1	Main group n =178	268	75.3	88	24.7	103	57.9	62	34.8	13	7.3
A	MSA n =92	115	62.5	69	37.5	34	37.0	47	51.1	eleven	11.9
B	MBA n =41	81	98.8	1	1.2	40	97.6	1	2.4	-	-
IN	DESA n =45	72	80.0	18	20.0	29	64.4	14	31.1	2	4.4
2	Control group n =172	299	86.9	45	13.1	131	76.2	37	21.5	4	2.3

Analysis of the distribution of genotypes of this polymorphism showed that the wild genotype C / C is more common in the healthy control group compared to the combined sample of patients 76.2% versus 57.9%, respectively; (χ^2 =13.2; P< 0.05; OR =0.4; 95% CI 0.27-0.68). These data indicate a protective effect of this genotype regarding the formation of angiodysplasia.

The heterozygous genotype C / G was more common in the main group compared to the control (34.8% versus 21.5%, respectively). Risk of developing cerebral vascular abnormalities when carrying this genotype, C / G is 1.9 times significantly higher (OR = 1.9; 95% CI 1.211-3.141) than in individuals who do not have this genotype with χ^2 = 7.6; P=0.001 [1, 3].

In the studied groups of patients and the control group, a significant difference in the frequency of homozygotes for the unfavorable allele 634 G was registered (7.3% and 2.3%, respectively). The risk of angiodysplasia formation and development migraine, as well as dyscirculatory encephalopathy in carriers of this genotype is 3.3 times significantly higher than in persons who do not have it (χ^2 = 4.7; P = 0.03; OR = 3.3; 95% CI 1, 05-10.36).

When comparing the distribution of frequencies of allelic and genotypic variants of the 634 G polymorphism of the VEGF gene depending on the frequency of cerebral vascular anomalies, we also identified a significant association of this polymorphism [1, 2].

Table 5 Distribution of frequencies of alleles and genotypes of the VEGF C 634 G marker V (main and control groups)

Alleles and genotypes	Frequency of alleles and genotypes in groups		Statistical difference
	Main	Control	
Allele C	268	299	χ^2 =15.4; P<0.05; OR =2.2; 95% CI 1.47-3.239
Allele G	88	45	
Genotype C / C	103	131	χ^2 =13.2; P<0.05; OR =0.4; 95% CI 0.27- 0.68
Genotype C / G	62	37	χ^2 =7.6; P=0.001; OR =1.9; 95% CI 1.211-3.141
Genotype G / G	13	4	χ^2 =4.7; P=0.03; OR =3.3; 95% CI 1.05- 10.36
Genotype C / G + G / G	75	41	χ^2 =13.2; P<0.05; OR =2.3; 95% CI 1.46- 3.68

Thus, the "favorable" allele 634 C was more common (Table 5) in the control group than in patients with subgroup 1 (86.9% versus 62.5%, respectively), and the G allele, on the contrary, was more common in the subgroup of patients with MSA, compared with the control group (37.5% vs. 13.1%, respectively). For allele 634G *the* odds ratio for developing migraine with vascular anomalies was *OR* = 4.0 (95% CI 2.58-6.145) with a significance level of χ^2 = 42.2; P < 0.05 (Table 6).

Table 6 Difference between alleles and genotypes of the polymorphic marker VEGF C 634 G in subgroup I and control sample

Alleles and genotypes	Frequency of alleles and genotypes in groups		Statistical difference
	subgroup A	Control	
Allele C	115	299	χ^2 =42.2; P<0.05 ; OR=4.0; 95% CI 2.58 - 6.145
Allele G	69	45	
Genotype C / C	34	131	χ^2 =39.3; P<0.05; OR =0.2; 95% CI 0.1059, 0.3179
Genotype C / G	47	37	χ^2 =24.2; P<0.05; OR =3.8; 95% CI 2.20-6.58
Genotype G / G	eleven	4	χ^2 =10.4; P =0.001; OR=5.7; 95% CI 1.762, 18.46
Genotype C / G + G / G	58	41	χ^2 =39.3; P<0.05; OR =5.4; 95% CI 3.146, 9.445

Heterozygous genotype C / G more common in the first subgroup-51.1%, versus 21.5% in the control group. The risk of developing vascular pathologies when carrying this genotype significantly increases by 3.8 times compared to other genotypes (OR = 3.8; 95% CI 2.20-6.58 with χ^2 = 24.2; P < 0. 05).

The unfavorable homozygous genotype G / G was also more common in group I compared with the control group (11.9% and 2.3%, respectively), indicating its predisposing effect on the risk of developing vascular anomalies with OR =5.7; 95% CI 1.762-18.46 with χ^2 =10.4 and P = 0.001 (Table 6). The frequency of combined genotypes in the subgroup of patients was also 5.4 times higher than in the control group (χ^2 =39.3; P< 0.05; OR =5.4; 95% CI 3.146, 9.445) [1.3] . No less interesting results were obtained during the comparison of subgroup II of patients, that is, the group of patients with MBASGM and the control group, Table 7.

Table 7 Differences in alleles and genotypes of the polymorphic marker VEGF C 634 G and the control group

Alleles and genotypes	Frequency of alleles and genotypes in groups		Statistical difference
	subgroup B	Control	
Allele C	81	299	χ^2 =9.7; P=0.002; OR =0.1; 95% CI 0.0111- 0.6041
Allele G	1	45	
Genotype C / C	40	131	χ^2 =9.6; P=0.002; OR =12.5; 95% CI 1.669- 93.9
Genotype C / G	1	37	χ^2 =8.2; P=0.004; OR =0.1; 95% CI 0.0121-0.685
Genotype G / G	-	4	χ^2 =1.0; P=0.3;
Genotype C / G + G / G	1	41	χ^2 =9.6; P=0.002; OR =0.1; 95% CI 0.0106- 0.5991

Thus, the 634 C allele occurs with a frequency of 98.8% in patients of group II and 86.9% in the control group. In patients of this subgroup, a low frequency of the unfavorable allele 634 G was observed , amounting to -1.2%, while in the control group its frequency was -13.1% (χ^2 =9.7; P = 0.002; OR = 0.1; 95% CI 0.01-0.60) [1].

As can be seen, this genotype in patients with migraine without cerebral vascular deformations was not significant in relation to the risk of morbidity. The mutant homozygous genotype G / G was practically not found in patients, whereas it occurred in the healthy control group - in 2.3% of cases (χ^2 = 1.0; P = 0.3). The combination of unfavorable genotypes C / G + G / G was also significantly more common in the control group than in the subgroup of patients (χ^2 =9.6; P =0.002; OR =0.1; 95% CI 0.0106-0. 5991) [1].

Table 8 Differences in alleles and genotypes of the polymorphic marker VEGF C 634 G in the III subgroup of Patients and the control group

Alleles and genotypes	Frequency of alleles and genotypes in groups		Statistical difference
	subgroup B	Control	
Allele C	72	299	χ^2 =2.7; P=0.1; OR =1.7; 95% CI 0.9079-3.039
Allele G	18	45	
Genotype C / C	29	131	χ^2 =2.5; P=0.1; OR =0.6; 95% CI 0.280-1.147
Genotype C / G	14	37	χ^2 =1.8; P=0.2; OR =1.6; 95% CI 0.795- 3.414
Genotype G / G	2	4	χ^2 =0.6; P=0.4; OR =1.9; 95% CI 0.346- 11.02
Genotype C / G + G / G	16	41	χ^2 =2.5; P=0.1; OR =1.8; 95% CI 0.87-3.56

The next stage of our work was a comparative analysis of the frequency distribution of alleles and genotypes of this polymorphism in the III subgroup of patients and the control sample (Table 8).

Here we can trace the following trend, in which the unfavorable allele 634 G of the VEGF gene actually has a predisposing effect on the development of various dysplasias, with chronic cerebrovascular accident (20% versus 13.1%, OR=1.7 95% CI 0.9079-3.039) without achieving statistical significance (χ^2 =2.7; P=0.1).

As can be seen from table 8, favorable homozygous The C / C genotype was not significantly more common in the control group than in patients with dyscirculatory encephalopathy (76.2% versus 64.4%, respectively, with χ^2 =2.5; P = 0.1; OR = 0.6; 95 % CI 0.280-1.147). The heterozygous genotype C / G was significantly more common in the subgroup of patients compared to the control group consisting of relatively healthy individuals (31.1% versus 21.5%, respectively, with χ^2 =1.8; P = 0.2; OR =1.6; 95% CI 0.795-3.414). The homozygous G / G genotype, as well as the heterozygous C / G genotype, was more common in patients compared to the control group (4.4% versus 2.3%, respectively; χ^2 =0.6; P = 0.4; OR = 1.9; 95% CI 0.346-11.02). As can be seen, this genotype also tends to increase the susceptibility to the risk of developing cerebral vascular pathology by 1.9 times [1].

Thus, the C 634 G polymorphism of the VEGF gene is associated with formation of angiodysplasia, development migraine, chronic cerebrovascular accident. Unfavorable G / G and C / G genotypes were significantly more common in patients with vascular pathologies than in the control group. The most unfavorable predisposing genotypic variant of the C 634 G polymorphism of the VEGF gene with respect to the increased risk of developing cerebral vascular pathology with the development of migraine was the homozygous G / G genotype.

As can be seen from table 9, non-closure of the circle of Willis , due to hypo /aplasia of the PSA or PSA, is associated with an unfavorable G/G genotype in 117 (70.9%) cases, and only in 26 (15.8%) cases with a combination of genotypes C/G+ G/G. The heterozygous genotype C/G in 18 (10.9%) was associated with openness Circle of Willis. The favorable C/C genotype in 4 (2.4%) patients was associated with openness Circle of Willis [1].

Table 9 The incidence of cerebral vascular anomalies depending on polymorphism C 634 G of the VEGF gene

Types of Anomalies/Genotype	C / C		C/G		G/G		C/G + G/G		Total	
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Hypo /aplasia PSA, PSA	4	2.4	18	10.9	117	70.9	26	15.8	165	49.8
Hypo /aplasia PA	0	0	8	14.0	26	45.6	23	40.4	57	17.2
Trifurcations of the ICA (posterior, anterior)	0	0	1	4.3	13	56.6	9	39.1	23	6.9
Fenestration	0	0		0	1	100	0	0	1	0.3
S- and C -shaped crimps	6	12.8	7	14.9	21	44.7	13	27.6	47	14.2
Kinking , coiling	2	5.3	4	10.5	24	63.1	8	21.1	38	11.5
Total	12	3.6	38	11.5	202	61.0	79	23.9	331	16.5

And hypo- and aplasia of PA were associated in 26 (45.6%) cases with the G/G genotype. against 23 (40.4%) C/G+ G/G genotype, and only 8 (14%) with C/G. Posterior trifurcations (anterior, posterior) of the ICA were associated - 13 (56.6%) with the G/G genotype and 9 (39.1%) with the combined genotype C/G+ G/G, only in 1 (4.3%) cases with the C/G genotype. Of the total number of examined patients with anomalies, only 47 (14.2%) patients had S- and C-shaped tortuosity, of which 21 (44.7%) cases had the G/G genotype, versus 13 (27.6%)) combined genotype, as well as in 7 (14.9%) C/G genotype, 6 (12.8%) with C/C genotype [1, 3].

Pathological deformities in the form of kicking or coiling were observed in 38 (11.5%) patients. Of these, these types of deformities in 24 (63.1%) cases were associated with the G/G genotype, and only 8 (21.1%) - with genotypes C/G + G/G, in 4 (10.5%) C/G, versus 2 (5.3%) C/C genotype.

Matrix metalloproteinases, in particular MMP3, MMP9, play an important role in the degradation of collagen, in particular collagen COL 4A1, which is involved in the formation of the vascular basement membrane, the degradation of which leads to vascular abnormalities. Therefore, the study of polymorphic variants of the MMP3 and MMP9 genes is key in the study of cerebral vascular abnormalities. The MMP3 gene has been studied in the Uzbek population for the first time [1, 5].

In the group of patients with migraine, the theoretically expected frequency of genotypes was: 6a/6a =0.49; 5a/6a =0.42; 5a/5a =0.09: observed: 6a/6a = 0.53; 5a/6a =0.33; 5a/5a =0.13. In the population sample, the theoretically expected frequency of genotypes was: 6a/6a =0.71; 5a/6a =0.26; 5a/5a =0.02: observed: 6a/6a =0.76; 5a/6a =0.26; 5a/5a =0.08. For this polymorphic marker, a study of the distribution of genotypes in both groups showed the presence of statistically significant deviations from RCV ($P < 0.05$), which was associated with a decrease in the observed heterozygosity 5a/6a and an increase in the frequency of the homozygous genotype 5a/5a in comparison with its expected values. The deviation from RCV in the studied groups of patients and controls can be explained by a significant deficiency of actual heterozygotes: 0.33/0.42 and 0.16/0.26, respectively (Tables 10 and 11). As is known, deviation from RCV due to a deficiency of observed heterozygotes against the expected one can be observed in the following cases:1) the polymorphism or locus under study is subject to selective selection; 2) in the studied populations or ethnic groups there is a high frequency of inbreeding; 3) with the Wahlund effect (pronounced genetic subdivision of a population into smaller subpopulations); 4) during a genetic - automatic process , accompanied by a change in the gene pool of the population from generation to generation, i.e., with genetic drift [1, 4]. Below are the results of a biometric analysis of gene diversity for the 5a/6a polymorphism of the MMP3 gene.

Table 10 Frequency distribution of genotypes polymorphism 5a/6a of the MMP3 gene V according to Hardy-Weinberg equilibrium in a group of patients

Gene	Polymorphism	Genotype	Genotype distribution		χ^2	R
			Expected	observable		
MMP3	5a/6a of the MMP3 gene	6a/6a	0.49	0.53	0.72	0.005
		5a/6a	0.42	0.33	3.35	
		5a/5a	0.09	0.13	3.9	

Table 11 Frequency distribution of genotypes of polymorphism 5a/6a of the MMP3 gene in accordance with the Hardy-Weinberg equilibrium in the control group

Gene	SNP	Genotype	Genotype distribution		χ^2	R
			Expected	Observable		
MMP3	5a/6a gene	6a/6a	0.71	0.76	0.628	<0.05
		5a/6a	0.26	0.16	6,745	
		5a/5a	0.02	0.08	18.11	

In the main group of patients, the expected and observed frequency of heterozygotes were equal to H_{exp} =0.42 and H_{obs} =0.33 [1, 5].

The D indicator turned out to be negative, which indicates a lack of heterozygotes with a high degree (D = -0.21). As can be seen, in the main group of patients, the value of expected heterozygosity is close to 0.5 ($0.42 < H_{exp} < 0.5$), which corresponds to the relatively high gene diversity of this polymorphism in the group of patients [1,4].

A different picture is observed in the control group, where the H_{exp} and $H_{obs\ values}$ were very low and corresponded to: 0.26 and 0.16. At the same time, the coefficient of the relative deviation of actual heterozygosity from the theoretical one had a negative value, indicating a deficiency of heterozygotes, and the degree of this deficiency is characterized as a high level (D = -0.4). The expected frequency among apparently healthy donors turned out to be very low, which corresponds to the relatively low gene diversity of the population for the 5a/6a polymorphism of the MMP3 gene ($0.25< H_{exp} < 0.3$). The decrease in heterozygosity in the group of conditionally healthy donors may have arisen due to an increase in the number of homozygotes for the major 6a allele. In addition, such a low frequency is probably a consequence of not very high fitness of this genotype in our population, table 12.

Table 12 Difference between expected and observed heterozygosity rates

Groups	h_{exp}	hobs	D *
Main group	0.42	0.33	-0.21
Control group	0.26	0.16	-0.4

D = (0.33-0.42) /0.42 = -0.21 for the main group :D = (0.16-0.26)/0.26 = -0.4 for the control group

A comparative analysis of the distribution of frequencies of allelic and genotypic variants of the polymorphic marker 5a/6a of the MMP3 gene among patients with cerebral vascular abnormalities and in the control sample revealed a higher concentration of the unfavorable allele 5a in patients compared to controls (30.1% versus 15.7% respectively) [1].

Table 13 Frequency distribution of alleles and genotypes of the 5a/6a polymorphism of the MMP3 gene in groups of patients

No.	Groups	Allele frequency				Genotype frequency					
		6a		5a		6a/6a		5a/6a		5a/5a	
		N	%	n	%	n	%	N	%	N	%
1	Main group n =178	249	69.9	107	30.1	95	53.4	59	33.1	24	13.4
A	MSA n =92	120	65.2	64	34.8	41	44.6	38	41.3	13	14.1
B	MBA n =41	68	82.9	14	17.1	thirty	73.2	8	19.5	3	7.3
IN	DESA n =45	61	67.8	29	32.2	24	53.3	13	28.9	8	17.8
2	Control group n =172	290	84.3	54	15.7	131	76.2	28	16.3	13	7.6

Note **:** Here and in other tables χ^2 - Pearson distributions (chi-square); P - level of significance; OR - relative risk; N - number of patients; n – number of alleles and genotypes.

The favorable allele 6a, on the contrary, predominated among healthy donors compared to the group of patients (84.3% versus 69.9%, respectively). At the same time, the difference in the distribution of alleles between patients and the control sample reaches a significant level of significance ($\chi^2 = 20.4$; $P < 0.05$; OR = 2.3; 95% CI 1.597-3.336), table 14.

Table 14 Difference between alleles and genotypes of polymorphism 5a/6a of the MMP3 gene V (main and control groups)

Alleles and genotypes	Frequency of alleles and genotypes in groups		Statistical difference
	Main	Control	
Allele 6a	249	290	χ^2 =20 , 4; P < 0.05 ; OR= 2,3 ; 95% CI 1.597- 3.336
Allele 5a	107	54	
Genotype 6a/6a	95	131	χ^2 = 19.9 ; P < 0.05 ; OR= 0.3 ; 95% CI 0.23-0.57
Genotype 6a/5a	59	28	χ^2 =13 , 3; P = 0.0003 ; OR= 2.5 ; 95% CI1 , 52 - 4 , 25
Genotype 5a/5a	24	13	χ^2 =3.2; P=0.07; OR =1.9; 95% CI 0.936- 3.88
Genotypes 6a/5a+5a/5a	83	41	χ^2 =20.0; P<0.05; OR =2.8; 95% CI 1.76- 4.413

Frequency distribution of genotypes of polymorphism 5a/6a of the MMP3 gene also revealed differences between the main group and the control sample (P< 0.05) . As can be seen from Table 15, in the main and control groups, carriers of the homozygous genotype 6a/6a (53.4% and 76.2%) are more common than genotypes 5a/6a (33.1% and 16.3%) and 5a/5a (13.4% and 7.6%), respectively.

Carriage of the unfavorable homozygous genotype 5a/5a was observed significantly more often in patients - 13.4% than in healthy individuals - 7.6%. When comparing the frequency of this genotype in patients and healthy people, statistically significant differences were obtained with an odds ratio equal to OR = 2.3 (95% CI 1.597-3.336) with a $\chi^{2\,level}$ = 20.4; P< 0.05 [1].

Among patients, the number of carriers of the wild genotype 6a/6a turned out to be significantly lower than in the control group (53.4% versus 76.2%, respectively) and these differences reached the level of threshold significance (χ^2 = 19.9; P < 0.05 ; OR =0.3; 95% CI 0.23-0.57). Concentrations of the heterozygous variant 5a/6a were significantly more often observed in the main group - 33.1% than in the control group - 16.3%, with χ^2 = 13.3; P=0.0003; OR =2.5; 95% CI 1.52. Homozygous genotype 5 a / 5 a also tends to have a predisposing risk effect for the formation of cerebral vascular pathology (23.4% versus 7.6%, respectively; χ^2 =3.2; P = 0.07; OR = 1.9 ; 95% CI 0.936-3.88).The frequency of the combined genotype 6 a /5 a +5 a /5 a in the patient group also turned out to be significantly higher compared to the control group (χ^2 = 20.0; P < 0.05; OR = 2.8; 95% CI 1.76-4.413) [1.5].

The results of the study of the relationship between this polymorphic locus and the incidence of angiodysplasia are presented in table 15.

A comparison of allele frequencies revealed that the wild allele 6a was more common in the healthy control group than in the 1st subgroup (84.3% versus 65.2%, respectively), while the concentration of the unfavorable allele 5a was high in the group of patients compared to the group healthy donors (30.1% vs. 15.7%, respectively). According to the calculated odds ratio, carriers of the 5a allele have a 2.9 times significantly higher risk of developing MSA than carriers of the 6a allele (χ^2 =25.2; P< 0.05; OR =2.9; 95% CI 1 .88-4.35) [1].

Table 15 Difference between alleles and genotypes of polymorphism 5a/6a of the MMP3 gene V subgroup of MSA patients and healthy controls

Alleles and genotypes	Frequency of alleles and genotypes in groups		Statistical difference
	1-group	Control	
Allele 6a	120	290	χ^2 =25 , 2; P < 0.05 ; OR= 2.9 ; 95% CI 1.88- 4.35
Allele 5a	64	54	
Genotype 6a/6a	41	131	χ^2 =26 , 4; P < 0.05 ; OR= 0.2 ; 95% CI 0.15-0.432
Genotype 6a/5a	38	28	χ^2 =20 , 0; P < 0.05 ; OR= 3.6 ; 95% CI2 , 02 - 6 , 46
Genotype 5a/5a	13	13	χ^2 =2.9; P=0.1; OR =2.0; 95% CI 0.891- 4.54
Genotypes 6a/5a+5a/5[a]	51	41	χ^2 =26.4; P<0.05; OR =4.0; 95% CI 2.31- 6.82

Genotype 6 a /5 a and genotypes 6 a /5 a +5 a /5 a were more common in the group of patients χ^2 =20.0; P<0.05; OR =3.6; 95% CI 2.02-6.46 and χ^2 =26.4; P<0.05; OR =4.0; 95% CI 2.31-6.82, respectively, which indicates an increased risk in the first case by 3.6 times, and in the other by 4 times.

Next, we analyzed the differences in the frequency of alleles and genotypes of the 5a/6a polymorphism of the MMP3 gene in the II subgroup of MSA patients and in the control group [1].

Table 16 Difference between alleles and genotypes of the polymorphic marker 5a/6a of the MMP3 gene in Subgroup B and control groups

Alleles and genotypes	Frequency of alleles and genotypes in groups		Statistical difference
	2-group	Control	
Allele 6a	68	290	χ^2 =0 , 1; P = 0.7 ; OR=1 ,1 ; 95% CI 0.58-2.106
Allele 5a	14	54	
Genotype 6a/6a	thirty	131	χ^2 =0 , 1; P = 0.7 ; OR= 0.8 ; 95% CI 0.393-1.852
Genotype 6a/5a	8	28	χ^2 =0 , 2; P = 0.6 ; OR= 1.2 ; 95% CI 0.5213-2.982
Genotype 5a/5a	3	13	χ^2 =0.003; P=0.9; OR =0.9; 95% CI 0.26- 3.55
Genotypes 6a/5a+5a/5a	eleven	41	χ^2 =0.2; P=0.7; OR =1.2; 95% CI 0.5399, 2.542

When comparing the frequencies of genotypes between subgroup II of patients and controls, the following distribution was revealed: wild homozygotes 6a/6a - 73.2% and 76.2%, heterozygotes 5a/6a - 19.5% and 16.3% and unfavorable homozygotes 5a/ 5a – 7.3% and 7.6%, respectively. In the patient group, the number of carriers of 6a and 5a alleles was 82.9% and 17.1%, while in the control group it was 84.3% and 15.7%, respectively. As can be seen, we did not obtain statistically significant differences in the distribution of frequencies of alleles and genotypes of the 5a/6a polymorphism of the MMP3 gene in the studied groups of patients and controls (χ^2 < 3.8; p>0.05). In a comparative analysis of the III subgroup of patients and the control group, statistically significant differences were revealed between them in the distribution of frequencies of alleles and genotypes of the polymorphic marker 5a/6a of the MMP3 gene (Table 17.)

In the course of a comparative analysis of the distribution of frequencies of alleles and genotypes of the polymorphic marker 5a/6a of the MMP3 gene among a subgroup of patients with DESA and the control sample, it was revealed that the risk factor for the formation of vascular pathology is the 5a allele and genotypes 5a/6a and 5a/5a, and the polymorphic option – 6a/6a [1].

Table 17 Differences in the distribution of alleles and genotypes of the polymorphic marker 5a/6a of the MMP3 gene in the main group and the control group

Alleles and genotypes	Frequency of alleles and genotypes in groups		Statistical difference
	subgroup B	Control	
Allele 6a	61	290	χ^2 = 12.6 ; P = 0.0004 ; OR= 2.5 ; 95% CI 1.50- 4.33
Allele 5a	29	54	
Genotype 6a/6a	24	131	χ^2 =9 , 1; P = 0.002 ; OR= 0.4 ; 95% C.I. 0.18 - 0.7 1
Genotype 6a/5a	13	28	χ^2 =3 , 7; P = 0.05 ; OR= 2.1 ; 95% CI 0.976, 4.473
Genotype 5a/5a	8	13	χ^2 =2.3; P=0.04; OR =2.6; 95% CI 1.02-6.842
Genotypes 6a/5a+5a/5[a]	21	41	χ^2 =9.1; P=0.002; OR =2.8; 95% CI 1.41- 5.533

Allele 5a was determined more often in the group of patients (32.2%) than in the control group (15.7%) while allele 6a *occurred* with a higher frequency in the control group (84.3%) compared to the subgroup of patients with DESA (67.8%). At the same time, these differences reached the level of statistical significance (χ^2 =12.6; P = 0.0004; OR = 2.5; 95% CI 1.50-4.33).

Homozygous genotype 6a/6a occurs in 53.3% of patients, while in the control group it was detected in 76.2%. Such differences turned out to be statistically significant (χ^2 =9.1; P=0.002; OR =0.4; 95% CI 0.18-0.71), which confirms the protectiveness of this genotype with respect to the formation of angiodysplasia with the development of dyscirculatory encephalopathy [1, 5].

Differences were revealed in the concentrations of combinations of unfavorable genotypes 6 a /5 a +5 a /5 a between the subgroup of patients and the control sample. This combination occurred 2.8 times significantly more often among patients (46.7%) than in the control group (23.8%), with χ^2 =9.1; P=0.002; OR =2.8; 95% CI 1.41-5.533.

As can be seen in table 18, genotypes of the MMP3 gene are mainly associated with pathological tortuosity. So, as with hypo /aplasia of PSA, PSA and PA, they were significantly less common: 37(15%) and 13(5.3%), respectively [1].

Table 18 Frequency of cerebral vascular anomalies depending on the polymorphic marker 5a/6a of the MMP3 gene

Types of anomalies/genotype	6a/6a		6a/5a		5a/5a		6a/5a+5a/5a		Total	
Hypo /aplasia PSA, PSA	-	6	16.2	8	21.6	23	62.2	37	15.0
Hypo /aplasia PA	-	1	7.7	4	30.8	8	61.5	13	5.3
Trifurcations of the ICA	-	2	13.4	5	33.3	8	53.3	15	6.1
S- and C -shaped crimps	-	8	9.2	26	29.8	53	61.0	87	35.5
Kk+Coiling	-	7	7.5	33	35.5	53		93	38.0
Total	-	24	9.8	76	31.0	145		245	100

4. Discussion and Conclusion

At the same time, hypo /aplasia of PSA, PSA and PA in 23 (62.2%): 8 (61.5%) cases, respectively, were associated with combined genotypes type 6a/5a+ 5a/5a, in 8 (21.6%) : 4 (30.8%) with the mutant 5a/5a genotype, only 6 (16.2%): 1 (7.7%), respectively, with the heterozygous genotype 6a/5a. In patients with ICA trifurcations , a total of 15 (6.1%) had various variants of MMP3 genotypes, and association with the combined genotype type 6a/5a+ 5a/5a occurred in the largest number of patients (8 (53.3%)); and with the mutant homozygous genotype 5a/5a in 5 (33.3%) cases, heterozygous S- and C -shaped tortuosity in 53 (61%) were associated with combined genotypes type 6a/5a+ 5a/5a, and the mutant homozygous genotype in 26 (29.8%) cases was associated with the above tortuosity, heterozygous genotype 6a/5a occurred in 8 (9.2%) cases. Pathological tortuosity in the form of kinking and coiling were also associated with combined genotypes in 53 (57%) cases, in 33 (35.4%) with the mutant homozygous 5a/5a variant and in 7 (7.5%) with the heterozygous variant [1, 4].

Summarizing the data obtained, we can conclude that the polymorphic marker 5a/6a of the MMP3 gene plays a significant role in the formation of cerebral vascular abnormalities with the development of migraine and dyscirculatory encephalopathy.

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