Health Scope. 2024 May; 13(2): e142145. https://doi.org/10.5812/healthscope-142145.

Published online: 2024 April 29. **Research Article**

Association of EPHA3 Gene Variation with Oral Hygiene in an Iranian Population

Hoshangh Rafighdoosth [1], Elaheh Hasanpour [1], Mohamad Hashemi [2], Gholamreza Bahari [2,3,**], Mohsen Taheri [4,5,*]

[1] Department of Anatomy, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[2] Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[3] Children and Adolescent Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
[4] Genetics of Non-communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
[5] Genetic Department, School of Medicine, Zahedan University of Medical Sciences, Zahedan,Iran

[*] Corresponding Author: Genetic Department, School of Medicine, Zahedan University of Medical Sciences, Zahedan,Iran. Email: amirt112@gmail.com
[**] Corresponding Author:Associate Professor of Clinical Biochemistry, Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran. Tel: +98-9153497890; Fax: +98-5433295796, Email: ghrb1333@gmail.com

Received 2023 October 22; **Revised** 2024 January 9; **Accepted** 2024 January 22.

Abstract

Background: Non-syndromic cleft lip with or without cleft palate (NSCL/P) is the most prevalent congenital birth anomaly. The EPHA3 gene is suggested to play a pivotal role in the development of oral clefts.
Objectives: This study aimed to evaluate the influence of EPHA3 gene polymorphisms on the risk of NSCL/P within an Iranian cohort.
Methods: We performed genotyping of the EPHA3 gene polymorphisms rs7650466, rs1398197, rs17801309, rs1054750, and rs7632427 in 150 NSCL/P patients and 152 healthy controls using PCR-RFLP, T-ARMS-PCR, and ARMS-PCR methods.
Results: The results indicated that the rs1398197 variation significantly reduced the risk of NSCL/P in a heterozygous codominant model (OR = 0.58, 95% CI = 0.36 - 0.94, P = 0.027, G/A vs. G/G), a dominant model (OR = 0.56, 95% CI = 0.35 - 0.89, P = 0.014, G/A + A/A vs. G/G), and at the allele level (OR = 0.62, 95% CI = 0.43 - 0.91, P = 0.014, A vs. G). The rs1054750 polymorphism showed a decreased risk of NSCL/P in codominant (OR = 0.62, 95% CI = 0.39 - 0.99, P = 0.047, T/C vs. T/T) and dominant models (OR = 0.62, 95% CI = 0.39 - 0.98, P = 0.042, T/C + C/C vs. T/T). The rs17801309 polymorphism was not associated with any risk or protection from NSCL/P. rs7650466 and rs7632427 were not polymorphic in the study sample.
Conclusions: Our findings suggest that variants of the EPHA3 gene may be linked with a reduced risk of NSCL/P.

Keywords: Polymorphism, Non-syndromic Cleft, NSCL/P, EPHA3

1. Background

Certainly, activities such as speaking, swallowing, and chewing require a healthy anatomical structure of the oral cavity. Defects like cleft palate (CP) in infants can cause food to enter the nasal cavity during feeding. This can lead to slurred speech, bad breath, and psychological consequences that significantly affect the child's family. Such complications can arise from chromosomal syndromes or occur in non-syndromic cases. Non-syndromic cleft lip with or without cleft palate (NSCL/P) is a universally common congenital anomaly among live births (1). The prevalence of NSCL/P varies across different ethnicities; it is highest in Asian and American Indian populations at approximately 1/500, about 1/1000 in Europeans, and lowest in Africans at around 1/2500 (2). Non-syndromic cleft lip with or without cleft palate often results in facial deformity and difficulties in speech and swallowing (3). Both environmental and genetic factors contribute to the susceptibility to NSCL/P (1, 4-6).

Non-syndromic cleft lip with or without cleft palate can be categorized into cleft lip only (CLO), cleft palate only (CPO), and cleft lip with cleft palate (CLP). Cleft lip only and CLP are considered variations of the same defect and are grouped together epidemiologically as cleft lip with or without cleft palate (CL/P) (7).

The EPHA3 gene, located on chromosome 3 at 3p11.1 and comprising 17 exons (also known as EK4, ETK, HEK, ETK1, HEK4, and TYRO4), belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family (8). EPHA3 is involved in several developmental processes including morphogenesis, cell adhesion, movement, contraction, and regulation of axon guidance (9-11). Animal studies have shown that B-type ephrin is highly expressed in the pre-fusion epithelium of the palatal shelves, suggesting its potential role as a candidate gene for CLP (12). Another study indicated that EPHA3 is highly expressed in palatal mesenchymal cells during palatal development (13).

The rs7632427 polymorphism, located in the 3´UTR of EPHA3, plays a controlling role in the progression of NSCL/P (14). Additionally, rs7632427 has been associated with NSCL/P (15). These studies suggest that EPHA3 is crucial in the development of the lip and palate and may participate in the pathogenesis of NSCL/P.

2. Objectives

We conducted this investigation to examine the association between EPHA3 variations and susceptibility to NSCL/P in a sample population from southeast Iran.

3. Methods

This case-control study included 150 patients with NSCL/P and 152 healthy subjects. The control samples consisted of unaffected, unrelated individuals without a family history of clefting, collected as randomly selected, population-based controls from Zahedan. All patients were diagnosed independently and were screened by a multidisciplinary team of specialists to exclude cleft-associated syndromes, such as DiGeorge, Stickler, Nager, and Van der Woude syndromes. The design of this investigation was based on previous studies (16). The project was approved by the local Ethics Committee of Zahedan University of Medical Sciences (IR.Zaums.REC.1398.122), and written informed consent was obtained from all participants or their parents. Blood samples from all participants were collected in tubes containing EDTA and stored at -20°C prior to DNA extraction. Genomic DNA was extracted using the salting-out method.

3.1. Genotyping

Genotyping of the variants was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), Tetra-ARMS, and ARMS-PCR methods. The primer sequences, annealing temperatures, restriction enzymes, and product sizes are displayed in Table 1. PCR was conducted using prime Taq premix (Genet bio, South Korea) according to the manufacturer's suggested procedure. Each 0.20 - milliliter PCR reaction tube contained 1 microliter of genomic DNA (~ 100 ng/mL), 1 microliter of each primer (10 µM), 10 microliters of 2X Prime Taq premix, and 7 microliters of ddH_2O for the PCR-RFLP and ARMS-PCR methods (and 5 microliters of ddH_2O for the Tetra-ARMS PCR method). The PCR conditions included an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 30 seconds at 95°C, annealing at the temperature listed in Table 1 for 30 seconds, and an extension at 72°C for 30 seconds, with a final extension of 72°C for 5 minutes. The PCR products (10 µL for PCR-RFLP) were digested by appropriate restriction enzymes as specified in "Table 1", analyzed by agarose gel electrophoresis, and visualized on a UV transilluminator.

3.2. Statistical Analysis

Data analysis was performed using the statistical package SPSS 20 software (IBM Corp., Armonk, NY, USA). The associations between EPHA3 variants and NSCL/P risk were evaluated by odds ratios (ORs) and 95% confidence intervals (CIs) under different genetic models. SNPstats software was utilized for haplotype

Table 1. The Primers Used for the Detection of EPHA3 Polymorphisms Using PCR-RFLP, ARMS-PCR or Tetra-ARMS Methods

Polymorphism EPHA3	Primer Sequence (5' – 3')	Method	Annealing, °C	Restriction Enzyme	Fragment, bp
rs7650466	F: TTTTGAAAAGATGTACCTGGTGGA	PCR-RFLP	58	DdeI	T allele = 233; C allele = 210 + 23
	R: TCTACAACAGATGAGCACTTCTG				
rs1398197	F (G allele): AGAAGCTATAGCCTACCGCCAG	ARMS-PCR	58	-	Product size = 211
	F (A allele): AGAAGCTATAGCCTACCGCCAA				
	R: ACCAGGAGCCACCCAGTTACAT				
rs17801309	F: GAAGGGGAGAACTTAGACAAGATGATT	PCR-RFLP	62	BstXI	G allele = 294; A allele = 263 + 31
	R: TGTCCAGACACCATTAAGCCAGTCACCAG				
rs1054750	FO: CATCAAACCTTCTTCTGGACCAAAG	Tetra-ARMS PCR	60	-	Control = 285; T allele = 200; C allele = 133
	RO: CGGTAGTCAGTACCTCAGATCTACCACTAA				
	FI (T allele): CACTGCAAGGAAATCTTCACGTGT				
	RI (C allele): GTGTCACAAGAACTGTACTCCCCG				
rs7632427	F: GCCTTTTCTTCAGTGTCTAACT	PCR-RFLP	56	BccI	T allele = 307; C allele = 190 + 117
	R: ACTCTTCACTTGCTTCACTCAT				

determination. P-values less than 0.05 were considered statistically significant.

4. Results

A total of 302 subjects, comprising 150 NSCL/P patients and 152 unrelated healthy subjects, were evaluated. The demographic characteristics of the subjects are shown in Table 2. Of the 150 patients, 54 had a cleft lip (CL), 51 had a CLP, and 45 had a CP. No statistically significant differences were found between the groups regarding sex and age (P = 0.352 and P = 0.101, respectively). Genotypic and allelic frequencies of EPHA3 gene polymorphisms are presented in Table 3. The findings showed that the rs1398197 polymorphism significantly decreased the odds of NSCL/P in a heterozygous codominant model (OR = 0.58, 95% CI = 0.36 - 0.94, P = 0.027, GA vs. GG), a dominant model (OR = 0.56, 95% CI = 0.35 - 0.89, P = 0.014, GA+AA vs. GG), and an allelic model (OR = 0.62, 95% CI = 0.43 - 0.91, P = 0.014, A vs. G). The rs1054750 variant was associated with protection against NSCL/P in codominant (OR = 0.62, 95% CI = 0.39 - 0.99, P = 0.047, TC vs. TT) and dominant models (OR = 0.62, 95% CI = 0.39 - 0.98, P = 0.042, TC + CC vs. TT). No significant association was detected among rs17801309 polymorphisms and NSCL/P. rs7650466 and rs7632427 were not polymorphic in the study. Stratified analysis was conducted according to CL, CLP, and CP (Table 4). The results suggested that the GA genotype.

Table 2. Demographic Characteristics of Cases and Controls [a]

Characteristics	Controls (n = 152)	CL/P (n = 150)	CL (n = 54)	CP (n = 45)	CLP (n = 51)	P-Value
Age (y)	9.09 ± 9.05	7.62 ± 5.55	8.74 ± 5.64	7.76 ± 5.58	6.31 ± 5.24	0.101
Sex						0.352
Male	85 (55.9)	92 (61.3)	34 (63.0)	27 (60.0)	31 (60.8)	
Female	67 (44.1)	58 (38.7)	20 (37.0)	18 (40.0)	20 (39.2)	

[a] Values are expressed as mean ± SD or No. (%).

Table 3. Genotypic and Allelic Frequencies of EPHA3 Genes Polymorphisms Among Cases and Controls and Their Association with Non-syndromic Cleft Lip with or Without Cleft Palate

Polymorphism EPHA3	Cases	Controls	OR (95%CI)	P-Value
rs1398197				
Codominant				
G/G	97 (64.7)	77 (50.7)	1	-
G/A	46 (30.7)	63 (41.4)	0.58 (0.36 - 0.94)	0.027
A/A	7 (4.6)	12 (7.9)	0.46 (0.17 - 1.23)	0.123
Dominant				
G/G	97 (64.7)	77 (50.7)	1	-
G/A + A/A	53 (35.3)	75 (49.3)	0.56 (0.35 - 0.89)	0.014
Recessive				
G/G + G/A	143 (95.4)	140 (92.1)	1	-

Polymorphism EPHA3	Cases	Controls	OR (95%CI)	P-Value
A/A	7 (4.6)	12 (7.9)	0.57 (0.22 - 1.49)	0.248
Allele				
G	240 (80.0)	217 (71.4)	1	-
A	60 (20.0)	87 (28.6)	0.62 (0.43 - 0.91)	0.014
rs17801309				
Codominant				
G/G	120 (80.0)	116 (76.3)	1	-
G/A	26 (17.3)	31 (20.4)	0.81 (0.45 - 1.45)	0.479
A/A	4 (2.7)	5 (3.3)	0.77 (0.20 - 2.95)	0.773
Dominant				
G/G	120 (80.0)	116 (76.3)	1	-
G/A + A/A	30 (20.0)	36 (23.7)	0.80 (0.46 - 1.39)	0.439
Recessive				
G/G + G/A	146 (97.3)	147 (96.7)	1	-
A/A	4 (2.7)	5 (3.3)	0.81 (0.21 - 3.06)	0.750
Allele				
G	266 (88.7)	263 (86.5)	1	-
A	34 (11.3)	41 (13.5)	0.82 (0.50 - 1.33)	0.422
rs1054750				
Codominant				
T/T	100 (66.7)	84 (55.2)	1	-
T/C	48 (32.0)	65 (42.8)	0.62 (0.39 - 0.99)	0.047
C/C	2 (1.3)	3 (2.0)	0.56 (0.09 - 3.43)	0.525
Dominant				
T/T	100 (66.7)	84 (55.2)	1	-
T/C + C/C	50 (33.3)	68 (44.8)	0.62 (0.39 - 0.98)	0.042
Recessive				
T/T + T/C	148 (98.7)	149 (98.0)	1	-
C/C	2 (1.3)	3 (2.0)	0.67 (0.11 - 4.08)	0.663
Allele				
T	248 (82.7)	233 (76.6)	1	-
C	52 (17.3)	71 (23.4)	0.88 (0.59 - 1.31)	0.526

[a] Values are expressed as No. (%) except otherwise indicated.

Table 4. Genotype and Allele Frequencies of EPHA3 Gene Polymorphisms in Subjects with Confidence Interval, Cleft Lip with Cleft Palate, and Cleft Palate

Polymorphism	Control	CL	OR (95%CI), P-Value	CLP	OR (95%CI), P-Value	CP	OR (95%CI), P-Value
rs1398197							
G/G	77 (50.7)	30 (55.6)	1	35 (68.6)	1	32 (71.1)	1
G/A	63 (41.4)	20 (37.0)	0.81 (0.42 - 1.57), 0.541	14 (27.5)	0.49 (0.24 - 0.99), 0.044	12 (26.7)	0.46 (0.22 - 0.96), 0.037
A/A	12 (7.9)	4 (7.4)	0.86 (0.26 - 2.86), 0.799	2 (3.9)	0.37 (0.08 - 1.73), 0.189	1 (2.2)	0.60 (0.06 - 5.59), 0.652
Allele							
G	217 (71.4)	80 (74.1)	1	84 (82.4)	1	76 (84.4)	1
A	87 (28.6)	28 (25.9)	0.87 (0.53 - 1.44), 0.592	18 (17.6)	0.53 (0.30 - 0.94), 0.028	14 (15.6)	0.46 (0.25 - 0.86), 0.013
rs17801309							
G/G	116 (76.3)	45 (83.3)	1	40 (78.5)	1	35 (77.8)	1
G/A	31 (20.4)	9 (16.7)	0.75 (0.33 - 1.70), 0.487	7 (13.7)	0.65 (0.27 - 1.60), 0.352	10 (22.2)	1.07 (0.48 - 2.40), 0.871
A/A	5 (3.3)	0 (0.0)	-	4 (7.8)	2.32 (0.59 - 9.07), 0.215	0 (0.0)	-
Allele							
G	263 (86.5)	99 (91.7)	1	87 (85.3)	1	80 (88.9)	1
A	41 (13.5)	9 (8.3)	0.58 (0.27 - 1.24), 0.159	15 (14.7)	1.11 (0.58 - 2.09), 0.757	10 (11.1)	0.80 (0.38 - 1.67), 0.555
rs1054750							
T/T	84 (55.2)	36 (66.7)	1	32 (62.7)	1	32 (71.1)	1
T/C	65 (42.8)	16 (29.6)	0.57 (0.29 - 1.12), 0.104	19 (37.3)	0.77 (0.40 - 1.48), 0.43	13 (28.9)	0.52 (0.26 - 1.08), 0.077
C/C	3 (2.0)	2 (3.7)	1.56 (0.25 - 9.71), 0.634	0 (0.0)	-	0 (0.0)	-
Allele							
T	233 (76.6)	88 (81.5)	1	83 (81.4)	1	77 (85.6)	1
C	71 (23.4)	20 (18.5)	0.95 (0.55 - 1.65), 0.866	19 (18.6)	0.96 (0.55 - 1.68), 0.89	13 (14.4)	0.71 (0.37 - 1.35), 0.291

[a] Values are expressed as No. (%) except otherwise indicated.

5. Discussion

The pathogenesis of NSCL/P is influenced by genetic and environmental factors (4, 17, 18). Previous studies have highlighted the role of the EPHA3 gene in susceptibility to NSCL/P, noting variations across different populations (14, 15, 19). This study aimed to evaluate the association between EPHA3 polymorphisms rs7650466, rs1398197, rs17801309, rs1054750, and rs7632427, and the odds of NSCL/P. Our results demonstrated that the rs1398197 and rs1054750 variants significantly reduced the odds of NSCL/P. However, no significant association was found between the rs17801309 polymorphism and NSCL/P in our investigation. Additionally, the rs7650466 and rs7632427 variants were not polymorphic in our study population.

The findings from our stratified analysis suggested that the GA genotype and A allele of the rs1398197 variant significantly decreased the odds of both CP and CLP. Pan et al. examined the association between six loci (rs7590268, rs7632427, rs12543318, rs1873147, rs8001641, and rs742071) and the risk of NSCL/P. They found that the rs7590268 variant was associated with an increased risk of NSCL/P, while rs7632427, rs12543318, and rs1873147 exhibited protective effects. No relationship was found between rs742071 and rs8001641 and the risk of NSCL/P in their study, underscoring the role of these genes in craniofacial development and their potential association with common orthopedic birth defects (15).

Chen et al. evaluated the impact of five SNPs in EPHA3 on the risk of NSCL/P and found that only the rs7650466 variant was associated with a decreased risk of NSCL/P. The other four SNPs showed no statistically significant differences between the NSCL/P and control groups in their study (20).

They hypothesized that EPHA3 plays a crucial role in the development of cranial and maxillofacial structures. Additionally, they found that this polymorphism could alter the binding site of miR - 2052 to the 3'-UTR of EPHA3. A decrease in binding capacity led to reduced expression of EPHA3 and a decreased incidence of NSCL/P (20). There are some limitations to this study, including the relatively small sample sizes. Another limitation is that we did not examine the biological functions of the polymorphisms. In summary, our results suggest that variations in the EPHA3 gene may contribute to NSCL/P susceptibility. Future large-scale, well-designed studies with diverse ethnicities are needed to confirm the role of EPHA3 gene polymorphisms in NSCL/P risk.

Acknowledgements

We thank the patients and healthy subjects who willingly participated in the study.

Footnotes

Authors' Contribution: HR was responsible for supervision of data collection, validation of data sources and contents, and interpretation of data. EH handled acquisition, data collection, and tabulation. MH was responsible for the concept and study design, supervision, and methodology. GB conceived and designed the study and drafted the manuscript. MT was involved in methodology, formal analysis, writing the original draft, and review and editing of the final draft.

Conflict of Interests Statement: All authors declared that they have no conflicts of interest.

Data Availability: All data of the manuscript will be provided upon reasonable request and approval by the ethics committee.

Ethical Approval: The study protocol was approved by the Ethics Committee of Zahedan University of Medical Sciences (IR.Zaums.REC.1398.122) .

Funding/Support: This study was funded by a dissertation grant from the Zahedan University of Medical Sciences.

Informed Consent: All patients signed the informed consent form before participation.

References

1. Sahin Uysal N, Sahin FI, Terzi YK. The impact of developmental genes in non-syndromic cleft lip and/or palate. *J Turk Ger Gynecol Assoc.* 2023;**24**(1):57-64. [PubMed ID: 36919534]. [PubMed Central ID: PMC10019015]. https://doi.org/10.4274/jtgga.galenos.2022.2021-10-7.
2. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev Genet.* 2011;**12**(3):167-78. [PubMed ID: 21331089]. [PubMed Central ID: PMC3086810]. https://doi.org/10.1038/nrg2933.
3. Silva MARD, Balderrama IDF, Wobeto AP, Werneck RI, Azevedo-Alanis LR. The impact of nonsyndromic cleft lip with or without cleft palate on oral health-related quality of life. *J Appl Oral Sci.* 2018;**26**(0). https://doi.org/10.1590/1678-7757-2017-0145.
4. Xu DP, Qu WD, Sun C, Cao RY, Liu DW, Du PG. A Study on Environmental Factors for Nonsyndromic Cleft Lip and/or Palate. *J Craniofac Surg.* 2018;**29**(2):364-7. [PubMed ID: 29283947]. https://doi.org/10.1097/SCS.0000000000004214.
5. Rafighdoost H, Poudineh A, Bahari G, Ghaffari H, Hashemi M. Association of Genetic Polymorphisms of GREM1 Gene with Susceptibility to Non-Syndromic Cleft Lip with or without Cleft Palate in an Iranian Population. *Fetal Pediatr Pathol.* 2020;**39**(5):409-

21. [PubMed ID: 31650875]. https://doi.org/10.1080/15513815.2019.1666329.

6. Niktabar SM, Aarafi H, Dastgheib SA, Noorishadkam M, Mirjalili SR, Lookzadeh MH, et al. Association of MTHFR 1298A > C Polymorphism with Susceptibility to Non-Syndromic Cleft Lip with or without Palate: A Case-Control Study and Meta-Analysis. *Fetal Pediatr Pathol.* 2021;**40**(1):1-17. [PubMed ID: 31682771]. https://doi.org/10.1080/15513815.2019.1683918.

7. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. *Am J Med Genet C Semin Med Genet.* 2013;**163C**(4):246-58. [PubMed ID: 24124047]. [PubMed Central ID: PMC3925974]. https://doi.org/10.1002/ajmg.c.31381.

8. Klein R. Eph/ephrin signalling during development. *Dev.* 2012;**139**(22):4105-9. [PubMed ID: 23093422]. https://doi.org/10.1242/dev.074997.

9. Janes PW, Slape CI, Farnsworth RH, Atapattu L, Scott AM, Vail ME. EphA3 biology and cancer. *Growth Factors.* 2014;**32**(6):176-89. [PubMed ID: 25391995]. https://doi.org/10.3109/08977194.2014.982276.

10. Janes PW, Nievergall E, Lackmann M. Concepts and consequences of Eph receptor clustering. *Semin Cell Dev Biol.* 2012;**23**(1):43-50. [PubMed ID: 22261642]. https://doi.org/10.1016/j.semcdb.2012.01.001.

11. Lawrenson ID, Wimmer-Kleikamp SH, Lock P, Schoenwaelder SM, Down M, Boyd AW, et al. Ephrin-A5 induces rounding, blebbing and de-adhesion of EphA3-expressing 293T and melanoma cells by CrkII and Rho-mediated signalling. *J Cell Sci.* 2002;**115**(Pt 5):1059-72. [PubMed ID: 11870224]. https://doi.org/10.1242/jcs.115.5.1059.

12. Agrawal P, Wang M, Kim S, Lewis AE, Bush JO. Embryonic expression of EphA receptor genes in mice supports their candidacy for involvement in cleft lip and palate. *Dev Dyn.* 2014;**243**(11):1470-6. [PubMed ID: 25073978]. [PubMed Central ID: PMC4404412]. https://doi.org/10.1002/dvdy.24170.

13. Xavier GM, Miletich I, Cobourne MT. Ephrin Ligands and Eph Receptors Show Regionally Restricted Expression in the Developing Palate and Tongue. *Front Physiol.* 2016;**7**:60. [PubMed ID: 26941654]. [PubMed Central ID: PMC4763095]. https://doi.org/10.3389/fphys.2016.00060.

14. Ludwig KU, Mangold E, Herms S, Nowak S, Reutter H, Paul A, et al. Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci. *Nat Genet.* 2012;**44**(9):968-71. [PubMed ID: 22863734]. [PubMed Central ID: PMC3598617]. https://doi.org/10.1038/ng.2360.

15. Pan Y, Han Y, Zhang H, Zhou L, Li D, Cai Q, et al. Association and cumulative effects of GWAS-identified genetic variants for nonsyndromic orofacial clefts in a Chinese population. *Environ Mol Mutagen.* 2013;**54**(4):261-7. [PubMed ID: 23536526]. https://doi.org/10.1002/em.21773.

16. Rafighdoost H, Hashemi M, Narouei A, Eskanadri-Nasab E, Dashti-Khadivaki G, Taheri M. Association between CDH1 and MSX1 gene polymorphisms and the risk of nonsyndromic cleft lip and/or cleft palate in a southeast Iranian population. *Cleft Palate Craniofac J.* 2013;**50**(5):e98-e104. [PubMed ID: 23231047]. https://doi.org/10.1597/12-144.

17. Bahrami R, Dastgheib SA, Niktabar SM, Amooee A, Lookzadeh MH, Mirjalili SR, et al. Association of BMP4 rs17563 Polymorphism with Nonsyndromic Cleft Lip with or without Cleft Palate Risk: Literature Review and Comprehensive Meta-Analysis. *Fetal Pediatr Pathol.* 2021;**40**(4):305-19. [PubMed ID: 31909686]. https://doi.org/10.1080/15513815.2019.1707916.

18. Huang L, Liang X, Ou Y, Tang S, He Y. Association between 20q12 rs13041247 polymorphism and risk of nonsyndromic cleft lip with or without cleft palate: a meta-analysis. *BMC Oral Health.* 2020;**20**(1):39. [PubMed ID: 32019513]. [PubMed Central ID: PMC7001214]. https://doi.org/10.1186/s12903-020-1003-2.

19. do Rego Borges A, Sa J, Hoshi R, Viena CS, Mariano LC, de Castro Veiga P, et al. Genetic risk factors for nonsyndromic cleft lip with or without cleft palate in a Brazilian population with high African ancestry. *Am J Med Genet A.* 2015;**167A**(10):2344-9. [PubMed ID: 26198054]. https://doi.org/10.1002/ajmg.a.37181.

20. Chen R, Guo S, Wang X, Mu Y, Duan E, Xu Y. Association of EPHA3 Gene Polymorphisms with Nonsyndromic Cleft Lip With or Without Cleft Palate. *Genet Test Mol Biomarkers.* 2018;**22**(7):420-4. [PubMed ID: 29932736]. https://doi.org/10.1089/gtmb.2017.0252.