MTHFR and MDR1 Gene Polymorphisms in Yakut Patients with Non-Syndromic Orofacial Clefts

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Abstract

Background: Non-syndromic malformations of the face, jaws, and teeth are quite frequent, and, often, serious diseases, representing one of the complex problems of maxillofacial surgery and surgical dentistry. The aim of our study was to investigate the relationship between the *MDR1* and *MTHFR* gene polymorphisms and non-syndromic cleft lip with or without cleft palate (NSCL/P) in the Yakut population in the Republic of Sakha (Yakutia).

Methods and Results: The sample of examined persons consisted of 60 children with NSCL/P. The NSCL/P group was divided into the CLP (cleft lip with cleft palate) subgroup (n=31), CLO (cleft lip only) subgroup (n=14), and CPO (cleft palate only) subgroup (n=15). The comparison group (control) included 174 healthy volunteers who did not have relatives with OFCs. The study of the *MDR1* rs1045642 SNP and the *MTHFR* rs1801133 SNP was performed by PCR and RFLP analysis.

Analysis of the frequency distribution of alleles and genotypes depending on the severity of clefts showed that the carriage of the TT homozygous genotype of the *MDR1* rs1045642 SNP was associated with significant risk for the development of NSCL/P (OR=2.52, 95% CI: 1.19-5.32, *P*=0.02). Analysis of the recessive model (TT vs CC + TC) also found a significant risk of NSCL/P with the TT genotype carriage (OR=2.20, 95% CI: 1.06-4.57, *P*=0.04). Analysis of the over-dominant model (TC vs TT + CC) showed that the heterozygous TC genotype had a protective effect (OR=0.41; 95% CI: 0.22-0.77, *P*=0.01) on the development of NSCL/P. Subgroup analysis according to NSCL/P subtypes (CLO, CPO and CLP) showed that the *MDR1* rs1045642 SNP was significantly associated with a high risk of CPO in three genetic models: heterozygous [(TT vs TC): OR=5.03; 95% CI: 1.55-16.32; *P*=0.01], recessive [(TT vs CC + TC): OR=3.96; 95% CI: 1.32-11.95; *P*=0.02], and over-dominant [(TC vs TT + CC): OR=0.23; 95% CI: 0.08-0.66; *P*=0.01].

Conclusion: A study of two SNPs in the *MDR1* and *MTHFR* genes revealed a statistically significant increased risk for NSCL/P in carriers of the TT genotype of the *MDR1* rs1045642 SNP. (*International Journal of Biomedicine*. 2021;11(4):576-580.)

Key Words: orofacial cleft • *MDR1* gene • *MTHFR* gene • single nucleotide polymorphism

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Abbreviations

CLP, cleft lip with cleft palate; CLO, cleft lip only; CPO, cleft palate only; *IRF6*, interferon regulatory factor 6; LD, linkage disequilibrium; *MTHFR*, methylenetetra-hydrofolate reductase; *MDR1*, multidrug resistance 1; NSCL/P, non-syndromic cleft lip with or without cleft palate; OFC, orofacial cleft; SNP, single nucleotide polymorphism.

Introduction

Non-syndromic malformations of the face, jaws, and teeth are quite frequent, and, often, serious diseases, representing one of the complex problems of maxillofacial surgery and surgical dentistry. According to Ushnitsky et al., in the structure of clefts, heredity accounts for $7.55 \pm 2.18\%$. Moreover, every eighth cleft lip and (or) palate is part of multiple congenital malformations.⁽¹⁾

The organ primordia of the maxillofacial system develop in the first trimester of pregnancy from the first and second branchial arches, as well as the frontal protrusion of the cerebral region. From the branchial arches, tissues of the maxillofacial and submandibular regions are formed, with the exception of the central part of the midface zone, which develops from the frontal protrusion of the cerebral region. The development of the fetal lips ends at about 5-6 weeks, and the palate closes at about 10 weeks of gestation.⁽²⁾ Disorders of the fusion of the secondary palate can be caused by an already existing cleft in the primary palate or can occur separately with a normally developed primary palate.

To clarify the etiology of congenital facial clefts, it is necessary to study how genetic susceptibility factors interact with environmental factors. In recent years, studies of genetic predisposition in the development of non-syndromic cleft lip with or without cleft palate (NSCL/P) have become widespread. Reliable associations of the *MDR1* and *MTHFR* gene variants with an increased risk for NSCL/P have been found in different populations.⁽³⁻⁵⁾

The *MTHFR* gene, encoding the synthesis of the MTHFR enzyme, is located on chromosome 1p36.3. MTHFR plays a key role in folic acid metabolism. The value of folate in the prevention of neural tube defects (NTDs) is well established,^(6,7) and recent studies showing hypomethylation of neural tissue in cases of NTD support this observation.⁽⁸⁻¹¹⁾

The rs1801133 SNP (also known as 677C>T) is localized in exon 4 of the *MTHFR* gene and is formed by the transition from cytosine (C) to thymine (T). The 222nd genetic code of the *MTHFR* gene changes from GCC to GTC, which leads to the replacement of alanine (Ala) with valine (Val) in the *MTHFR* polypeptide. Animal studies have shown that reducing the formation of methionine from homocysteine plays a key role in the development of neural tube defects. A number of studies investigated the relationship between the polymorphisms of the *MTHFR* gene and OFCs, but with mixed results.^(3,5,10)

Functional SNPs in the *MDR1* gene can affect the expression and activity of transport proteins located on the apical and basolateral surfaces of syncytiotrophoblast and placental capillary endothelial cells. These proteins are able to remove toxins or drugs from the environment that enter the mother's body, into the mother's bloodstream, and can lead to an altered response of the fetus on xenobiotics and a subsequent increase in the risk of complex genetic disorders or birth defects.⁽⁴⁾ Pels et al. suggest that the rs1045642 SNP (also known as 3435C>T) has an adaptive significance, or is linked to other polymorphic sites that have an adaptive significance.⁽¹²⁾

The aim of our study was to investigate the relationship between the *MDR1* and *MTHFR* gene polymorphisms and NSCL/P in the Yakut population in the Republic of Sakha (Yakutia).

Materials and Methods

The experimental part of the work on the genotyping of the *MDR1* rs1045642 SNP and the *MTHFR* rs1801133 SNP was carried out in the Department of Molecular Genetics at YSC CMP. For the study, we used DNA samples from the collection of biomaterials of the YSC CMP (Project "The Genome of Yakutia"; No. USE_507512).

The sample of examined persons consisted of 60 children (29 girls and 31 boys) with NSCL/P. The NSCL/P group was divided into the CLP subgroup (n=31), CLO subgroup (n=14), and CPO subgroup (n=15). According to indications, a cytogenetic examination was performed to exclude chromosomal pathology in this group of children. The comparison group (control) included 174 healthy volunteers (128 women and 46 men) who did not have relatives with OFCs.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Genomic DNA samples were isolated from the peripheral blood leukocytes using a commercial kit for DNA isolation Excell biotech (Yakutsk, Russia). The study of the *MDR1* rs1045642 SNP and the *MTHFR* rs1801133 SNP was performed by PCR and RFLP analysis. Primer sequences, conditions for amplification, restriction pattern, restriction enzymes, and the lengths of the restoration fragments are presented in Table 1.

Table 1.

The primers and restriction enzymes used for detection of *MDR1* and *MTHFR* SNPs using PCR-RFLP method

Gene / RefSNP ID	Primers	AT	RE	RFL
<i>MDR1</i> rs1045642	F: 5'-TTGATGGCA AAGAAATAAAGC-3' R: 5'-CTTACATTA GGCAGTGACTCG-3'	54°C	DpnI	CC: 130,76 bp CT: 206,130,76 bp TT: 206 bp
<i>MTHFR</i> rs1801133	F: 5'-TGGGGTCAG AAGCATATCAGTCA-3' R: 5'-CTGGAAGA ACTCAGCGAAC-3'	62°C	TaqI	CC: 497 bp TC: 497,271,226 bp TT: 271,226 bp

AT, annealing temperature; RE, restriction enzyme; RFL - restriction fragment length; bp, base pair

Genotypes were determined by analyzing the sizes of the resulting fragments by gel electrophoresis on a 4% agarose gel with ethidium bromide in a standard Tris-acetate buffer at 120V for 1 hour. Restriction products were visualized using a UV gel documentation system (Vilber Lourmat, France).

Statistical analysis was performed using Microsoft Excel 2010. Differences in the allele and genotype distribution between the groups were assessed by χ^2 -test with Yates correction or Fisher's exact test, when appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The following genetic models and test were analyzed: allelic model: D compared with d (d - the minor allele); genotypic tests – homozygous model (DD compared with dd), heterozygous model (Dd compared with dd); recessive model (dd compared with Dd + DD); dominant model (dd+Dd compared with DD); over-dominant model (DD+dd compared with Dd); co-dominant model (DD compared with Dd, compared with dd). A probability value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Among 60 OFC patients, CLP was found in 31(51.7%) cases, CLO in 14(23.3%) cases, and CPO in 15(25%) cases.

Table 2.

Results of allelic and genotype analysis

Gene	Group	CC	CT	TT	C	T	χ^2	<i>P</i> -value	Genotypes		Alleles	
									HWE	<i>P</i> -value	HWE	<i>P</i> -value
MTHFR rs1801133	CLP	69.4	30.6	0	0.847	0.153	1.171	0.279				
	M	73.7	26.3	0	0.868	0.132	0.436	0.509	NA	NA	0.40	0.841
	F	64.7	35.3	0	0.824	0.176	0.781	0.377				
	CLO	50	50	0	0.750	0.250	0.889	0.346				
	M	50	50	0	0.750	0.250	0.667	0.414	NA	NA	0.444	0.505
	F	50	50	0	0.750	0.250	0.222	0.637				
	CPO	68.8	31.3	0	0.844	0.156	0.549	0.459				
	M	50	50	0	0.750	0.250	0.667	0.414	NA	NA	0.395	0.530
	F	80	20	0	0.900	0.100	0.123	0.725				
	NSCL/P	66.7	33.3	0	0.833	0.167	2.400	0.121				
	M	64.5	35.5	0	0.823	0.177	1.442	0.230	NA	NA	0.07	0.935
	F	69.0	31.0	0	0.845	0.155	0.978	0.323				
	Control	70.1	28.2	1.7	0.842	0.158	0.588	0.443				
	M	78.3	19.6	2.2	0.880	0.120	0.230	0.632	2.306	0.316	1.026	0.311
	F	67.2	31.3	1.6	0.828	0.172	1.224	0.269				
Gene	Group	CC	CT	TT	C	T	χ^2	<i>P</i> -value	Genotypes		Alleles	
									HWE	<i>P</i> -value	HWE	<i>P</i> -value
MDR1 rs1045642	CLP	11.1	63.9	25.0	0.431	0.569	3.303	0.690				
	M	5.3	68.4	26.3	0.395	0.605	3.544	0.60	1.396	0.498	0.169	0.681
	F	17.6	58.8	23.5	0.471	0.529	0.554	0.457				
	CLO	37.5	62.5	0	0.688	0.313	1.653	0.199				
	M	50	50	0	0.750	0.250	0.667	0.414	NA	NA	0.97	0.755
	F	0	100	0	0.500	0.500	2.000	0.157				
	CPO	18.8	43.8	37.5	0.406	0.594	0.139	0.710				
	M	16.7	16.7	66.7	0.250	0.750	1.852	0.174	3.810	0.149	1.045	0.307
	F	20	60	20	0.500	0.500	0.400	0.527				
	NSCL/P	16.7	58.3	25.0	0.458	0.542	1.834	0.176				
	M	16.1	54.8	29.0	0.435	0.565	0.412	0.521	0.563	0.755	0.113	0.737
	F	17.2	62.1	20.7	0.483	0.517	1.710	0.191				
	Control	9.7	77.1	13.1	0.483	0.517	51.917	0				
	M	10.9	73.9	15.2	0.478	0.522	10.645	0.01	0.344	0.842	0	0.983
	F	9.4	78.1	12.5	0.484	0.516	40.720	0				

F, female; M, male. χ^2 -test with Yates correction

As known, the prevalence of OFC varies according to gender and the nature of the cleft. For example, Mossey et al.⁽¹³⁾ found a predominance of CLP in men, with a sex ratio (M/F) of 1.81(95% CI:1.75–1.86) and the opposite ratio for CPO – 0.93(95% CI:0.89–0.96); however, one Danish study failed to find a significant predominance of women with cleft palate.⁽¹⁴⁾ In our study, among CLP cases, boys predominated slightly (51.7%). Analysis of the gender distribution found that CLO was more common in boys (57.2%) and CPO in girls (66.7%). Our study is comparable to the results obtained by Tafazzoli and Shahryari.⁽¹⁵⁾

NSCL/P has a multifactorial etiology that includes both genetic and environmental factors, and several genes have been associated with this malformation.⁽¹⁶⁾

More than 40% of OFC children had a family history of the disease. Since the mechanism of occurrence of each type of cleft is different, we compared the genetic variants of the *MDR1* rs1045642 SNP and the *MTHFR* rs1801133 SNP in patients with CLP, CLO, and CPO. Our analysis showed (Table 2) no

significant differences in the allele and genotype frequencies between all subgroups. We also did not find differences in the frequency distribution of alleles and genotypes between boys and girls. As a result, we compared these subgroups with the comparison group regardless of gender.

We found no significant association between the carriage of the unfavorable allele T of the *MTHFR* rs1801133 SNP and OFC in all subgroups (Table 3).

Analysis of the frequency distribution of alleles and genotypes depending on the severity of clefts showed that

Table 3.

Association of the *MDR1* (rs1045642) and *MTHFR* (rs1801133) polymorphisms with the development of NSCL/P, CLP, CLO, CPO

MTHFR (rs1801133)				
NSCL/P and subgroup	NSCLP	CLP	CLO	CPO
MAF / Associated risk alleles	T			
Control genotypes	122/49/3			
Sample size (case/control)	60/174	36/174	8/174	16/174
Case genotypes CC/TC/TT	40/20/0	25/11/0	4/4/0	11/5/0
Allelic model (T vs C) (95% CI) P-value	OR = 1.06 (0.61-1.86) 0.82	OR = 0.96 (0.47-1.94) 0.91	OR = 1.78 (0.55-5.71) 0.36	OR = 0.99 (0.36-2.67) 0.98
Dominant model (TC + TT vs CC) (95% CI) P-value	OR = 1.17 (0.63-2.20) 0.62	OR = 1.03 (0.47-2.25) 0.94	OR = 2.35 (0.56-9.74) 0.25	OR = 1.07 (0.35-3.22) 0.91
Reverse heterozygous model (TC vs CC) (95% CI) P-value	OR = 1.24 (0.66-2.34) 0.50	OR = 1.10 (0.50-2.40) 0.82	OR = 2.41 (0.60-10.35) 0.22	OR = 1.13 (0.37-3.43) 0.83
Over-dominant model (TC vs TT + CC) (95% CI) P-value	OR = 1.28 (0.68-2.40) 0.45	OR = 1.12 (0.51-2.45) 0.77	OR = 2.55 (0.61-10.60) 0.20	OR = 1.16 (0.38-3.51) 0.79
MDR1 (rs1045642)				
MAF / Associated risk alleles	T			
Control genotypes	17/135/23			
Sample size (case/control)	60/175	36/174	8/174	16/174
Case genotypes CC/TC/TT	10/35/15	4/23/9	3/5/0	3/7/6
Allelic model (C vs T) (95% CI) P-value	OR = 1.10 (0.73-1.67) 0.64	OR = 1.23 (0.74-2.06) 0.42	OR = 0.42 (0.14-1.25) 0.11	OR = 1.36 (0.65-2.85) 0.40
Homozygous model (TT vs CC) (95% CI) P-value	OR = 1.11 (0.40-3.06) 0.84	OR = 1.66 (0.44-6.31) 0.45	NA	OR = 1.48 (0.32-6.77) 0.61
Heterozygous model (TT vs TC) (95% CI) P-value	OR = 2.52 (1.19-5.32) 0.02	OR = 2.30 (0.94-5.58) 0.08	NA	OR = 5.03 (1.55-16.32) 0.01
Reverse heterozygous model (TC vs CC) (95% CI) P-value	OR = 0.44 (0.19-1.05) 0.07	OR = 0.72 (0.22-2.35) 0.60	OR = 0.21 (0.05-0.96) 0.06	OR = 0.29 (0.07-1.24) 0.12
Recessive model (TT vs CC + TC) (95% CI) P-value	OR = 2.20 (1.06-4.57) 0.04	OR = 2.20 (0.92-5.27) 0.09	NA	OR = 3.96 (1.32-11.95) 0.02
Dominant model (TC + TT vs CC) (95% CI) P-value	OR = 0.54 (0.23-1.25) 0.16	OR = 1.16 (0.37-3.68) 0.80	OR = 0.18 (0.04-0.82) 0.04	OR = 0.47 (0.12-1.80) 0.30
Over-dominant model (TC vs TT + CC) (95% CI) P-value	OR = 0.41 (0.22-0.77) 0.01	OR = 0.52 (0.24-1.13) 0.11	OR = 0.49 (0.11-2.16) 0.36	OR = 0.23 (0.08-0.66) 0.01

MAF, minor allele frequency

the carriage of the TT homozygous genotype of the *MDR1* rs1045642 SNP was associated with significant risk for the development of NSCL/P (OR=2.52, 95% CI: 1.19-5.32, $P=0.02$).

Analysis of the recessive model also found a significant risk of NSCL/P with the TT genotype carriage (OR=2.20, 95% CI: 1.06-4.57, $P=0.04$).

Analysis of the over-dominant model (TC vs TT + CC) showed that the heterozygous TC genotype had a protective effect (OR=0.41, 95% CI: 0.22-0.77, $P=0.01$) on the development of NSCL/P.

Subgroup analysis according to NSCL/P subtypes (CLO, CPO and CLP) showed that the *MDR1* rs1045642 SNP was significantly associated with a high risk of CPO in three genetic models (Table 3): heterozygous [(TT vs TC): OR=5.03, 95% CI: 1.55-16.32, $P=0.01$], recessive [(TT vs CC + TC): OR=3.96, 95% CI: 1.32-11.95, $P=0.02$], and over-dominant [(TC vs TT + CC): OR=0.23, 95% CI: 0.08-0.66, $P=0.01$].

Conclusion

The present case-control study of two SNPs in the *MDR1* and *MTHFR* genes revealed a statistically significant increased risk for NSCL/P in carriers of the TT genotype of the *MDR1* rs1045642 SNP.

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Competing Interests

The authors declare that they have no competing interests.

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