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# Association of MSX1 gene polymorphisms and maxillary lateral incisor agenesis in Non-syndromic cleft lip and/or palate individuals

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A B S T R A C T

*Background:* To investigate the association between MSX1 gene polymorphisms and maxillary lateral incisor agenesis with and without Non Syndromic Cleft lip and palate (NSCL/P) in a South Indian population.
*Methods:* The study sample was divided into four groups: Group I: Twenty five Cleft lip and palate (NSCL/P) subjects with unilateral/bilateral congenitally missing maxillary lateral incisors, without agenesis of any other permanent teeth, Group II: Twenty five individuals with NSCL/P and a full complement of teeth, Group III: Twenty five non-cleft individuals with unilateral/bilateral congenitally missing maxillary lateral incisors and Control group: Twenty five healthy individuals with normal teeth development and without orofacial defects. MSX1 gene polymorphisms rs12532, rs1042484 and rs11726039 were genotyped by Sanger sequencing method with pre designed primer using ABI 3730 DNA sequencer. Associations were tested using Pearson Chi-square analysis.
*Results:* The three SNPs, rs12532, rs1042484 and rs11726039 of MSX1 gene exhibited polymorphism. rs12532 was associated with reduced risk of maxillary lateral incisor agenesis in NSCL/P subjects. rs1042484 had significant association with the NSCL/P without maxillary lateral incisor agenesis (full complement of teeth) subjects. rs11726039 showed significant association with NSCL/P with or without maxillary lateral incisor agenesis.
*Conclusion:* The present study on a South Indian population revealed a significant association between the MSX1 gene polymorphisms and tooth agenesis in NSCL/P subjects. Keywords: MSX1, non syndromic cleft lip and palate, lateral incisor agenesis.

## 1. Introduction

Nonsyndromic cleft lip with/without cleft palate (NSCL/P) is the most common orofacial defect in humans.[1] During the early stages of embryogenesis, the incomplete separation of nasal and oral cavities characterizes NSCL/P.[2] According to the World Health Organization (WHO), the prevalence of NSCL/P varies globally, ranging from 3.4 to 22.9 per 10,000 births for CL/P and 1.3–25.3 per 10,000 births for cleft palate only. The incidence of NSCL/P varies among the studies conducted in different geographical locations, ethnicity, race, gender, and socioeconomic status. [3] According to the Indian Council of Medical Research (ICMR) task force, 15 % of instances with CL/P cases were found to have a familial connection whereas, the other 85 % of cases did not involve familial history. [4]

NSCL/P is a multifactorial disorder caused by genetic and environmental factors that act during the facial developmental of organogenesis stage. Further, changes in the growth factors, extracellular matrix (ECM), and cell adhesion molecules during the cell specification and morphogenesis may also play an essential role in orofacial development. Epidemiological studies have also linked tobacco, alcohol consumption, and multivitamin supplementation to NSCL/P. [5]

Recent studies have identified several susceptible loci and the genetic polymorphisms of more than ten genes linked with the risk of NSCL/P. [6,7] These include genes such as *PAX9*, *IRF6*, *SATB2*, *TBX22*, and *MSX*. [8] Muscle segment homobox gene 1 (*MSX 1,* 14p16.1) encodes a protein of 297 amino acids that functions as a transcriptional repressor during embryogenesis and contributes to craniofacial and dental development of the mesenchyme.[9] Further, the role of the *MSX1* gene in maxillary lateral incisor agenesis has been established.[10] However, due to the heterogeneity of the disease, the genetic polymorphism for NSCL/P remains largely inconclusive. So, the role of MSX1 in subjects with NSCL/P and maxillary lateral incisor agenesis traits would shed further light on the role of MSX1. Therefore, the present study investigated the association between MSX1 gene polymorphisms and maxillary

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lateral incisor agenesis with/without NSCL/P in a South Indian population.

## 2. Methods

The Institutional Ethics Committee approval was obtained before commencing the study (REF: CSP/16/NOV/52/287). The inclusion criteria were non syndromic patients in the age group of 14–35 years. The exclusion criteria were patients only with cleft lip, supernumerary teeth or multiple tooth agenesis. The sample size calculation was estimated using an incidence of 47.5 %, with 10 % absolute error and 95 % confidence interval. This provided requirement of 23 subjects per group, leading to a total of 92 subjects. Considering an attrition rate of 8 %, 25 patients for each of the four groups were selected. Informed consent/assent was obtained from these 100 patients or parents. (if the patients were less than 18 years)

Group I (NSCL/P with missing maxillary laterals): Twenty five subjects with NSCL/P with unilateral/bilateral congenitally missing maxillary lateral incisors, without agenesis of any other permanent teeth.
Group II (NSCL/P with no missing teeth): Twenty five subjects with NSCL/P and a full complement of teeth.
Group III (Non cleft with missing laterals): Twenty five non-cleft subjects with unilateral/bilateral congenitally missing maxillary lateral incisors.
Group IV (Control group): Twenty five healthy individuals with normal teeth development and without orofacial defects.

Table 1 provides the general characteristics of the patients. The age (mean ± SD) was 16.3 ± 5.6 years for Group I, 22.4 ± 5.4 years for Group II, 15.8 ± 4.9 years for Group III and 21 ± 4.5 years for group IV. Group I had 11 males and 14 females, Group II had eight males and 17 females, Group III had 15 males and 10 females and Group IV had had seven males and 18 females.

## 3. SNP selection and genotyping

The three (rs1042484, rs12532, and rs11726039)tagging single nucleotide polymorphisms (tag SNPs) covering *MSX1* gene from the release 2.0 Phase II data of the HapMap Project (www.hapmap.org) were selected using the *pairwise* tagging method.[11] The tag SNPs were chosen according to the following criteria: $r^2 \geq 0.8$ and minor allele frequency of ≥5 % in the Gujarati Indians in Houston (GIH) population.[12] Prior to saliva sample collection, the subjects were advised not to consume any food or liquids for a minimum of 30 min prior. The subjects were asked to hold the saliva in their mouths for a minute and were then requested to spit it out into the container. Three ml of unstimulated saliva sample was collected from the study participants. The human genomic DNA was isolated from the saliva using Norgen's Saliva DNA Isolation Kit (Cat. RU45400-NB). Further, the DNA quality and quantity were estimated by using NanoDrop ND-1000 Spectrophotometer (Thermo Scientific NanoDrop Technologies, Wilmington, DE). Sanger DNA sequencing (Big Dye Termination Kit-Applied bioscience-Cleveland Ohio, United States of America) based on the use of fluorescently labeled dideoxynucleotides (ddNTPs) in addition to the normal nucleotides (NTPs) which is found in the DNA was used to assess the samples. MSX1 gene polymorphisms were genotyped by the Sanger sequencing method with pre designed primer using ABI 3730 DNA sequencer. DNA molecules were labeled with multiple fluorescent dyes. Genetic, dominant, recessive, and allelic models were performed to find the association between the MSX1 gene polymorphisms and the four groups.

**Table 1**
Demographics of the four groups.

| | | |
|---|---|---|
| Group I (N = 25) NSCL/P with missing laterals | Mean Age | 16.3 ± 5.6 years |
| | Male | 11 |
| | Female | 14 |
| Group II (N = 25) | Mean Age | 22.4 ± 5.4 years |
| NSCL/P with no missing teeth | Male | 8 |
| | Female | 17 |
| Group III (N = 25) | Mean Age | 15.8 ± 4.9 years |
| Non Cleft with missing laterals | Male | 15 |
| | Female | 10 |
| Group IV - Control (N = 25) | Mean Age | 21 ± 4.5years |
| | Male | 7 |
| | Female | 18 |

## 4. Statistical analysis

Clinical and biochemical data were reported as means and standard deviations (SD). Allele and genotype frequencies were established by gene counting and compared by 2 × 2 and 2 × 3 contingency tables, respectively. Hardy-Weinberg exact test was performed for each marker to evaluate whether the observed genotype frequency distribution would be consistent with the expected outcome of the Hardy –Weinburg equilibrium for genotyping error and also to identify any potential issues with SNP or gene that may explain the observed variation. Chi-square analysis with one degree of freedom and a Monte Carlo simulation test using the HWSIM program were performed. Values of $p < 0.05$ were considered statistically significant.

## 5. Results

### 5.1. Role of MSX1 rs12532

The frequency of minor allele was 18 in Group 1 and four in Group 4 showing an increase in NSCL/P with maxillary incisor agenesis subjects. The frequencies were in Hardy-Weinburg equilibrium. The AG genotype was similar in both the groups. ($p = 0.1$). As there was no GG mutant homozygous genotype in the Control (Group 4) group, the computation of Odds Ratio was not possible. The genotypes in the dominant model failed to show association with Group 1. ($p = 0.07$) The allelic model showed an association ($p = 0.03$) and thus contributed to the reduced risk of maxillary incisor agenesis in NSCL/P subjects. ($p = 0.03$) The model however failed to show association between maxillary incisor agenesis in non-cleft subjects. ($p = 0.64$) For Group 2, the genotypes in the dominant model and allelic groups showed a significant association. ($p = 0.02$ and $p = 0.01$ respectively) The genotypes, dominant model and alleles contributed to the reduced risk of maxillary lateral incisor agenesis in NSCL/P subjects. For Group 3, the genotypes in the dominant model and allelic group did not show a significant association. ($p = 0.63$ and $p = 0.64$ respectively) The genotypes, dominant model and alleles did not contribute to the risk of maxillary lateral incisor agenesis in non-cleft subjects.

### 5.2. Role of MSX1 rs1042484

The frequency of minor allele was 18 in Group 1 and eight in Group 4 indicating the presence of more mutant allele in Group 1. The frequencies deviated from Hardy-Weinburg equilibrium. The AG genotype was similar in both the groups. ($p = 0.56$) The GG mutant homozygous was equally distributed in Groups 1 and 4. ($p = 0.27$) The genotypes in the dominant and recessive models failed to show association with Group 1. ($p = 0.43$ and $p = 0.29$ respectively) The allelic model also failed to show an association ($p = 0.13$) The genotypes, dominant and recessive models and alleles did not contribute to the risk of NSCL/P with maxillary lateral incisor agenesis. For Group 2, the genotypes in the dominant model and allelic group failed to show a significant association. ($p = 0.35$ and $p = 0.15$ respectively) The allelic model showed a significant association with NSCL/P with full complement of teeth. ($p = 0.04$). The genotypes, dominant, recessive models and alleles did not

contribute to the reduced risk of maxillary lateral incisor agenesis in NSCL/P subjects. The allelic group contributed to the risk of NSCL/P with full complement of teeth. For Group 3, the genotypes in the dominant and recessive model did not show a significant association. (p = 1) The allelic model also did not show significance. The genotypes, dominant model and alleles did not contribute to the risk of maxillary lateral incisor agenesis in non-cleft subjects.

### 5.3. Role of MSX1 rs11726039

The frequency of minor allele was 16 in Group 1 and four in Group 4 indicating an increased presence of more mutant allele in Group 1. The frequencies were in from Hardy-Weinburg equilibrium. The TC genotype was similar in both the groups. (p = 0.89) As there was no CC mutant homozygous genotype in the Control (Group 4) group, the computation of Odds Ratio was not possible. The genotypes in the dominant model failed to show association with Group 1. (p = 0.22) The allelic model showed a significant association with NSCL/P with maxillary lateral incisor agenesis (p = 0.04) and therefore contributed to the risk of NSCL/P with maxillary lateral incisor agenesis. The allelic model showed a significant association with NSCL/P with full complement of teeth. (p = 0.01). For Group 3, the genotypes in the dominant model did not show a significant association. (p = 0.63) The allelic model also did not show significance. (p = 0.39)The genotypes, dominant model and alleles did not contribute to the risk of maxillary lateral incisor agenesis in non-cleft subjects (Tables 3 and 4).

## 6. Discussion

The association between MSX1 gene polymorphisms and maxillary lateral incisor agenesis with and without Non Syndromic Cleft lip and palate (NSCL/P) in a South Indian population was evaluated. MSX1 gene polymorphisms rs12532, rs1042484 and rs11726039 were genotyped. Genetic polymorphism can be observed in allelic heterogeneity. While genes code for specific proteins alleles are changed versions of the same gene.

Several previous studies have been investigated the role of the MSX1 gene polymorphisms in the pathogenesis of NSCL/P in different human populations.[13–15] The main finding of this study is that the MSX1 gene polymorphisms (rs1042484, rs12532, and rs11726039) showed a significant association with tooth agenesis with/without NSCL/P either in allelic, genetic, and/or dominant models (Table 2). Similarly, a study conducted on 28 Korean NSCL/P subjects and 98 subjects with CLP, revealed that the MSX1-rs12532 polymorphisms were significantly associated with tooth agenesis.[16] Further, the A allele at MSX1-rs3821949 was strongly correlated with a considerably higher risk of NSCL/P. The A allele at rs3821949 also increased the risk of NSCLP, while the G allele was under-represented.[16] Fallin et al. observed eight single nucleotide polymorphisms of the *MSX1* gene and reported that the rs3821949, and rs12532 was associated with NSCL/P.[14] The results are in accordance with a case-control study from the south Indian population analyzed the five SNPs of the MSX1 gene (rs11726039, rs868257, rs6446693, rs1907998, and rs6832405) with 173 patients NSCL/P and 176 controls revealed significant association between MSX1 rs11726039 and NSCL/P. [17] A recent meta-analysis covered 12 SNPs from the MSX1 gene revealed that the eight MSX1 SNPs were associated with risk of NSCL/P. [18] Contradictory to this, studies on Brazilian [19,20,] European,[21] Chinese, [13,22,23] and populations failed to find a significant association between the MSX1 gene polymorphisms and NSCL/P. The diverse findings in literature and the variance with the findings of our study reinforces the complex genetic and environmental interplay in the development of NSCL/P.

The findings of this research might provide valuable insights into the impact of genetic diversity in MSX1 gene polymorphisms on tooth agenesis of NSCL/P within the south Indian population. However, several factors are to be considered when interpreting the results obtained in this study. First, it is crucial to assess the MSX1 genotype and allelic distribution in non-cleft subjects, both with and without tooth agenesis to definitively establish the exact role of SNPs in tooth agenesis. Second, it is necessary to confirm the functional effects of these SNPs. Further studies on orofacial cleft development and the effects of gene to gene interactions, environmental factors and the role of ethnicity are warranted.

The main limitation of this study was that it was restricted to a South Indian population. The other limitation is the constraint of the limited sample size. There was no data on the consanguinity or other genetic factors which could be involved especially in NSCL/P. However, the division into four separate groups would be a major strength and novelty of the study.

## 7. Conclusion

The present study revealed a significant association between the MSX1 gene polymorphisms and tooth agenesis of NSCL/P among the south Indian population. Thus the need for genetic counseling for not only the surgical management of NSCL/P but also the early diagnosis and management of the missing maxillary lateral incisor would be prudent for the discerning clinician.

## IEC

The Institutional Ethics Committee approval was obtained before commencing the study (REF: CSP/16/NOV/52/287).

## Patient guardian consent obtained

Patient guardian consent obtained.

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**Table 2**
Association analysis between the four groups studied and rs1042484. (MAF: Minor Allele Frequency, HWE-p: Hardy Weinberg Equilibrium p value).

| SNP | Control (C) | Group (G1) | Group 2 (G2) | Group 3 (G3) | P value C Vs G1 | P value C Vs G2 | P value C Vs G3 |
|---|---|---|---|---|---|---|---|
| AA | 22 (88) | 19 (76) | 18 (72) | 22 (88) | Reference | Reference | Reference |
| AG | 2 (8) | 3 (12) | 3 (12) | 2 (8) | 0.56 | 0.53 | 1 |
| GG | 1 (4) | 3 (12) | 4 (16) | 1 (4) | 0.27 | 0.14 | 1 |
| AG + GG | 3 (12) | 6 (24) | 7 (28) | 3 (12) | 0.27 | 0.18 | 1 |
| GG | 1 (4) | 3 (12) | 4 (16) | 1 (4) | Reference | Reference | Reference |
| AA + AG | 24 (96) | 22 (88) | 21 (84) | 24 (96) | 0.30 | 0.18 | 1 |
| A | 46 (92) | 41 (82) | 39 (78) | 46 (92) | Reference | Reference | Reference |
| G | 4 (8) | 9 (18) | 11 (22) | 4 (8) | 0.13 | 0.04 | 1 |
| MAF | 8 | 18 | 22 | 8 | NA | NA | NA |
| HWE-p | 0.02 | 0.00 | 0.00 | 0.02 | NA | NA | NA |

**Table 3**
Association analysis between the four groups studied and **rs12532**. (MAF: Minor Allele Frequency, HWE-p: Hardy Weinberg Equilibrium p value).

| AA | 23 (92) | 18 (72) | 16 (64) | 22 (88) | Reference | Reference | Reference |
|---|---|---|---|---|---|---|---|
| AG | 2 (8) | 5 (20) | 8 (32) | 3 (12) | 0.18 | **0.03** | 0.64 |
| GG | 0 | 2 (8) | 1 (4) | 0 | 0.12 | 0.24 | NA |
| AG + GG | 2 (8) | 7 (28) | 9 (36) | 3 (12) | 0.07 | **0.02** | 0.64 |
| GG | 0 | 2 (8) | 1 (4) | 0 | Reference | Reference | Reference |
| AA + AG | 25 (100) | 23 (92) | 24 (96) | 25 (100) | 0.15 | 0.31 | NA |
| A | 48 (96) | 41 (82) | 40 (80) | 47 (94) | Reference | Reference | Reference |
| G | 2 (4) | 9 (18) | 10 (20) | 3 (6) | **0.03** | **0.01** | 0.65 |
| MAF | 4 | 18 | 20 | 0 | NA | NA | NA |
| HWE-p | 0.83 | 0.11 | 1 | 0.75 | NA | NA | NA |

**Table 4**
Association analysis between the four groups studied and **rs11726039**. (MAF: Minor Allele Frequency, HWE-p: Hardy Weinberg Equilibrium p value).

| TT | 23 (92) | 20 (80) | 18 (72) | 22 (84) | Reference | Reference | Reference |
|---|---|---|---|---|---|---|---|
| TC | 2 (8) | 2 (8) | 4 (16) | 2 (11.5) | 0.89 | 0.30 | 0.97 |
| CC | 0 | 3 (12) | 3 (12) | 1 (3.8) | 0.07 | 0.06 | 0.31 |
| TC + CC | 2 (8) | 5 (20) | 7 (28) | 4 (15.3) | 0.221 | 0.07 | 0.41 |
| CC | 0 | 3 (12) | 3 (12) | 1 (3.8) | Reference | Reference | Reference |
| TT + TC | 25 (100) | 22 (88) | 22 (88) | 24 (96) | 0.07 | 0.07 | 0.31 |
| T | 48 (96) | 42 (84) | 40 (80) | 47 (90) | Reference | Reference | Reference |
| C | 2 (4) | 8 (16.0) | 10 (20) | 5 (9.6) | **0.04** | **0.01** | 0.26 |
| MAF | 4 | 16 | 20 | 8 | NA | NA | NA |
| HWE-p | 0.83 | 0.00 | 0.01 | 0.02 | NA | NA | NA |


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## Declaration of competing interest

The authors declare that they have no conflicts of interest.

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## References


1. Rahimov F, Jugessur A, Murray JC. Genetics of nonsyndromic orofacial clefts. *Cleft Palate Craniofac J*. 2012;49:73–91.
2. Hammond NL, Dixon MJ. Revisiting the embryogenesis of lip and palate development. *Oral Dis*. 2022 Jul;28(5):1306–1326.
3. Ahmed MK, Bui AH, Taioli E. Epidemiology of cleft lip and palate. In: *Designing Strategies for Cleft Lip and Palate Care*. Intech Open; 2017 Mar 22.
4. Indian Council of Medical Research (ICMR) Task Force Project. *Division of Non-communicable Diseases*. 2016. New Delhi.
5. 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; 8, 592271.
6. Ebadifar A, Hamedi R, KhorramKhorshid HR, Kamali K, Moghadam FA. Parental cigarette smoking, transforming growth factor-alpha gene variant and the risk of orofacial cleft in Iranian infants. *Iran J Basic Med Sci*. 2016;19(4):366–373.
7. Zucchero TM, Cooper ME, Maher BS, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *New Eng J Med*. 2004;351(8): 769–780.
8. Nakatomi M, Wang XP, Key D, et al. Genetic interactions between Pax9 and Msx1 regulate lip development and several stages of tooth morphogenesis. *Dev Biol*. 2010; 340(2):438–449.
9. Mitchell LE, Murray JC, O'Brien S, Christensen K. Evaluation of two putative susceptibility loci for oral clefts in the Danish population. *Am J Epidemiol*. 2001;153 (10):1007–1015.
10. Boeira Junior BR, Echeverrigaray S. Dentistry and molecular biology: a promising field for tooth agenesis management. *Tohoku J Exp Med*. 2012;226(4):243–249.
11. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinform*. 2005;21(2):263–265.
12. 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*. 2015;526(7571):68–74.
13. Huang YQ, Ma J, Ma M, et al. Association between MSX1 variants and oral clefts in Han Chinese in western China. *DNA Cell Biol*. 2011;30(12):1057–1061.
14. Fallin MD, Hetmanski JB, Park J, et al. Family-based analysis of MSX1 haplotypes for association with oral clefts. *Gen Epidemiol*. 2003;25(2):168–175.
15. Singh VP, Ramu D. Association of MSX1 799 G>T variant with nonsyndromic cleft lip/palate in South Indian adolescent patients. *Int J Paediatr Dentistry*. 2012;22(3): 228–231.
16. Kim NY, Kim YH, Park JW, Baek SH. Association between MSX1 SNPs and nonsyndromic cleft lip with or without cleft palate in the Korean population. *J Kor Med Sci*. 2013 Apr;28(4):522–526.
17. Gurramkonda VB, Syed AH, Murthy J, Lakkakula BV. Evidence of the involvement of the polymorphisms near MSX1 gene in nonsyndromic cleft lip with or without cleft palate. *Int J Pediatr Otorhinolaryngol*. 2015;79(7):1081–1084.
18. Tasanarong P, Pabalan N, Tharabenjasin P, Jarjanazi H. MSX1 gene polymorphisms and nonsyndromic cleft lip with or without palate (NSCL/P): a meta-analysis. *Oral Dis*. 2019;25(6):1492–1501.
19. Machado RA, Messetti AC, de Aquino SN, et al. Association between genes involved in craniofacial development and nonsyndromic cleft lip and/or palate in the Brazilian population. *Cleft Palate Craniofac J*. 2016;53(5):550–556.
20. Paranaiba LM, de Aquino SN, Bufalino A, et al. Contribution of polymorphisms in genes associated with craniofacial development to the risk of nonsyndromic cleft lip and/or palate in the Brazilian population. *Med Oral, Patol Oral Cirugía Bucal*. 2013 May 1;18(3):e414–e420.
21. Mossey PA, Little J, Steegers-Theunissen R, et al. Genetic interactions in nonsyndromic orofacial clefts in europe-EUROCRAN study. *Cleft Palate Craniofac J*. 2017;54(6):623–630.
22. Ma L, Xu M, Li D, et al. A miRNA-binding-site SNP of MSX1 is Associated with NSOC Susceptibility. *J Dent Res*. 2014;93(6):559–564.
23. Hong Y, Pan W, Yu L, et al. Association of MSX1 genetic polymorphisms with nonsyndromic cleft lip with or without cleft palate in a Uyghur population in Xinjiang, China. *Int J Clin Exp Med*. 2017;10(1):1115–1120.