BRITISH MEDICAL JOURNAL

British Medical Journal Volume-2, No 4
10.5281/zenodo.7361130
British Medical Journal
Volume 2, No 4., 2022
Internet address: http://ejournals.id/index.php/bmj
E-mail: info@ejournals.id
Published by British Medical Journal
Issued Bimonthly
3 knoll drive. London. N14 5LU United Kingdom
+44 7542 987055
Chief Editor
Dr. Fiona Egea

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British Medical Journal Volume-2, No 4
10.5281/zenodo.7361130

PECULIARITIES OF THE GENETIC VARIANT OF MDR1(C3435T) POLYMORPHISM IN NON-SYNDROME CONGENITAL MAULTS OF THE MAXILLOFACIAL REGION

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Abstract: The features of MDR1 (C3435T) polymorphism in congenital malformations of the maxillary region of Uzbekistan were studied. The results of a comparative assessment of differences in the distribution of allele and genotype frequencies for the polymorphic gene MDR 1 (C3435T) among the studied groups of patients with HPVLO and healthy people were characterized by the absence of their statistical significance. In this regard, based on the results of the study, we cannot state that the polymorphism of the MDR1 gene (C3435T) is involved in the formation of HPVLO in general, and therefore this polymorphic gene cannot be considered as an independent genetic marker that increases the likelihood of developing HPVLO in Uzbekistan.

Keywords: congenital malformations of the maxillary region, genetic polymorphism MDR1 (C3435T), allele, frequency, genotype, carrier proportion, isolated cleft palate (Q 35), isolated cleft lip (Q 36), combined cleft palate and lip (Q 37).

Relevance. Congenital malformations of the maxillary region (HPL) are a very complex group of multifactorial diseases, which include isolated cleft palate (Q 35), isolated cleft lip (Q 36) and combined cleft palate and lip (Q 37) [12,13]. In the mechanisms of development of these defects, many aspects still remain not fully disclosed [8,10,11]. At the same time, molecular genetic studies of a number of leading experts have shown that in the initiation of pathological processes that contribute to the onset of HPVLO, a special contribution belongs to various genetic polymorphisms of xenobiotics involved in the metabolism [7,9,14,15].

MDR 1 gene is of particular interest [6]. It is known that the MDR 1 gene, being the gene of the xenobiotic detoxification system, is expressed in the plasma membranes of cells and organs, encodes a cellular transmembrane P-glycoprotein, which removes a wide range of xenobiotic compounds from cells [1,2,5]. The most common polymorphic variant of the MDR 1 gene with a replacement in the DNA sequence of the cytosine nucleotide (major allele C) at position 3435 with thymine

(minor allele T) of the gene (MDR 1 C3435T) with the formation of genotypic C/C variants; S/T and T/T [3,4].

Changes in the structure of the MDR 1 gene are accompanied by disturbances in its activity, which is fraught with the launch of complex pathological processes, which are the basis for the development of HPVLO [3], which served as the basis for conducting studies to assess the contribution of the MDR 1 (C3435T) polymorphism to the formation of HPVLO in Uzbekistan.

Material and methods. The study involved 105 children (mean age 6.5±1.8 years) with HPVLO (the main group of HPVLO) living on the territory of the Republic of Uzbekistan, who were under observation at the clinic of the Tashkent State Dental Institute in the period from 2019 to 2022. In accordance with the international classification of diseases of the 10th revision (ICD 10), all children with HPVLO (n = 105), depending on the nosology, are divided into three groups: Q 35 (n = 35) - children with cleft palate; Q 36 (n =33) - children with cleft lip; Q 37 (n = 37) - children with cleft palate and lips. The compared control group consisted of 103 healthy children with no history of congenital malformations, comparable in place of residence, age and gender with the main group of children with HPVLO.

Molecular genetic studies of the features of the polymorphic gene MDR 1 (C3435T) were carried out in the laboratory of molecular genetics, cytogenetics and FISH of the Republican Specialized Scientific and Practical Medical Center for Hematology (RSNPMCG, Republic of Uzbekistan, Tashkent). In accordance with the generally accepted method, DNA was isolated from blood leukocytes and the C3435T polymorphism of the MDR 1 gene was studied (Rotor Gene Q, Quagen, Germany). The results were processed using the statistical program " OpenEpi 2009, Version 9.2".

Results and discussion. Analysis of the distribution of expected and observed genotype frequencies according to the genetic polymorphism MDR 1 (C3435T) in groups of patients with HPVLO and healthy people showed their correspondence to the canonical distribution according to the Hardy-Weinberg equilibrium (HWB) ($p>0.05$).

Examining the features of the occurrence of the polymorphic variant of the MDR 1 gene (C3435T) in the main group of patients with HPVLO (n = 105), it was found that the carriage of the main (C) and minor (T) alleles differs little from their shares in the compared control group (n = 103). So, their values, respectively, for the studied groups were 73.8% (n =155) versus 72.3% (n =149) and 26.2% (n =55) versus 27.7% (n =57), respectively. At the same time, the carriage of C/C, C/T and T/T genotypes in the main group of patients with HPVLO was determined in 56.2% (n = 59), 35.2% (n = 37) and 8.6% (n = 9) cases, respectively, while in the control group their proportions were 51.5% (n =53), 41.7% (n =43) and 6.8 % (n =7), respectively (Table 1).

Table 1

Distribution of frequencies of alleles and genotypes of polymorphism C 3435 T in the MDR 1 gene in HPVLO patients and healthy controls

18	Group	Allele frequency	Frequency distribution of genotypes

		FROM		T		S/S		S/T		T/T	
		n	%	N	%	n	%	n	%	n	%
I	Main HPVLO group, n=105	155	73.8	55	26.2	59	56.2	37	35.2	9	8.6
II	Q35 (cleft palate), n=35	51	72.9	19	27.1	12	57.1	11	31.4	4	11.4
III	Q36 (cleft lip), n=33	48	72.7	18	27.3	18	54.5	12	36.4	3	9.1
IV	Q37 (cleft palate and lip), n=37	56	75.7	18	24.3	21	56.8	14	37.8	2	5.4
V	Control group, n=103	149	72.3	57	27.7	53	51.5	43	41.7	7	6.8

The data show that among the subjects of the main group, the frequency of the wild C/C genotype and the mutant T/T genotype is slightly higher than those in the control, but the proportion of carriers of the heterozygous C/T genotype was less detected among patients with HPVLO.

Assessing the features of the occurrence of variants of the polymorphic gene MDR 1 (C3435T) in the group of patients with Q35 (n=35) and Q36 (n=33) in relation to the major and minor alleles, their carriage was found in 72.9% (n=51) and 72.7% (n =48), as well as 27.1% (n=19) and 27.3% (n=18) cases, respectively. However, among patients with Q37 (n=37), in relation to all the groups considered, the frequency of the C allele was determined somewhat more often (75.7%), and the T allele, naturally, somewhat less frequently (24.3%).

Along with these features, when determining the frequencies of genotypes, it was found that in the group with Q35, the C/C, C/T and T/T variants were detected in 57.1% (n=20), 31.4% (n=11) and 11.4% (n= 4) cases, respectively; in the group with Q36 in 54.5% (n=18), 36.4% (n=12) and 9.1% (n=3) cases, respectively, and in the group with Q37 in 56.8% (n=21), 37.8% (n= 14) and 5.4% (n=2) cases, respectively. Meanwhile, the analysis shows that the highest frequency of the mutant homozygous T/T genotype was determined among patients with cleft palate (Q35), and the lowest in the group of patients with combined cleft palate and lip (Q37). Therefore, from the above results, it is obvious to us that there are more significant differences in the proportion of the distribution of the minor T/T genotype according to the MDR 1 (C3435T) polymorphism in the groups with Q35 and Q36 studied by the main group compared with the control group.

10.5281/zenodo.7361130

To assess the degree of participation of allelic and genotypic variants of the MDR 1 (C3435T) gene polymorphism in the pathogenesis of HPVLO, a comparative analysis of the differences in their distribution between all the studied groups was further carried out.

In the main group of patients with HPVLO, compared with the control, the T allele was statistically insignificantly less frequently registered less than once (26.2% vs. 27.7%; $\chi 2$=0.1; P=0.8; OR=0.9; 95% CI: 0.6-1.43). At the same time, the main C/C genotype, on the contrary, although not statistically significant, was still 1.2 times higher among HPVLO patients (56.2% vs. 51.5%; $\chi 2$=0.5; P=0.5; OR=1.2; 95% CI: 0.7 -2.09) similar in control. The heterozygous variant of the C/T genotype, compared with healthy ones, was registered less than once among patients (35.2% versus 41.7%; $\chi 2$=0.9; P=0.4; OR=0.8; 95% CI: 0.43-1.33), while the mutant T/T genotype was determined more often by 1.3 times (8.6% vs. 6.8%; $\chi 2$=0.2; P=0.7; OR=1.3; 95% CI: 0.46-3.58), respectively, without reaching statistical significance (Table 2).

Comparing differences in the distribution of polymorphism of the MDR 1 gene (C3435T) between groups of patients with Q 35 and healthy individuals revealed no significant differences in the carriage of the allelic variant T (27.1% vs. 27.7%; χ^2 <3.84; P=0.95; O R =1.0; 95%CI: 0.53-1.79) . In addition, despite the excess of the frequency of carriage of the main C / C genotype by 1.3 times (57.1% vs. 51.5%; χ 2 < 3.84 ; P = 0.6; O R = 1.3; T 1.8 times (11.4% vs. 6.8%; χ^2 =0.8; P=0.4; O R =1.8; 95% CI: 0.49-6.36), as well as a lower frequency of the heterozygous C / T genotype (31.4% vs. 41.7% ; χ^2 \u003d 1.2; P \u003d 0.3; O R \u003d 0.6; 95% CI: 0.28-1.44) in the group of patients with Q 35, the differences compared to control values did not differ in statistical significance (Table 2).

table 2

Analysis of the association of C3435T polymorphism in the MDR1 gene with the risk of HPVLO formation (differences compared with control)

Study Groups	Alleles and genotypes	Statistical difference compared to control					
		RR	95%CI:	OR	95%CI:	χ^2	p (confidence)
I -o main group HPVLO , (n=105)	FROM	1.0	0.67-1.57	1.1	0.7-1.66	0.1	0.8
	T	1.0	0.64-1.49	0.9	0.6-1.43		
	S/S	1.1	0.64-1.86	1.2	0.7-2.09	0.5	0.5
	S/T	0.8	0.48-1.48	0.8	0.43-1.33	0.9	0.4
	T/T	1.3	0.52-3.07	1.3	0.46-3.58	0.2	0.7
II - group c Q35, (n= 35)	FROM	1.0	0.41-2.46	1.0	0.56-1.89	0.0	0.95
	T	1.0	0.74-1.34	1.0	0.53-1.79		
	S/S	1.1	0.36-3.46	1.3	0.58-2.72	0.3	0.6
	S/T	0.8	0.22-2.57	0.6	0.28-1.44	1.2	0.3
	T/T	1.7	0.32-8.71	1.8	0.49-6.36	0.8	0.4

III - group c Q36, (n= 33)	FROM	1.0	0.4-2.54	1.0	0.55-1.9	0.0	0.95
	T	1.0	0.74-1.33	1.0	0.53-1.83		
	S/S	1.1	0.33-3.41	1.1	0.52-2.49	0.1	0.8
	S/T	0.9	0.26-2.94	0.8	0.35-1.79	0.3	0.6
	T/T	1.3	0.19-9.44	1.4	0.34-5.61	0.2	0.7
IV – group c Q3 7, (n= 37)	FROM	1.0	0.42-2.58	1.2	0.65-2.2	0.3	0.6
	T	1.0	0.71-1.29	0.8	0.46-1.55		
	S/S	1.1	0.37-3.3	1.2	0.58-2.64	0.3	0.6
	S/T	0.9	0.3-2.78	0.8	0.39-1.84	0.2	0.7
	T/T	0.8	0.07-9.3	0.8	0.16-3.94	0.1	0.8

A similar pattern was observed in the difference in allelic and genotypic variants of the MDR 1 (C3435T) gene polymorphism between groups of patients with Q 36 and healthy individuals. Thus, a statistically insignificant difference was found in the carriage of the allelic variant T (27.3% versus 27.7%; χ 2 <3.84; P=0.95; O R =1.0; 51.5%; χ^2 <3.84; P=0.8; O R =1.1; 95% CI: 0.52-2.49) and heterozygous C/T genotype (36.4% vs. 41.7%; χ^2 =0.3; P=0.6; O R = 0.8; 95%CI: 0.35-1.79). At the same time, in relation to the homozygous minor T/T genotype, although very weak, there was a tendency to increase its frequency by 1.4 times compared to that in the control group (9.1% vs. 6.8%; χ^2 <3.84; P= 0.7; O R =1.4; 95% CI: 0.34-5.61) (Table 2).

Differences in the carriage of allelic and genotypic variants of the MDR 1 (C3435T) gene polymorphism between groups of patients with Q 37 and healthy individuals again did not reach statistical significance. For example, in the group of patients compared with the control in the carriage of the minor allelic variant T (24.3% vs. 27.7%; χ^2 <0.3; P=0.6; O R =0.8; 95% CI: 0.46-1.55) , heterozygous genotype C/ T (37.8% vs. 41.7%; χ^2 =0.3; P=0.6; O R = 0.8; 95% CI: 0.39-1.84) and homozygous minor T/T genotype (5.4% vs. 6.8%; χ^2 <3.84; P =0.8; O R =0.8; 95%CI: 0.16-3.94) the differences were less than one (Table 2).

The next stage of the study was aimed at assessing the degree of difference in the frequencies of distribution of variants of alleles and genotypes of the MDR 1 (C3435T) gene polymorphism between groups of patients with Q35 and Q36, in which the differences for the minor allele T were one (27.1% vs. 27.3%; χ^2 < 3.84; P=0.99; O R =1.0; 95% CI: 0.47-2.12), for the C/C genotype - 1.1 in favor of the group with Q 35 (57.1% vs. 54.5%; χ^2 <3.84; P=0.9; O R \u003d 1.1 ; 95% CI: 0.43-2.9) , for the variant of the C / T genotype - less than one (31.4% vs.) and for the mutant T/T genotype - 1.3 (11.4% versus 9.1%; χ^2 =0.1; P=0.8; O R =1.3; 95% CI: 0.27-6.24) .

of alleles and genotypes of the MDR 1 gene polymorphism (C3435T) between the groups of patients with Q35 and Q37 was also characterized by the absence of differences, where the differences for the minor allele T were 1.2 (27.1% vs. 24.3%; χ^2 =0.1; P=0.7; O R = 1.2; 95% CI: 0.55-2.45) , for the C/C genotype - 1.0 (57.1%

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versus 56.8%; χ^2 <3.84; P=0.98; O R =1.0 variant of the C/T genotype - less than one (31.4% versus 37.8%; χ^2 =0.3; P=0.6; O R =0.8; 95% CI: 0.28-1.99). However, in relation to the mutant T/T genotype, there was a slight upward trend among the group with Q 35 (11.4% vs. 5.4%; χ^2 =0.9; P=0.4; O R =2.3; 95%CI: 0.4-12.7).

Differences in the distribution of frequencies of alleles and genotypes of the MDR 1 gene polymorphism (C3435T) between groups of patients with Q36 and Q37 did not reach statistical significance. So, if the differences for the minor allele T were 1.2 (27.3% versus 24.3%; χ 2 = 0.2; P=0.7; O R =1.2; 56.8%; χ^2 <3.84; P=0.9; O R =0.9; 95% CI: 0.36-2.35) and C/T (36.4% vs. 37.8%; χ^2 <3.84; P=0.9; O R = 0.8; 95%CI: 0.36-2.48). At the same time, the carriage of the mutant genotype T/T was 1.8 times higher among patients with Q 36 (9.1 % vs. but still lacking statistical significance.

Conclusion. Thus, the results of a comparative assessment of differences in the distribution of allele and genotype frequencies for the polymorphic gene MDR 1 (C3435T) among the studied groups of patients with HPVLO and healthy people were characterized by the absence of their statistical significance. So, in the main group of patients with HPVLO, compared with the control, the differences for the minor allele T (χ^2 =0.1; P=0.8) and the heterozygous variant of the C/T genotype (χ^2 =0.9; P=0.4) did not reach one , while the proportion of the mutant T/T genotype among patients was 1.3 times higher (χ^2 =0.2; P=0.7) . In groups of patients with Q 35, Q 36 and Q 37, compared with the control, the absence of statistical significance of the revealed differences was found both in the distribution of the minor allele T (Q 35- χ^2 <3.84; P=0.95; O R =1.0; Q 36 - χ^2 <3.84; P=0.95; O R =1.0; Q 37 - χ^2 <0.3; P=0.6; O R =0.8), as well as C/T genotypes (Q 35 - χ^2 <3.84; P=0.95; O R \u003d 1.0; Q 36 - χ^2 \u003d 0.3; P \u003d 0.6; O R \u003d 0.8; Q 37 - χ^2 \u003d 0.3; P \u003d 0.6; O R \u003d 0.8) and T / T (Q 35 - χ^2 \u003d 0.8; P=0.4; O R =1.8; Q 36 - χ^2 <3.84; P=0.7; O R =1.4; Q 37 - χ^2 <3.84; P=0.8; About R = 0.8).

No statistical significance was found when assessing the distribution frequencies of alleles and genotypes of the MDR 1 gene polymorphism (C3435T) between groups of patients with Q35 and Q36 (for T - χ^2 <3.84; P=0.99; O R =1.0; for the C/T genotype - χ^2 =0.2; P=0.7; O R = 0.8 and for the T/T mutant genotype - χ^2 =0.1; P=0.8; O R = 1.3); Q35 and Q37 (for T - χ^2 \u003d 0.1; P \u003d 0.7; O R \u003d 1.2; for the C / T genotype - χ^2 \u003d 0.3; P \u003d 0.6; O R \u003d 0.8 and for the mutant genotype T / T - χ^2 =0.9; P=0.4; O R =2.3) as well as Q36 and Q37 (for T - χ^2 =0.2; P=0.7; O R =1.2; for the C/T genotype - χ^2 <3.84; P=0.9; O R =0.8 and for the mutant genotype T/T – χ^2 =0.4; P=0.6; O R =1.8).

In this regard, based on the results of the study, we cannot state that the polymorphism of the MDR1 gene (C3435T) is involved in the formation of HPVLO in general, and therefore this polymorphic gene cannot be considered as an independent genetic marker that increases the likelihood of developing HPVLO in Uzbekistan.

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