ODOVTOS
International Journal of Dental Sciences

UNIVERSIDAD DE COSTA RICA FOd Facultad de Odontología

https://revistas.ucr.ac.cr/index.php/rOdontos

BASIC RESEARCH:

Genetic Analysis of Methylenetetrahydrofolate Reductase (MTHFR) Gene of Polymorphism (rs1801133) in Patients with Non-Syndromic Cleft lip and Palate in the South Indian Population. A Preliminary Case Control Study

Análisis genético del polimorfismo (rs1801133) del gen metilentetrahidrofolato reductasa (MTHFR) en pacientes con labio y paladar hendido no sindrómico en la población del sur de la India: estudio preliminar de casos y controles

A. Sumathi Felicita[1] https://orcid.org/0000-0003-2002-0140
Kamala Devi S.[1] https://orcid.org/0009-0002-8740-7104
Vijayashree Priyadharshini Jayaseelan[1] https://orcid.org/0000-0001-7884-5466

[1]Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai- 600077, India.

Correspondence to: A. Sumathi Felicita - sumifeli@hotmail.com

Received: 7-II-2026 Accepted: 7-III-2026

ABSTRACT: To identify if an association exists between MTHFR gene polymorphism (rs1801133) and non-syndromic cleft lip (NSCLP) and palate in patients belonging to the South Indian population. 25 patients with NSCLP and 25 healthy patients as controls were enrolled in the study. Genotyping of rs1801133 polymorphism was performed with PCR and amplification refractory mutation system. The primers used were MTHFR-Forward: 5'- TGCTGTTGGAAGGTGCAAGAT - 3', MTHFRR1: 5' - GCGTGATGATGAAATCGG - 3' and MTHFRR2: 5' - GCGTGATGATGAAATCGA - 3'. Cycling was carried out with an annealing temperature of 58 degree C for 30 seconds Genotype and allele frequency distributions in both the groups were compared using Chi-square test. The frequency of the GA was 100% without GG and AA genotype in group 1 (case group). The allele frequency in group 2 (control group) was found to be GG=24% and GA=76%. The GG homozygous genotype was absent in the case group, whereas the AA homozygous genotype was absent in boths groups. There was a statistically significant deviation from Hardy Weinberg Equilibrium with a p value of <0. 00001 and <0.0022 in the case group control group respectively. Both the groups showed a deviation in Hardy- Weinberg equilibrium depicting an evolving genotyping variant in the South Indian population. Classification of the genotypes based on genetic models such as dominant, recessive or additive did not present any significant association of the polymorphism marker with disease status.

KEYWORDS: MTHFR gene; rs1801133; Non syndromic; Cleft lip and palate; Polymorphism; Genetic variation.

RESUMEN: El objetivo de este estudio fue identificar si existe una asociación entre el polimorfismo del gen MTHFR (rs1801133) y el labio y paladar hendido no sindrómico (LPHNS) en pacientes pertenecientes a la población del sur de la India. Se incluyeron en el estudio 25 pacientes con LPHNS y 25 individuos sanos como grupo control. La genotipificación se realizó mediante PCR y el sistema de mutación refractaria a la amplificación (ARMS). Los cebadores utilizados fueron: MTHFR-Forward: 5'- TGCTGTTGGAAGGTGCAAGAT - 3', MTHFRR1: 5' - GCGTGATGATGAAATCGG - 3' y MTHFRR2: 5' - GCGTGATGATGAAATCGA - 3'. El proceso de amplificación se llevó a cabo con una temperatura de alineamiento de 58 °C durante 30 segundos. Las distribuciones de frecuencia de genotipos y alelos en ambos grupos se compararon mediante la prueba de chi-cuadrado. La frecuencia del genotipo GA fue del 100%, sin presencia de los genotipos GG ni AA en el grupo 1 (grupo de casos). En el grupo 2 (grupo control), la frecuencia alélica fue GG = 24% y GA = 76%. El genotipo homocigoto GG estuvo ausente en el grupo de casos, mientras que el genotipo homocigoto AA estuvo ausente tanto en ambos grupos. Se observó una desviación estadísticamente significativa del equilibrio de Hardy-Weinberg, con valores de $p < 0,00001$ en el grupo de casos y $p < 0,0022$ en el grupo control. La distribución de frecuencia del polimorfismo génico entre los dos grupos no pudo evaluarse debido a la ausencia de los genotipos GG y AA. Ambos grupos mostraron una desviación del equilibrio de Hardy-Weinberg, lo que sugiere una posible variación genotípica en evolución en la población estudiada. La clasificación de los genotipos basada en modelos genéticos como dominante, recesivo o aditivo no evidenció una asociación significativa del marcador polimórfico con el estado de la enfermedad.

PALABRAS CLAVE: Gen MTHFR; rs1801133; No sindrómico; Labio y paladar hendido; Polimorfismo; Variación genética.

INTRODUCTION

Cleft lip and palate is one of the most common congenital defects in humans especially in infants. They are classified as syndromic and non-syndromic (1,2). Non syndromic cleft contributes to a greater proportion of reported cases compared to syndromic cleft (3). Syndromic cleft lip and palate may be associated with abnormalities of various organs (4). There are several complications associated with cleft lip and palate. Patients with cleft lip and palate may be associated with difficulties in eating, talking and hearing. They may be associated with financial, social, and psychological burden on the family (2,5). The prevalence of non-syndromic cleft lip and palate is different among different ethnic groups with a greater occurrence among the Asian and American population compared to the African population (6).

The etiology of non-syndromic cleft lip and palate is multi-factorial. Some of the common factors that may be associated with the etiology of non-syndromic cleft lip and palate are smoking and alcohol consumption during pregnancy, nutrient inadequacies, and administration of drugs that may have teratogenic effect (2,7). Although several gene mutations have been associated with non-syndromic cleft lip and palate, one of the most predominant genetic influences in the development of non-syndromic cleft lip and palate is the methylenetetrahydrofolate reductase (MTHFR) gene mutation. The annealing between dihydrofolate and S-adenosyl methionine can alter the activity of MTHFR gene (8). The location of the MTHFR gene for coding of the MTHFR enzyme is on chromosome 1 and location p36.3 in the human genome. There is variability in the DNA sequence inside this gene resulting in polymorphism (9).

There are several polymorphisms associated with the MTHFR gene. Of these C677T and A1298C polymorphisms are the most prevalent. These mutations can reduce gene activity resulting in an accumulation of homocysteine. The presence of both the polymorphisms has been suggested to increase the severity of expression of NSCLP (10). These polymorphisms result in a low MTHFR action perhaps with higher homocysteine or lower plasma folate levels (11,12). The process is complex and involves factors related to folate digestion and encoding of key proteins of folate and methionine digestion (8, 13, 14). Methylenetetrahydrofolate reductase results in folate digestion of homocysteine, which catalyzes the decrease of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, circulatory form of folate and the carbon benefactor for the remethylation of homocysteine to methionine (15). The 5-10-methylenetetrahydrofolate reductase (MTHFR) gene is also essential for cell homeostasis because it is vital to the one-carbon cycle, which involves methionine and folate digestion and protein, DNA, and RNA union. The MTHFR gene maintains the methionine and homocysteine equilibrium (16).

Hence, the present study was contemplated to evaluate the influence of the MTHFR C677T gene polymorphism in the development of non-syndromic cleft lip and palate in patients belonging to the South Indian population.

The null hypothesis was that there is no association between non syndromic cleft lip and palate and MTHFR C677T gene polymorphism (rs1801133) in the South Indian population. The aim of the study was to identify the association between MTHFR C677T gene polymorphism (rs1801133) and non-syndromic cleft lip and palate in patients belonging to the South Indian population.

MATERIALS AND METHODS

This was a case control study. The study was performed in the department of orthodontics of our institution. The study was approved by the scientific review board of our university with reference number SRB/SDC/FACULTY/22/ORTHO/052. It was further reviewed and approved by the ethical board with reference number IHEC/SDC/FACULTY/22/ORTHO/581.

100 patients who reported to the cleft and craniofacial unit of our university were screened. Patients with cleft lip and palate without associated syndromes or familial history of cleft lip and palate were included in the study. Patients with a familial history of systemic diseases except diabetes, deleterious oral habits such as smoking, tobacco chewing, pan chewing associated with lesions like leukoplakia, erythroplakia or oral submucous fibrosis were excluded from the study. Patient who received systemic antibiotic treatment during pregnancy or lactation were excluded from the study.

25 patients met the inclusion criteria and were included in the study. 25 healthy individuals without cleft lip and palate were included as control. This study consisted of a total of 50 participants with 25 individuals each in group 1 (case group) and group 2 (control group).

A total of 2 ml venous blood was collected from the antecubital fossa from all patients and transferred into ethylenediaminetetraacetic acid-coated vacutainers. The collected samples were sent to the laboratory for further genetic analysis. Genotyping of rs1801133 polymorphism was performed with polymerase chain reaction (PCR) and amplification refractory mutation system

(ARMS). The primers used were MTHFR-Forward: 5'- TGCTGTTGGAAGGTGCAAGAT - 3', MTHFRR1: 5' - GCGTGATGATGAAATCGG - 3' and MTHFRR2: 5' - GCGTGATGATGAAATCGA - 3'.Cycling was carried out with an annealing temperature of 58 degree C for 30 seconds. Agarose gel electrophoretogram showed allele specific amplification of rs1801133 polymorphism for the MTHFR gene. Each genomic DNA sample amplified with both the primer sets. F+R1 and F+R2 were designated as heterozygous. Samples showing amplification with any one of the primer set namely F+R1 which was specific for G allele or F+R2 which was specific for A allele were designated as either GG homozygous wild-type or AA homozygous variant respectively (Figure 1). The data obtained was tabulated and subjected to statistical analysis.

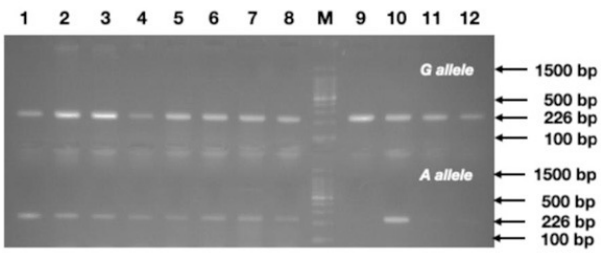

Figure 1. Agarose gel electrophoretogram showing allele specific amplification of polymorphism (rs1801133) spanning the MTHFR gene.

STATISTICAL ANALYSIS

Statistical analysis was done with SPSS software version 17.0 (SPSS; IBM, USA). The Hardy–Weinberg equilibrium (HWE) was tested in each group using Chi Square test. Allele ratio and genotype distribution of NSCLP patients and healthy controls were analyzed. P value ≤ 0.05 was considered to be statistically significant.

RESULTS

50 patients, 25 in each group were evaluated. Patients in group 1 had a mean age of 4.99±5.2 years and patients in group 2 had a mean age of 23.24±6.8 years. The ancestral allele associated with MTHFR gene polymorphism (rs1801133) was the G allele and the variant allele was the A allele with the minor allele frequency 0f 0.50 (https://asia.ensembl.org/Homo_sapiens/Variation/Explore?db=core;r=1:11795821-11796821;v=rs1801133;vdb=variation;vf=122492).

Agarose gel electrophoretogram showed amplification of MTHFR gene with amplicon spanning the polymorphic site (rs1801133) of the MTHFR gene with a size of 226 bp (Figure 1).The frequency of the GA was 100% with no GG and AA genotype in group 1 (case group) (Table 1). The allele frequency in group 2 (control group) was found to be GG=24% and GA=76%. The GG homozygous genotype was absent in the case group, whereas the AA homozygous genotype was absent in both the case and the control group (Table 1). Both the study groups showed a deviation in Hardy- Weinberg equilibrium (Table 1). There was a statistically significant deviation from Hardy Weinberg Equilibrium with a p value of <0. 00001 and <0.0022 in the case group control group respectively (Table 1). The frequency distribution of the gene polymorphism between the two groups could not be tested since the frequency of GG and AA genotype was zero. Classification of the genotypes based on genetic models such as dominant, recessive or additive (allelic) did not present any significant association of the polymorphism marker with the disease status {dominant gene (OR: 0.0588, CI: 0.0031 to 1.1086 p= 0.0586), recessive gene (OR: 1.0000, CI: 0.0191 to 52.3653 p=1.0000), allele (OR: 0.6129, CI: 0.2765 to 1.3584 p=0.2280)} (Table 2). The null hypothesis was accepted.

The comparison of allele frequencies revealed the distribution of G and A alleles of the control in the present study matched the frequencies of the European population (Figure 2). The frequency of

the ancestral G allele was greater than the American population but less than the global, African, East Asian and South Asian population (Figure 2).

Table 1. Genotype frequencies of MTHFR gene polymorphism (rs1801133) among the cases and controls.

Groups	GG (%)	GA (%)	AA (%)	G	A	HWE (p value)*
Case (N=25)	0 (0)	25(100)	0(0)	0.50	0.50	<0. 00001
Control (N=25)	6(24)	19(76)	0(0)	0.62	0.38	<0.0022

*For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom.

Table 2. Overall genotype distribution of the MTHFR gene polymorphism (rs1801133) in cases and controls.

Dominant				
Genotypes	**Case**	**Control**	**Unadjusted OR [95% CI]**	**P value**
GG	0	6	0.0588 [0.0031 to 1.1086]	0.0586
GA+AA	25	19		
Recessive				
GG+GA	25	25	1.0000 [0.0191 to 52.3653]	1.0000
AA	0	0		
Allele				
G	25	31	0.6129 [0.2765 to 1.3584]	0.2280
A	25	19		

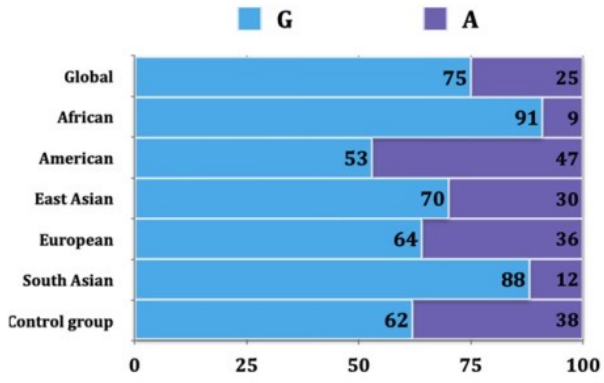

Figure 2. The graph depicts the allele frequency of MTHFR gene polymorphism (rs1801133) in various study population compared to the present study group (Ensembl database).

DISCUSSION

MTHFR gene has a wide range of clinical implications. It has been shown to be strongly associated with essential hypertension, celiac disease, type I diabetes mellitus, and other autoimmune diseases (17,18,19). Climate, diet and pathogen load has been suggested to have a selective influence in different ethnic groups resulting in allele frequency variation around the world (20).

From the present study, genotype frequencies of MTHFR gene polymorphism (rs1801133) among the cases and controls was statistically significant with P<0.00001. The Hardy Weinberg equilibrium showed a significant deviation in both the study group and the control group suggestive of an evolving genotyping variant in the South Indian

population. However, classification of the genotypes based on genetic models such as dominant, recessive or additive (allelic) did not present any significant association of the polymorphism marker with non-syndromic cleft lip and palate in the present study.

Earlier studies in the South Indian population with DNA sequence analysis of the C 677T MTHFR gene polymorphism showed that the polymorphism was dormant in 16% of the sample of NSCLP cases (21). The C677T MTHFR gene variant was a minor risk factor (21,22) and may not be a genetic marker for NSCLP in the South Indian population (21). All the above findings were similar to the findings of the present study. However, one study found a significant association between MTHFR (rs1801133) and NSCLP and considered it presence a risk factor in the Indian population (23).

There was no association of MTHFR C677T and non-syndromic cleft lip and palate in the Japanese population with no significant differences in the frequencies of MTHFR C677T gene polymorphisms between the patients and controls in the Japanese population with no transmission equilibrium or linkage equilibrium among the cases (24).

Similarly, there was a low association of C677T polymorphism of the MTHFR gene and a risk of development of non-syndromic cleft lip and palate in the Moroccan population (25).

In the Mexican population, maternal folic acid intake during the periconceptional period and the TT genotype compared to the CC genotype of the MTHFR C677T polymorphism in children independently reduced the risk of NSCLP (26).

A decreased risk of NSCLP was observed in patients from Tibet, Bangladesh, and Iran, presenting the C677T variant at MTHFR gene (27).

In the Iranian population, there were several sstudies that found an association between NSCLP and MTHFR 677TT genotype with increased risk of for the development of orofacial clefts (28, 29). It was found that the risk of development of NSCLP was higher when the mothers didn't use folic acid (29). In the Iranian population, there was a significant difference in the rates of the C677T mutation when affected patients and their fathers were compared with the control group. There was no significant difference between the mothers and the control group (30). However, one study found no association between genetic polymorphism of MTHFR c.677C>T and the risk of NSCLP in the Iranian population (31).

There was no evidence of association between rs1801133 of MTHFR gene and cleft palate only in the Italian and Asian population and there was difference in the genetic background between non syndromic cleft lip and palate and cleft palate only (32).

In the Chilean population offspring and maternal genotypes for MTHFR c.677C>T variant was strongly associated with NSCLP (33). In Shanxi Province of China, MTHFR C677T gene polymorphism was found to be associated with the development of NSCLP (34).

A meta-analysis of literature showed that fetal MTHFR 677 C>T polymorphism may be is significantly associated with NSCLP and the risk may be greater among some population groups compared to others (35). MTHFR 677 C>T polymorphism has been found to contribute to the development of NSCLP in Caucasian, Brazilian, Turkish, and Indian populations, but not in Asian, Chinese, and US-American population (35).

Meta-analysis of 22 studies with 3724 NSCLP cases and 5275 controls to establish the association of the MTHR C677T polymorphism with NSCLP around the world suggested that MTHFR

C677T polymorphism is significantly associated with non-syndromic orofacial cleft (36) although it may not have a significant effect on the South Indian population according to the present study.

Treatment of cleft lip and palate starts at a very early age and consists of surgery, orthodontic treatment, feeding therapy, genetic evaluation and speech and hearing therapy (37-42), facial esthetics can be assessed with several predetermined parameters (43). The recent development in the treatment of cleft lip and palate involves the use of artificial intelligence (44) and stem cell for bone regeneration at the cleft site (45). The study can be performed in another centre with similar settings. Future studies with a larger sample size can be contemplated.

CONCLUSION

The present study showed that there was no association between MTHFR C667T gene polymorphism (rs1801133) and non-syndromic cleft lip and palate in the South Indian population. However, a deviation for the Hardy Weinberg distribution showed an evolving population.

AUTHOR CONTRIBUTION STATEMENT: Conceptualization and design: V.P.J.; Literature Review: A.S.F.; Methodology and validation:, V.P.J.; Formal analysis: V.P.J. and A.S.F.; Investigation and data collection: A.S.F.; Resources: K.D.S.; Data analysis and interpretation: V.P.J. and A.S.F.; Writing – original draft preparation: A.S.F.; Writing – review & editing: A.S.F.; Fund acquisition: K.D.S.

REFERENCES

1. Farronato G., Cannalire P., Martinelli G., Tubertini I., Giannini L., Galbiati G., Maspero C. Cleft lip and/or palate: review. Minerva Stomatol. 2014; 63: 111-26.
2. Vyas T., Gupta P., Kumar S., Gupta R., Gupta T., Singh H.P. Cleft of lip and palate: A review. J Family Med Prim Care. 2020; 9: 2621-25.
3. Martinelli M., Palmieri A., Carinci F., Scapoli L. Non-syndromic Cleft Palate: An Overview on Human Genetic and Environmental Risk Factors. Front Cell Dev Biol. 2020; 20: 592271.
4. Venkatesh R. Syndromes and anomalies associated with cleft. Indian J Plast Surg. 2009; 42: S51-5.
5. De Cuyper E., Dochy F., De Leenheer E., Van Hoecke H. The impact of cleft lip and/or palate on parental quality of life: A pilot study. Int J Pediatr Otorhinolaryngol. 2019: 109598.
6. Putri F.A., Pattamatta M., Anita S.E.S., Maulina T. The Global Occurrences of Cleft Lip and Palate in Pediatric Patients and Their Association with Demographic Factors: A Narrative Review. Children (Basel). 2024; 11: 322.
7. Murray J.C. Gene/environment causes of cleft lip and/or palate. Clinical genetics. 2002; 61: 248-56.
8. Abdollahi-Fakhim S., Asghari Estiar M., Varghaei P., Alizadeh Sharafi M., Sakhinia M., Sakhinia E. Common Mutations of the Methylenetetrahydrofolate Reductase (MTHFR) Gene in Non-Syndromic Cleft Lips and Palates Children in North-West of Iran. Iran J Otorhinolaryngol. 2015; 27: 7-14.
9. Davis W.H. Development of a high-throughput genotyping assay for detection of functional polymorphisms involved in homocysteine metabolism and the methylation process implicated in multiple sclerosis (Doctoral dissertation, Stellenbosch: Stellenbosch University).
10. Lupi-Herrera E., Soto-López M.E., Lugo-Dimas A.J., Núñez-Martínez M.E., Gamboa R., Huesca-Gómez C., Sierra-Galán L.M., Guarner-Lans V. Polymorphisms C677T and A1298C of MTHFR Gene: Homocysteine Levels and Prothrombotic Biomarkers in

Coronary and Pulmonary Thromboembolic Disease. Clin Appl Thromb Hemost. 2019; 25: 1076029618780344.
11. Zarembska E., Ślusarczyk K., Wrzosek M. The Implication of a Polymorphism in the Methylenetetrahydrofolate Reductase Gene in Homocysteine Metabolism and Related Civilisation Diseases. Int J Mol Sci. 2023; 25: 193.
12. Blanton S.H., Henry R.R., Yuan Q., Mulliken J.B., Stal S., Finnell R.H., Hecht J.T. Folate pathway and nonsyndromic cleft lip and palate. Birth Defects Res A Clin Mol Teratol. 2011; 91: 50-60.
13. Li Q., Xu L., Jia X., Saleem K., Zaib T., Sun W., Fu S. SNPs in folate pathway are associated with the risk of nonsyndromic cleft lip with or without cleft palate, a meta-analysis. Biosci Rep. 2020; 27; 40: BSR20194261.
14. Zheng Y., Cantley L.C. Toward a better understanding of folate metabolism in health and disease. J Exp Med. 2019; 216: 253-266.
15. Friso S., Choi S.W., Girelli D., Mason J.B., Dolnikowski G.G., Bagley P.J., Olivieri O., Jacques P.F., Rosenberg I.H., Corrocher R., Selhub J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A. 2002; 99: 5606-11.
16. Zarembska E., Ślusarczyk K., Wrzosek M. The Implication of a Polymorphism in the Methylenetetrahydrofolate Reductase Gene in Homocysteine Metabolism and Related Civilisation Diseases. International Journal of Molecular Sciences. 2024; 25: 193.
17. Fabbri E., Rustignoli L., Muscari A., Puddu G.M., Guarino M., Rinaldi R., Minguzzi E., Caio G., Zoli M., Volta U. Recurrent ischemic strokes in a young celiac woman with MTHFR gene mutation. World J Gastroenterol. 2012; 18: 3472-6.
18. Meng Y., Liu X., Ma K., Zhang L., Lu M., Zhao M., Guan M.X., Qin G. Association of MTHFR C677T polymorphism and type 2 diabetes mellitus (T2DM) susceptibility. Mol Genet Genomic Med. 2019;7: e1020.
19. Lu M., Peng K., Song L., Luo L., Liang P., Liang Y. Association between Genetic Polymorphisms in Methylenetetrahydrofolate Reductase and Risk of Autoimmune Diseases: A Systematic Review and Meta-Analysis. Dis Markers. 2022: 4568145.
20. Hancock A.M., Rienzo A.D. Detecting the Genetic Signature of Natural Selection in Human Populations: Models, Methods, and Data. Annu Rev Anthropol. 2008; 37: 197-217.
21. Desai A.L., Dinesh M.R., Amarnath B.C., Dharma R.M., Akshai K.R., Prashanth C.S. Association of Transforming Growth Factor Alpha and Methylenetetrahydrofolate reductase gene variants with nonsyndromic cleft lip and palate in the Indian population. Contemp Clin Dent. 2014; 5: 329-33.
22. Murthy J., Gurramkonda V.B., Karthik N., Lakkakula B.V. MTHFR C677T and A1298C polymorphisms and risk of nonsyndromic orofacial clefts in a south Indian population. Int J Pediatr Otorhinolaryngol. 2014; 78: 339-42.
23. Avasthi K.K., Agarwal A., Agarwal S. Association of MTHFR, BMP4, TGFA and IRF6 Polymorphisms with Non-Syndromic Cleft lip and Palate in North Indian Patients. Avicenna J Med Biotechnol. 2022; 14: 175-180.
24. Komiyama Y., Koshiji C., Yoshida W., Natsume N., Kawamata H. 5,10-Methylenetetrahydrofolate reductase (MTHFR) C677T/A1298C polymorphisms in patients with nonsyndromic cleft lip and palate. Biomed Rep. 2020; 6: 57.
25. Rafik A., Rachad L., Kone A.S., Nadifi S. MTHFR C677T polymorphism and risk of nonsyndromic cleft lip with or without cleft palate in the Moroccan population. Appl Clin Genet. 2019; 7: 51-54.

26. Estandia-Ortega B., Velázquez-Aragón J.A., Alcántara-Ortigoza M.A., Reyna-Fabian M.E., Villagómez-Martínez S., González-Del Angel A. 5,10-Methylenetetrahydrofolate reductase single nucleotide polymorphisms and gene-environment interaction analysis in non-syndromic cleft lip/palate. Eur J Oral Sci. 2014; 122: 109-13.
27. Martinelli M., Girardi A., Cura F., Nouri N., Pinto V., Carinci F., Morselli P.G., Salehi M., Scapoli L. Non-syndromic cleft lip with or without cleft palate in Asian populations: Association analysis on three gene polymorphisms of the folate pathway. Arch Oral Biol. 2016; 61: 79-82.
28. Ebadifar A., Ameli N., Khorramkhorshid H.R., Salehi Zeinabadi4 M., Kamali K., Khoshbakht T. Incidence Assessment of MTHFR C677T and A1298C Polymorphisms in Iranian Non-syndromic Cleft Lip and/or Palate Patients. J Dent Res Dent Clin Dent Prospects. 2015; 9: 101-4.
29. Ebadifar A., KhorramKhorshid H.R., Kamali K., Salehi Zeinabadi M., Khoshbakht T., Ameli N. Maternal Supplementary Folate Intake, Methylenetetrahydrofolate Reductase (MTHFR) C677T and A1298C Polymorphisms and the Risk of Orofacial Cleft in Iranian Children. Avicenna J Med Biotechnol. 2015; 7: 80-4.
30. Abdollahi-Fakhim S., Asghari Estiar M., Varghaei P., Alizadeh Sharafi M., Sakhinia M., Sakhinia E. Common Mutations of the Methylenetetrahydrofolate Reductase (MTHFR) Gene in Non-Syndromic Cleft Lips and Palates Children in North-West of Iran. Iran J Otorhinolaryngol. 2015; 27: 7-14.
31. Jahanbin A., Hasanzadeh N., Abdolhoseinpour F., Sadr-Nabavi A., Raisolsadat M.A., Shamsian K., Mohajertehran F., Kianifar H. Analysis of MTHFR Gene C.677C>T and C.1298A>C Polymorphisms in Iranian Patients with Non-Syndromic Cleft Lip and Palate. Iran J Public Health. 2014; 43: 821-7.
32. Carinci F., Palmieri A., Scapoli L., Cura F., Borelli F., Morselli P.G., Nouri N., Abdali H., Gianni A.B., Russillo A., Docimo R., Martinelli M. Non-syndromic cleft palate: Association analysis on three gene polymorphisms of the folate pathway in Asian and Italian populations. Int J Immunopathol Pharmacol. 2019; 33: 2058738419858572.
33. Ramírez-Chau C., Blanco R., Colombo A., Pardo R., Suazo J. MTHFR c.677C>T is a risk factor for non-syndromic cleft lip with or without cleft palate in Chile. Oral Dis. 2016; 22: 703-8.
34. Nan X., Liu M., Yuan G. Relationship between genetic polymorphism of MTHFR C677T and nonsyndromic cleft lip with or without cleft palate in Shanxi Province of China]. Zhonghua Zheng Xing Wai Ke Za Zhi. 2014; 30: 265-9.
35. Amooee A., Dastgheib S.A., Niktabar S.M., Noorishadkam M., Lookzadeh M.H., Mirjalili S.R., Heiranizadeh N., Neamatzadeh H. Association of Fetal MTHFR 677C>T Polymorphism with Non-Syndromic Cleft Lip with or without Palate Risk: A Systematic Review and Meta-Analysis. Fetal Pediatr Pathol. 2021; 40: 337-353.
36. Rai V. Strong Association of C677T Polymorphism of Methylenetetrahydrofolate Reductase Gene with Nosyndromic Cleft Lip/Palate (nsCL/P). Indian J Clin Biochem 2018; 33: 5-15.
37. Natarajan P.G., Mahipathy S.R., Nalabothu P. Anatomic Subunit Cleft Lip Repair: New Repair Concept Based on Nostril Sill and Philtrum-White Roll Junction. Journal of Craniofacial Surgery. 2024; 35:2463-5.
38. Alkadhi O.H., Alotaibi L.H., Alrashoud R.R., Almutairi M.H., Al Matar H.A., Mallineni S.K. Effect of Maxillary Expansion on the Maxillary

Arch Width in Patients with Bilateral Cleft Palate: A Review. Children. 2023; 10: 762.
39. da Silva Filho, O.G. · Teles, S.G. · Ozawa, T.O. Secondary bone graft and eruption of the permanent canine in patients with alveolar clefts: literature review and case report. Angle Orthod. 2000; 70:174-178
40. Tindlund, R.S. · Rygh, P. · Boe, O.E. Orthopedic protraction of the upper jaw in cleft lip and palate patients during the deciduous and mixed dentition periods in comparison with normal growth and development. Cleft Palate Craniofac J. 1993; 30:182-194
41. Robin N.H., Baty H., Franklin J., Guyton F.C., Mann J., Woolley A.L., Waite P.D., Grant J. The multidisciplinary evaluation and management of cleft lip and palate. South Med J. 2006; 99: 1111-20.
42. Frederick R., Hogan A.C., Seabolt N., Stocks R.M.S. An Ideal Multidisciplinary Cleft Lip and Cleft Palate Care Team. Oral Dis. 2022; 28: 1412-1417.
43. Anishya D., Nagesh S. Assessment of Nasal Aesthetic Parameters in Patients with Unilateral Cleft Lip and Palate–A Retrospective Study. The Cleft Palate Craniofacial Journal. 2024; 11:10556656241261908.
44. Jaber M., Alshikh Ali A.M., El Saleh R.I., Prasad P. The Use of Stem Cells in Bone Regeneration of Cleft Lip and Palate Patients: A Systematic Review. Journal of Clinical Medicine. 2024; 13: 5315.
45. Tovani-Palone M.R. Management of orofacial clefts in times of artificial intelligence: advances and challenges. European Archives of Paediatric Dentistry. 2024; 31: 1-2.