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FEATURES OF MTHFR (A1298C) ALLELIC POLYMORPHISM AMONG CHILDREN WITH CONGENAL DEFECTS OF THE MAXILLOFACIAL AREA IN UZBEKISTAN

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Abstract

The features of allelic polymorphism of the MTHFR gene (A1298C) were studied among children with congenital defects of the maxillofacial region (CDMR) in Uzbekistan. The study of the genetic polymorphism of MTHFR (A1298C) was carried out by analyzing DNA samples using Real Time PCR. The results of the study made it possible to establish that, based on the polymorphism of the MTHFR gene (A1298C), among carriers of the minor allele C and the mutant genotype C/C, the risk of developing Q35 increases by 1.6 times (χ^2=2.5; P=0.2) and 3.2 times (χ^2=2.7; p =0.1) respectively. Meanwhile, a statistically significant connection with an increased risk of Q36 formation was established in carriers of the minor allele C by 2.0 times (χ^2=5.4; p=0.025), with a tendency to increase its development in carriers of the heterozygous genotype A/C by 2.1 times (χ^2=3.6 ; p=0.1). In addition, there was a statistically significant increase in the frequency of occurrence of the minor allele C and the heterozygous genotype A/C in the group of children with Q36 compared to those in the group with Q37 by 2.6 times (χ^2=6.2; P=0.025) and 2.8 times (χ^2=4.5 ; P=0.05) may indicate their contribution to increasing the risk of Q36 formation.

Therefore, the MTHFR gene polymorphism (A1298C) can be considered as a genetic predictor of an increased risk of developing CDMR (Q35 and Q36) in Uzbekistan.

Keywords: CDMR, cleft palate, cleft lip, cleft palate and lip, polymorphism of the MTHFR gene (A1298C), allele, frequency, genotype, carrier fraction, increased risk of formation.

Actuality

It is known that among congenital malformations, the problem of congenital malformations of the maxillofacial region (CDMR) is attracting increasing attention and interest of researchers around the world [5,6]. This interest is due to their widespread and high prevalence, the severity of clinical manifestations, which

inevitably lead to disruption of adaptation, an increase in the percentage of disability and a decrease in the patient's quality of life [7]. The pathogenesis of CDMR is based on complex disorders that arise as a result of the interaction of a combination of external and internal environmental agents [3,8,11,12,17].

The causal and pathogenetic mechanisms of the formation of this complex group of pathologies remain completely unclear to date [4,15]. Meanwhile, progress in medical science associated with the widespread use of genetic research today allows us to better understand the mechanisms of initiation of many pathologies, including CDMR. In particular, modern research is aimed at assessing the significance of a number of genetic polymorphisms involved in the formation of complex disorders that contribute to an increased risk of developing CDMR [9,19,24]. Among the most significant polymorphic genes that have a certain contribution to the initiation of the onset of CDMR, an important place is given to the genes of the folate cycle, namely the MTHFR gene, which encodes an intracellular enzyme involved in the conversion of homocysteine to methionine [14,20,26]. Replacement of adenine (A) with cytosine (C) at position 1298 of the DNA section of the MTHFR gene leads to the formation of a polymorphic variant of the MTHFR gene (A1298C) with a change in its biochemical properties and a decrease in the activity of the corresponding enzyme, which directly increases the likelihood of developing defects in fetal formation [2, 23].

Taking these facts into account, a number of studies have been conducted to assess the significance of the polymorphic variant of the MTHFR gene (A1298C) in the mechanisms of CDMR formation [10,25]. At the same time, the available research results in this area are distinguished by their inconsistency [1,21].

In turn, diverse views in this regard serve as the basis for additional research to study the contribution of the polymorphic variant of the MTHFR gene (A1298C) to the mechanisms of CDMR formation.

Material and Methods

The study was conducted with the participation of 105 children with CDMR (main CDMR group) with a median age of 6.5±1.8 years, living in the territory of the Republic of Uzbekistan, who were observed in the clinic of the Tashkent State Dental Institute in the period from 2019 to 2022. In accordance with the International Classification of Diseases, 10th revision (ICD 10), all children with CDMR (n=105), depending on the nosology, are divided into three groups: Q35 (n=35) – children with cleft palate; Q36 (n=33) – children with cleft lip; Q37 (n=37) – children with cleft palate and lip. The comparison control group consisted of 103 healthy children

without a history of congenital defects, matched by place of residence, age and gender with the main group of children with CDMR.

Molecular genetic studies were carried out in the laboratory of molecular genetics, cytogenetics and FISH of the Republican Specialized Scientific and Practical Medical Center of Hematology (RSSPMCH, Republic of Uzbekistan, Tashkent). In accordance with the generally accepted method, DNA was isolated from blood leukocytes and using test systems "Litech" (Russia) using the "Applied Biosystems" 2720 (USA) system, a study (SNP-PCR) of the polymorphism of the MTHFR gene (A1298C) was carried out by staging polymerase chain reaction (PCR) in Real Time mode (Rotor Gene Q, Quagen, Germany). Mathematical processing of the results was carried out using the statistical program “OpenEpi 2009, Version 9.2”.

Results and Discussion

Among children with CDMR (n=105) and the control group (n=103), an analysis was carried out of the correspondence of the observed and expected genotypic frequencies of the MTHFR polymorphism (A1298C) to the Hardy-Weinberg equilibrium (HW, p>0.05), which was characterized by the absence of deviations from the HW (p>0.05).

Features of the distribution of the polymorphic variant of the MTHFR gene (A1298C) in the main group of CDMR patients (n=105) were characterized by a slightly lower frequency of occurrence of the major allele A (70.5% versus 76.7%) and a higher frequency of the minor allele C (29.5% versus 23.3%) than in the control group group (n=103). In addition to these features, differences were also found in the frequency distribution of all three genotype variants. In particular, the wild A/A genotype among CDMR patients was found in 41.8% (n=51), the heterozygous A/C genotype in 43.8% (n=46) and the mutant C/C genotype in 7.6% (n=8) of cases. At the same time, in the compared control group, the proportion of similar ones was 57.3% (n=59), 38.8% (n=40) and 3.9% (n=4), respectively (Table 1).

Table 1 Distribution of allele and genotype frequencies of the MTHFR genetic polymorphism (A1298C) in groups of patients with CDMR and healthy controls

№	Group	Allele frequency				Genotype frequency					
		A		C		A/A		A/C		C/C	
		n	%	n	%	n	%	n	%	n	%
I	CDMR main group, n=105	148	70.5	62	29.5	51	48.6	46	43.8	8	7.6
II	Q35 (cleft palate), n=35	47	67.1	23	32.9	16	45.7	15	42.9	4	11.4
III	Q36 (cleft lip), n=33	41	62.1	25	37.9	11	33.3	19	57.6	3	9.1
IV	Q37 (cleft palate and lip), n=37	60	81.1	14	18.9	24	64.9	12	32.4	1	2.7
V	Control group, n=103	158	76.7	48	23.3	59	57.3	40	38.8	4	3.9

Based on the results obtained, the fact of the predominance of the frequencies of occurrence of unfavorable alleles and genotypes among patients with CDMR in the general group is obvious.

By studying the distribution features of the polymorphic MTHFR gene (A1298C) separately, depending on the nosology, interesting facts were established. Thus, in the group of patients with Q35 (n=35) and Q36 (n=33), the proportions of occurrence of the major allele A were the smallest (67.1% and 62.1%, respectively) with the highest occurrence of the minor allele C (32.9% and 37.9%, respectively. Together with this, in comparison with all other groups, the frequencies of the major A/A genotype in groups with Q35 (45.7%) and Q36 (33.3%) also turned out to be the lowest. Accordingly, the carriage of heterozygous A/C (42.9% and 57.6%) and mutant C/C (11.4% and 9.1%) genotypes were most often determined in these two groups.

In the group of patients with Q37, alleles A and C were determined in 81.1% and 18.9% of cases, respectively, and genotypes A/A, A/C and C/C in 64.9%, 32.4% and 2.7% of cases, respectively.

Thus, the results of studying the characteristics of the distribution of alleles and genotypes of the polymorphic gene MTHFR (A1298C) in the studied groups of patients and healthy controls differed from each other by an increase in the frequency of occurrence of unfavorable alleles (C) and genotypes (A/C and C/C) in the main group with CDMR due to their increase in groups of patients with Q35 and Q36, which may be associated with their contribution to the increased risk of developing these two pathologies.

To clarify this assumption, the severity of differences in the distribution of allelic and genotypic variants of the MTHFR gene polymorphism (A1298C) between the groups of patients and controls was statistically analyzed.

During the comparative analysis between the main and control groups, it was revealed that the minor allele C was statistically insignificantly registered more often among patients of the main group with CDMR by 1.4 times (29.5% versus 23.3%; χ^2=2.1; P=0.2; OR=1.4; 95%CI : 0.89-2.14). At the same time, statistically insignificant differences were revealed between the frequencies of the wild genotype A/A and the heterozygous genotype A/C (56.2% versus 51.5%; χ^2=0.5; P=0.5; OR=1.2; 95%CI: 0.7-2.09) and 1.2 times (56.2% versus 51.5%; χ^2=0.5; P=0.5; OR=1.2; 95%CI: 0.7-2.09).

Along with these features in this group of patients, it is important to emphasize the absence of a significant difference in the frequency of the mutant genotype C/C,

which turned out to be 2.0 times higher compared to the control (7.6% versus 3.9%; χ^2=1.3; P=0.3; OR=2.0 ; 95%CI: 0.61-6.85) (Table 2).

Consequently, the results of a comparative analysis of differences in the distribution of frequencies of alleles and genotypes of the polymorphic gene MTHFR (A1298C) between the main group of patients with CDMR and healthy ones showed a lack of statistical significance (χ^2<3.84; p>0.05).

Differences in the carriage of alleles and genotypes for the MTHFR gene polymorphism (A1298C) in the main group of patients with CDMR compared to the control were detected due to differences in the groups with Q35 and Q36. In this regard, a comparative assessment of the differences between these groups of patients and controls was further carried out.

The results of comparison between groups of patients with Q35 and healthy ones revealed the presence of a statistically insignificant increase in the unfavorable C allele among patients by 1.6 times (32.9% versus 23.3%; χ^2=2.5; p=0.2; OR=1.6; 95%CI: 0.89-2.91) and a tendency to increase the risk of developing this pathology by 3.2 times among carriers of the mutant C/C genotype (11.4% versus 3.9%; χ^2=2.7; p=0.1; OR=3.2; 95%CI: 0.8-12.68). At the same time, although the proportion of the heterozygous genotype A/C also turned out to be 1.2 times higher among patients (42.9% versus 38.8%; χ^2=0.2; p=0.7; OR=1.2; 95%CI: 0.54-2.57), it was still detected the difference was not statistically significant.

Table 2 Analysis of the association of MTHFR gene polymorphism (A1298C) with the risk of developing CDMR

Study groups	Alleles and genotypes	The difference is statistical compared to control					
		RR	95% CI:	OR	95% CI:	χ^2	p (confidence)
I - main group CDMR, (n=105)	A	0.9	0.62-1.36	0.7	0.47-1.12	2.1	0.2
	C	1.1	0.68-1.74	1.4	0.89-2.14		
	A/A	0.8	0.5-1.44	0.7	0.41-1.22	1.6	0.3
	A/C	1.1	0.67-1.91	1.2	0.7-2.13	0.5	0.5
	C/C	2.0	0.85-4.51	2.0	0.61-6.85	1.3	0.3
II – group with Q35, (n=35)	A	0.9	0.38-1.99	0.6	0.34-1.12	2.5	0.2
	C	1.1	0.81-1.62	1.6	0.89-2.91		
	A/A	0.8	0.26-2.46	0.6	0.29-1.35	1.4	0.3
	A/C	1.1	0.36-3.41	1.2	0.54-2.57	0.2	0.7
	C/C	2.9	0.67-13	3.2	0.8-12.68	2.7	0.1
III – group c Q36, (n=33)	A	0.8	0.36-1.84	0.5	0.28-0.9	**5.4**	**0.025**
	C*	1.2	0.87-1.76	2.0	1.12-3.61		
	A/A*	0.6	0.17-2.04	0.4	0.17-0.84	**5.7**	**0.025**
	A/C	1.5	0.4-4.82	2.1	0.97-4.7	3.6	0.1

	C/C	2.3	0.39-13.9	2.5	0.55-11.2	1.4	0.3
IV – group c Q37, (n=37)	A	1.1	0.39-2.87	1.3	0.67-2.53	0.6	0.5
	C	0.9	0.69-1.29	0.8	0.4-1.49		
	A/A	1.1	0.36-3.56	1.4	0.63-3.0	0.6	0.5
	A/C	0.8	0.26-2.69	0.8	0.34-1.67	0.5	0.5
	C/C	0.7	0.02-22.6	0.7	0.08-6.28	0.1	0.8

Statistically significant differences in the distribution of allelic and genotypic variants for the MTHFR gene polymorphism (A1298C) were found between groups of patients with Q36 and healthy individuals. First of all, this was manifested by a statistically significant difference in the proportion of allele C, which was 2.0 times higher among patients with Q36 than in controls (37.9% versus 23.3%; χ^2=5.4; p=0.025; OR=2.0; 95%CI: 1.12- 3.61).

Moreover, compared with the control in the group of patients with Q36, a tendency was found to increase the proportion of the heterozygous genotype A/C by 2.1 times (57.6% versus 38.8%; χ^2=3.6; p=0.1; OR=2.1; 95%CI: 0.97- 4.7) and an insignificant increase in the frequency of the mutant genotype C/C by 2.5 times (9.1% versus 3.9%; χ^2=1.4; p=0.3; OR=2.5; 95%CI: 0.55-11.2).

All these data show the presence of a contribution of unfavorable allele (C) and genotype (A/C) of the polymorphic MTHFR gene (A1298C) to the mechanisms of Q36 formation.

Differences in the carriage of allelic and genotypic variants of the MTHFR gene polymorphism (A1298C) between the groups of patients with Q37 and healthy individuals were not statistically significant. Thus, among patients, compared with controls, carriage of the minor allelic variant C (18.9% versus 23.3%; χ^2=0.5; P=0.6; OR=0.8; 95%CI: 0.4-1.49), heterozygous genotype A/C (32.4% versus 38.8%; χ^2=0.5; P=0.5; OR=0.8; 95%CI: 0.34-1.67) and homozygous minor genotype C/C (2.7% versus 3.9%; χ^2<3.84; p=0.8; OR=0.7; 95%CI: 0.08-6.28) differences were less than one.

Further assessing the degree of differences in the distribution frequencies of variant alleles and genotypes of the MTHFR gene polymorphism (A1298C) between the groups of patients with Q35 and Q36, we were unable to identify their statistical significance in relation to the minor allele C (32.9% versus 37.9%; χ^2<3.84; P= 0.6; OR=0.8; 95%CI: 0.4-1.62), as well as genotypes A/C (42.9% versus 57.6%; χ^2=1.5; P=0.3; OR=0.6; 95%CI: 0.21-1.44) and C /C (11.4% vs. 9.1%; χ^2<3.84; P=0.8; OR=1.3; 95%CI: 0.27-6.24).

However, differences in the frequencies of alleles and genotypes of the polymorphic gene MTHFR (A1298C) in the group with Q35 compared to the Q37 group were

characterized by a tendency to increase the unfavorable allele C in the group by 2.1 times (32.9% versus 18.9%; χ^2=3.7; P=0.1; OR =2.1; 95%CI: 0.98-4.48), with a statistically insignificant decrease in the frequencies of the major genotype A/A (45.7% versus 64.9%; χ^2=2.7; P=0.2; OR=0.5; 95%CI: 0.18-1.17) and mutant variant of genotype C/C by 4.6 times (11.4% versus 2.7%; χ^2=2.1; P=0.2; OR=4.6; 95%CI:0.59-36.73).

The results of the study prove that the minor allele C and genotype C/C of the MTHFR gene polymorphism (A1298C) increase the risk of developing Q35.

Statistically significant differences in the distribution of allele frequencies and genotypes of the MTHFR gene polymorphism (A1298C) differed between the groups of patients with Q36 and Q37. Thus, the frequency of the minor allele C in the group of patients with Q36 was statistically significantly higher than that in the group with Q37 by 2.6 times (37.9% versus 18.9%; χ^2=6.2; P=0.025; OR=2.6; 95%CI: 1.23-5.55). At the same time, the heterozygous genotype A/C increased the risk of Q36 formation by 2.8 times (57.6% versus 32.4%; χ^2=4.5; P=0.05; OR=2.8; 95%CI: 1.08-7.41), reaching statistical significance, while for the mutant genotype with an increase in its frequency by 3.6 times (9.1% versus 2.7%; χ^2=1.3; P=0.3; OR=3.6; 95%CI: 0.41-31.98) it did not reach a significant character compared to that in the group with Q37 (Table .3).

Table 3 Differences in the frequency of allelic and genotypic variants of the MTHFR polymorphism (A1298C) between groups of patients with Q36 and Q37

Alleles and genotypes	Number of alleles and genotypes examined				χ^2	P	RR	95%CI	OR	95%CI
	Q36		Q37							
	n	%	n	%						
A	41	62.1	60	81.1	6.2	0.025	0.8	0.4-1.47	0.4	0.18-0.81
C*	25	37.9	14	18.9	**6.2**	**0.025**	**1.3**	**0.54-3.15**	**2.6**	**1.23-5.55**
A /A*	11	33.3	24	64.9	6.9	0.01	0.5	0.17-1.51	0.3	0.1-0.72
A /C*	19	57.6	12	32.4	**4.5**	**0.05**	**1.8**	**0.6-4.77**	**2.8**	**1.08-7.41**
C /C	3	9.1	1	2.7	1.3	0.3	3.4	0.99-11.4	3.6	0.41-31.98

Thus, the results of the study, compared with the control group, show that among carriers of the mutant C/C genotype for the MTHFR gene polymorphism (A1298C), there is a tendency to increase the risk of Q35 formation by 3.2 times (χ^2=2.7; p=0.1). At the same time, a statistically significant connection with an increased risk of Q36 formation was established in carriers of the minor allele C by 2.0 times (χ^2=5.4; p=0.025), with a tendency to increase its development in carriers of the heterozygous genotype A/C by 2.1 times (χ^2=3.6; p=0.1).

In addition, there was a statistically significant increase in the frequency of occurrence of the minor allele C and the heterozygous genotype A/C in the group of children with Q36 compared to those in the group with Q37 by 2.6 times (χ^2=6.2; P=0.025) and 2.8 times (χ^2=4.5 ; P=0.05) prove their association with an increased risk of Q36 formation.

Conclusion

Congenital malformations of the maxillofacial region (CDMR) are complex pathologies with a mechanism of formation that is not fully understood [13,16]. Meanwhile, today there are opinions about the contribution of polymorphic genes of the folate cycle to the initiation of CDMR [22]. In this regard, of particular interest to researchers is the study of the contribution of MTHFR (A1298C) to the initiation of fetal malformations, ultimately leading to the onset of CDMR [3,17,18]. However, researchers are divided in their opinion, some provide results on the presence of an association of MTHFR (A1298C) with an increased risk of developing CDMR, others on its absence [1,21].

Given these conflicting results, we thought it would be interesting to study the association of the MTHFR polymorphic variant (A1298C) with an increased risk of CDMR formation in Uzbekistan. In our studies, in comparison with the control group, it was found that according to the polymorphism of the MTHFR gene (A1298C) among carriers of the minor allele C and the mutant genotype C/C, the risk of developing Q35 increases by 1.6 times (χ^2=2.5; P=0.2) and 3.2 times (χ^2=2.7; p=0.1) respectively. Meanwhile, a statistically significant connection with an increased risk of Q36 formation was established in carriers of the minor allele C by 2.0 times (χ^2=5.4; p=0.025), with a tendency to increase its development in carriers of the heterozygous genotype A/C by 2.1 times (χ^2=3.6 ; p=0.1). In addition, there was a statistically significant increase in the frequency of occurrence of the minor allele C and the heterozygous genotype A/C in the group of children with Q36 compared to those in the group with Q37 by 2.6 times (χ^2=6.2; P=0.025) and 2.8 times (χ^2=4.5 ; P=0.05) may indicate their contribution to increasing the risk of Q36 formation.

Therefore, the MTHFR gene polymorphism (A1298C) can be considered as a genetic predictor of an increased risk of developing CDMR (Q35 and Q36) in Uzbekistan.

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