Original Article

Associations between the risk of tooth agenesis and single-nucleotide polymorphisms of *MSX1* and *PAX9* genes in nonsyndromic cleft patients

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ABSTRACT

Objective: To investigate the association between the risk of tooth agenesis and single-nucleotide polymorphisms (SNPs) of *MSX1 and PAX9* genes in nonsyndromic cleft patients.

Materials and Methods: The subjects were 126 Korean nonsyndromic cleft patients. Tooth agenesis type (TAT) was classified as none (0); cleft area (1); cleft area + other area (2); and other area (3) based on agenesis of the maxillary lateral incisor (MXLI) and another tooth within or outside the cleft area. TAT was further grouped into two subcategories (0 and 1) and four subcategories (0, 1, 2, and 3). Three SNPs of *MSX1* and 10 SNPs of *PAX9* were investigated using Fisher's exact test and logistic regression analysis.

Results: Although the association between genotype distribution of *PAX9*-rs7142363 and TAT was significant ($P < .05$ in four subcategories), genotypic odds ratios (GORs) of SNPs in each TAT were not meaningful. However, for *MSX1*-rs12532 *and PAX9*-rs2073247, associations between genotypic distribution and TAT were significant ($P < .01$ in four subcategories and $P < .05$ in two subcategories; $P < .01$ in two subcategories, respectively). In cleft area, GORs of MXLI agenesis in genotypes GA of *MSX1*-rs12532 and CT of *PAX9*-rs2073247 were increased by 3.14-fold and 4.15-fold compared with genotype GG of *MSX1-rs12532* and CC of *PAX9*-rs2073247, respectively ($P <$. 01; $P < .05$). In cleft area + other area, the GOR of agenesis of MXLI and another tooth in genotype AA of *MSX1-rs12532* was increased by fivefold compared with genotype GG ($P < .05$).

Conclusion: Genetic disturbances of *MSX1 and PAX9* genes are associated with tooth agenesis within and outside the cleft area. (*Angle Orthod.* 2013;83:1036–1042.)

KEY WORDS: SNP; MSX1; PAX9; Tooth agenesis; Nonsyndromic cleft; Association analysis

INTRODUCTION

Because development of the oral cleft and formation of the tooth germ are closely related in terms of timing and anatomic position, dental anomalies including tooth agenesis both within and outside the cleft area have been reported to be more frequent in persons with nonsyndromic cleft lip with or without cleft palate (NS-CL ± P) than in individuals with nonsyndromic cleft lip with or without cleft palate (NS-CL ±P) than in the non-cleft individuals.[1–6] Tooth agenesis in cleft patients affects esthetics, function, and periodontal health; causes collapse of the dental arch; and creates psychosocial problems. Therefore, an interdisciplinary approach is required to allow patients to receive more effective and efficient treatment.

The *MSX1* and *PAX9* genes are known to contribute to tooth agenesis of the posterior teeth and the maxillary lateral incisor.[7–14] The *MSX1* genes with a homeodomain and the *PAX9* genes with a paired domain encode transcription factors that are essential for craniofacial and dental development of the mesenchyme.[15–19] Generally, mutations in *MSX1* and *PAX9*

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Accepted: April 2013. Submitted: February 2013.
Published Online: May 29, 2013

cause loss of function because of haploinsufficiency and reduce the amount of functional protein available to maintain tooth development; this results in abnormalities in odontogenesis, such as arrest of the tooth bud.[15–19]

To date, only a few studies have addressed the genetic basis of oral cleft with or without tooth agenesis in humans.[20–22] Van den Boogaard et al.[20] and Liang et al.[21] suggested that tooth agenesis and oral cleft were associated with nonsense mutations of *MSX1*, such as Ser104stop in exon 1 in a Dutch family and Q189X in exon 2 in a Chinese family, respectively. However, Liang et al.[21] also reported that sequence analysis of *PAX9* did not reveal mutation in any of the affected individuals studied. Modesto et al.[22] investigated single-nucleotide polymorphisms (SNPs) in *MSX1* of CL±P with or without tooth agenesis compared with non-cleft individuals and reported that the 101C>G variant occurred more frequently in patients with both NS-CL±P and tooth agenesis, whereas the *6C>T variant was found more often in those with NS-CL±P. However, these studies have several limitations, such as small sample size, inclusion of non-cleft individuals, or no classification of tooth agenesis within and outside the cleft area.

Given that not all patients with NS-CL±P have tooth agenesis, it is necessary to investigate the genetic basis of NS-CL±P with tooth agenesis compared with NS-CL±P without tooth agenesis. The *MSX1* and *PAX9* genes are known to contribute to both cleft and tooth agenesis.[16,18,20–23] However, no study has investigated whether tooth agenesis within the cleft area is associated with isolated genetic disturbance or local tissue defect as part of clefting.

Therefore, the purpose of this study was to investigate the association between the risk of tooth agenesis and the SNPs of *MSX1* and *PAX9* genes in Korean nonsyndromic cleft patients. The null hypothesis was that there was no significant association between genotypic distribution of SNPs in the *MSX1* and *PAX9* genes and tooth agenesis type (TAT) in patients with cleft lip and alveolus (CLA) and cleft lip and palate (CLP).

MATERIALS AND METHODS

The study samples consisted of 126 Korean nonsyndromic cleft patients (82 males and 44 females; 28 patients with CLA and 98 patients with CLP). Subjects with longitudinal serial records and panoramic radiographs were selected from the Department of Orthodontics, Seoul National University Dental Hospital (SNUDH). The study protocol was approved by the Institutional Review Board at SNUDH (IRB CRI-G07002).

Subjects were classified according to cleft type and the status and location of missing teeth. Diagnosis of nonsyndromic CLA and CLP was made through clinical inspections by highly trained orthodontists. Tooth agenesis was identified from serial panoramic radiographs based on the age of the subject and considering the fact that the mean delay in tooth formation of the cleft children was approximately 4 to 6 months relative to that of non-cleft children.[1,24] Regardless of size and morphology, any permanent tooth on either side of the alveolar cleft between the maxillary central incisor and canine was considered existence of the maxillary lateral incisor (MXLI).[25,26]

TAT was divided into none; cleft area only (missing of the MXLI within the cleft area only), cleft area + other area (missing of the MXLI within the cleft area and another maxillary tooth outside the cleft area), and other area only (missing of another maxillary tooth outside the cleft area only) (Figure 1). The status of those who were missing another maxillary tooth outside the cleft area did not include agenesis of the maxillary third molar. TAT was further grouped into two subcategories (none and cleft area only) and four subcategories (none, cleft area only, cleft area + other area, and other area only).

Peripheral venous blood samples of patients were collected at SNUDH after obtaining written informed consent. Genomic DNA samples were extracted from peripheral venous blood lymphocytes by the protein precipitation method using a commercial DNA extraction kit (Quiagen Inc, Valencia, Calif) and were genotyped using the VeraCode Technology program (Illumina Inc, San Diego, Calif) at SNP Genetics Inc (Seoul, Korea).

SNP markers located from 2kb ~ 5′ to 2kb ~ 3′ of the *MSX1* and *PAX9* genes were obtained from literature review and the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). Three SNP markers of the *MSX1* gene (rs3821949, rs12532, and rs4464513) and 10 SNP markers of the *PAX9* gene (rs2295221, rs7142363, rs2073247, rs17104928, rs17176643, rs11156925, rs17104939, rs17104944, rs17104965, and rs1884213) with minor allele frequency (MAF) greater than 1% in the Japanese population were selected using the Web-based program, TAG SNP selection (TagSNP; http://snpinfo.niehs.nih.gov/guide.htm#snptag).[27–29]

Fisher's exact test was used to investigate the correlation between TAT and genotypic distribution of SNPs in *MSX1* and *PAX9* genes. Logistic regression analysis was performed to calculate the genotypic odds ratio of SNPs in *MSX1* and *PAX9* genes according to the genotypes in each TAT.

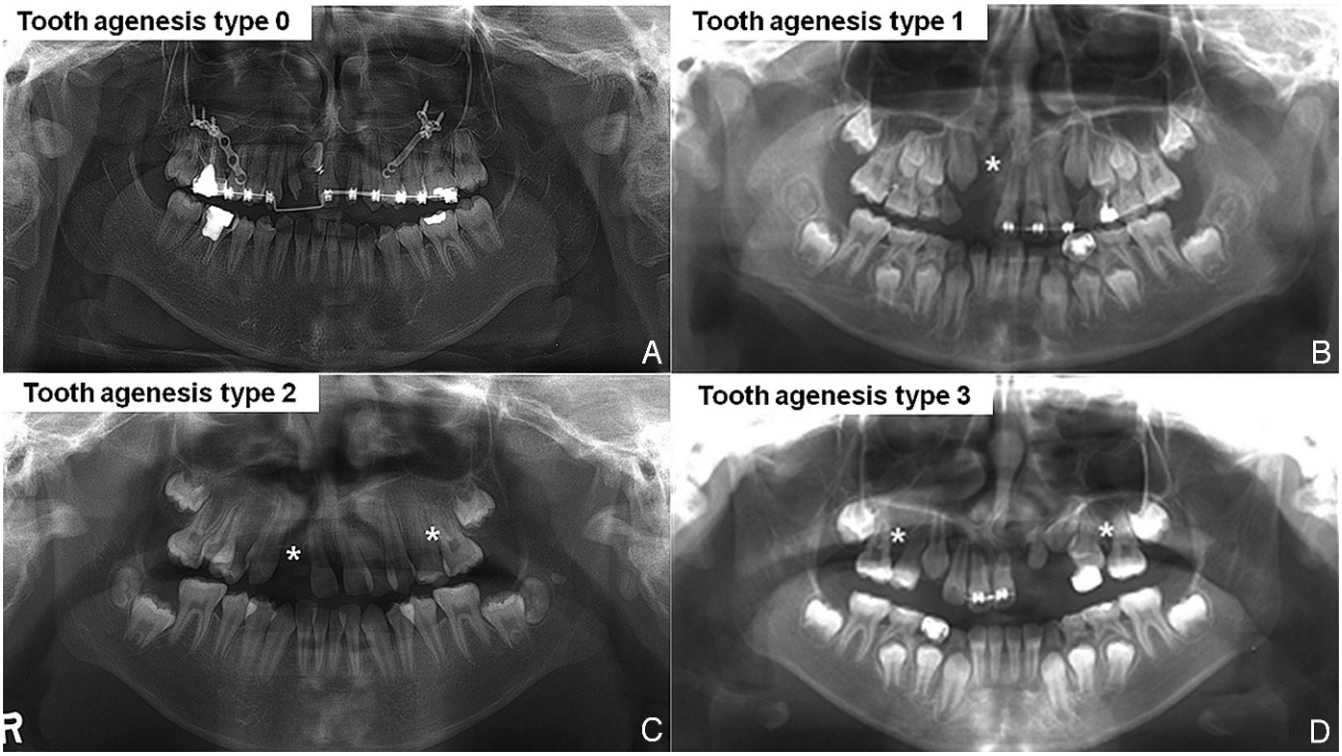

Figure 1. A. Tooth agenesis type (TAT)-0 'None (no missing tooth)' with bilateral cleft lip and palate (CLP) **B.** TAT-1 'Cleft area only [missing of the maxillary lateral incisor (MXLI) within the cleft area only]' with unilateral CLP. **C.** TAT-2 'Cleft area + Other area (missing of MXLI within the cleft area and another maxillary tooth outside the cleft area)' with unilateral CLP. **D.** TAT-3 'Other area only (missing of another maxillary tooth outside the cleft area only)' with unilateral CLP. An asterisk represents a missing tooth.

RESULTS

Demographic Information for Cleft Type, TAT, and Gender

Among 126 Korean patients with NS-CL±P (82 males and 44 females; 28 patients with CLA and 98 patients CLP), TAT distribution was cleft area only (39.7%), cleft area + other area (15.9%), other area only (8.7%), and none (35.7%) (Table 1).

Distribution of Tooth Agenesis

The most frequently missing teeth were the MXLI (73.6%) and the maxillary second premolar (16.5%) (Table 2).

Association Between TAT and SNPs of *MSX1* and *PAX9* Genes

In the four subcategories of none, cleft area only, cleft area + other area, and other area only, *MSX1*-rs12532

Table 1. Demographic Information Regarding Cleft Type, Tooth Agenesis Type, and Gender[a]

		CLA (n = 28)				CLP (n = 98)				
	Cleft Type	UCLA (Right)	UCLA (Left)	BCLA	Subtotal	UCLP (Right)	UCLP (Left)	BCLP	Subtotal	Sum (n = 126)
Tooth agenesis type	None	5	12	2	19 (67.85%)	7	13	6	26 (26.53%)	45 (35.71%)
	Cleft area only	2	3	1	6 (21.43%)	10	19	15	44 (44.90%)	50 (39.68%)
	Cleft area + other area	0	2	0	2 (7.14%)	4	10	4	18 (18.37%)	20 (15.87%)
	Other area only	1	0	0	1 (3.57%)	3	6	1	10 (10.20%)	11 (8.73%)
Gender	Male	8	8	2	18 (64.29%)	14	29	21	64 (65.31%)	82 (65.08%)
	Female	0	9	1	10 (35.71%)	10	19	5	34 (34.69%)	44 (34.92%)

[a] CLA indicates cleft lip and alveolus; UCLA, unilateral cleft lip and alveolus; BCLA, bilateral cleft lip and alveolus; CLP, cleft lip and palate; UCLP, unilateral cleft lip and palate; BCLP, bilateral cleft lip and palate; none, no missing teeth; cleft area only, missing of the maxillary lateral incisor within the cleft area only; cleft area + other area, missing of the maxillary lateral incisor within the cleft area and missing of another maxillary tooth outside the cleft area; other area only, presence of the maxillary lateral incisor within the cleft area and missing of another maxillary tooth outside the cleft area.

Table 2. Distribution of the Tooth Agenesis of the Maxillary Dentition[a]

	Central Incisor	Lateral Incisor	Canine	First Premolar	Second Premolar	First Molar	Second Molar	Sum
CLA	0	9 (64.29%)	1 (7.14%)	2 (14.29%)	2 (14.29%)	0	0	14
CLP	6 (5.60%)	80 (74.77%)	1 (0.93%)	0	18 (16.82%)	2 (1.87%)	0	107
Sum	6 (4.96%)	89 (73.55%)	2 (1.65%)	2 (1.65%)	20 (16.53%)	2 (1.65%)	0	121

[a] CLA indicates cleft lip and alveolus; CLP, cleft lip and palate.

and *PAX9*-rs7142363 showed a significant association with tooth agenesis ($P < .01$ and $P < .05$, respectively, Table 3). In the two subcategories of none and cleft area only, *MSX1*-rs12532 and *PAX9*-rs2073247 were significantly associated with tooth agenesis ($P < .05$ and $P < .01$, respectively, Table 3). These three candidate SNPs (*MSX1*-rs12532, *PAX9*-rs7142363, and *PAX9*-rs2073247) were further examined to assess genotypic distribution and genotypic odds ratios (GORs).

Genotypic Distribution and GORs of *MSX1-rs12532* According to TAT

For *MSX1*-rs12532, there was a significant association between genotypic distribution and TAT in both the four subcategories and the two subcategories ($P < .01$ and $P < .05$, respectively, Table 4). The GOR of MXLI agenesis in genotype GA of *MSX1*-rs12532 was significantly increased 3.14-fold in cleft area only compared with genotype GG (95% confidence interval [CI] = 1.32–7.48, $P < .01$;Table 5). The GOR of MXLI and another maxillary tooth agenesis in genotype AA of *MSX1*-rs12532 was also significantly increased fivefold in cleft area + other area compared with genotype GG (95% CI = 1.04–23.98, $P < .05$, Table 5).

Table 3. Association Between Tooth Agenesis Type and Single-Nucleotide Polymorphisms (SNPs) of the *MSX1* and *PAX9* Genes[a]

		Tooth Agenesis Type (*P*-Value)	
Gene	SNP	Four Subcategories	Two Subcategories
MSX1	rs3821949	.2437	.3076
	rs12532	**.0021****	**.0304***
	rs4464513	.3696	.6388
PAX9	rs2295221	.2966	.7669
	rs7142363	**.0365***	.0845
	rs2073247	.0993	**.0092****
	rs17104928	.6522	.1780
	rs17176643	.7837	.6483
	rs11156925	.6328	.5405
	rs17104939	.4138	.7656
	rs17104944	.5758	.3139
	rs17104965	.0541	.7156
	rs1884213	.2535	.8971

[a] Fisher's exact test was performed. * $P < .05$; ** $P < .01$. The four subcategories are none, cleft area only, cleft area + other area, and other area only; the two subcategories are none and cleft area only.

Genotypic Distribution and GORs of *PAX9-rs7142363* and *rs2073247* According to TAT

Although the association between genotype distribution of *PAX9*-rs7142363 and TAT was not significant in the two subcategories ($P > .05$, Table 6), this association was significant in the four subcategories ($P < .05$, Table 6). However, *PAX9*-rs2073247 showed the opposite tendency: the genotypic distribution of *PAX9*-rs2073247 exhibited a significant association with TAT in the two subcategories ($P < .01$, Table 6), but not in the four subcategories ($P > .05$, Table 6).

Although the GORs of genotypes of *PAX9*-rs7142363 in each TAT were not meaningful (Table 7), the GOR of tooth agenesis of MXLI in genotype CT of *PAX9*-rs2073247 was significantly increased 4.15-fold in cleft area only compared with genotype CC (95% CI = 1.43–12.05, $P < .01$, Table 7).

DISCUSSION

Because mutations in *PAX9* or *MSX1* are known to cause agenesis of the posterior teeth[7–10,17] and MXLI,[11–14] we categorized NS-CL±P samples into four types of tooth agenesis—none (0), cleft area only (1), cleft area + other area (2), and other area only (3)—and grouped them into two subcategories (0 and 1) and four subcategories (0, 1, 2, and 3).

In this study, *MSX1*-rs12532 showed a significant association with tooth agenesis in both the four subcategories and the two subcategories of TAT ($P < .01$ and $P < .05$, respectively, Table 4). MXLI agenesis was significantly higher in patients with genotype GA compared with those with genotype GG (3.14-fold, $P < .01$, Table 5). In addition, agenesis of MXLI and another maxillary tooth was significantly higher in patients with genotype AA compared with those with genotype GG (fivefold, $P < .05$, Table 5). These findings suggest that this SNP might be associated with agenesis of MXLI and another maxillary tooth.

However, the results of this study were somewhat different from those of previous studies. In three patients with sporadic nonsyndromic oligodontia, Pawlowska et al.[11] observed SNPs at *MSX1*-rs12532 in a patient with presence of MXLI and SNP at *MSX1*-rs8670 in a patient with absence of MXLI. Because

Table 4. Genotypic Distributions of *MSX1*-rs12532 According to Tooth Agenesis Type in Four Subcategories and Two Subcategories[a]

	MSX1-rs12532			*P*-Value	
Tooth Agenesis Type	GG	GA	AA	Four Subcategories	Two Subcategories
None	25 (55.56%)	17 (37.78%)	3 (6.67%)	**.0021****	**.0304***
Cleft area only	15 (30.0%)	32 (64.00%)	3 (6.00%)		
Cleft area + other area	10 (50.00%)	4 (20.00%)	6 (30.00%)		NA
Other area only	3 (27.27%)	6 (54.55%)	2 (18.18%)		NA

[a] Fisher's exact test was performed. * $P < .05$; ** $P < .01$; NA, not applicable.

these SNPs may be relatively common, they concluded that these polymorphisms would not be expected to have any pronounced phenotypic effect.[11] In addition, although Paixão-Côrtes et al.[13] found three polymorphic sites in the untranslated region of *MSX1* exon 2 (rs8670, rs1095, and rs12532), there was no statistical difference in allele and genotype distributions between patients and control subjects. The polymorphism at rs8670 was not included in the present study, but it has previously been observed in individuals with MXLI agenesis.[11,14] However, because this polymorphism at rs8670 is also relatively common, additional genes might be involved in this phenotype.[14] The discordance between previous studies and this study seem to originate from differences between oligodontia patients and cleft patients.

For *PAX9*-rs7142363, a significant association was found in the four subcategories of TAT ($P < .05$, Table 6) but not in the two subcategories (Table 6). However, a meaningful GOR was not demonstrated in either category (Table 7), suggesting that this SNP might not be related to MXLI agenesis. However, the low percentage of genotype CA in the other area only compared with that of the cleft area only (9.1% vs 60.0%, respectively, Table 6) suggests a possible association between this SNP and TAT in cleft patients (GOR = 2.21, $P = .0803$ in cleft area only; GOR = 0.15, $P = .0844$ in other area only, compared with none, Table 7). These results indicate that statistical power might be increased as the sample size increases. Therefore, further studies are needed to investigate the relationship between *PAX9*-rs7142363 and MXLI agenesis using a larger sample size.

In contrast, *PAX9*-rs2073247 showed a significant association in the two subcategories of TAT ($P < .01$, Table 6), but not in the four subcategories (Table 6). In particular, the frequency of genotype CT was significantly increased in cleft area only (4.15-fold, $P < .01$, Table 7). These results indicate that *PAX9*-rs2073247 might be a potential candidate marker for MXLI agenesis susceptibility in Korean cleft patients. However, *PAX9*-rs2073247 and other polymorphisms located adjacent to this locus in the promoter region are also known to be associated with third molar agenesis. Saito et al.[30] and Bianchi et al.[31] reported that the CC genotype of the *PAX9*-rs2073247 showed a positive association with third molar agenesis. Peres et al.[32] also suggested that the GT haplotype of *PAX9*-rs 2073244 and rs2076246 polymorphisms was associated with hypodontia, in most cases including third molar agenesis. Therefore, further studies are needed to investigate the relationship between *PAX9*-rs2073247 and MXLI agenesis.

In previous studies on the association between NS-CL±P and SNPs of the *MSX1* and *PAX9* genes, the A allele at *MSX1*-rs3821949 was associated with a significantly increased risk of NS-CL±P (GOR = 1.64, 95% CI = 1.03–2.63, $P < .05$, additive model)[28] and the G/A heterozygote at *PAX9*-rs17104928 showed a significant association with NS-CL±P (GOR = 2.88, 95% CI = 1.42 into 5.84, $P < .01$).[29] However, in the present study, these two SNPs of *MSX1* and *PXA9* did not show any significant association with tooth agenesis (Tables 3 through 7), whereas *MSX1*-rs12532 and *PAX9*-rs7142363 and rs2073247 did. These findings suggest that occurrence of oral cleft or tooth agenesis might depend on

Table 5. Genotypic Odds Ratios (GORs) of Genotypes GA and AA Compared with Genotype GG *of MSX1-rs12532* in Each Tooth Agenesis Type[a]

		MSX1-rs12532		
Tooth Agenesis Type	Genotypic Odds Ratio	GG (Intercept)	GA	AA
None	–	–	–	–
Cleft area only	GOR (95% CI)	–	**3.14 (1.32, 7.48)**	1.67 (0.3, 9.34)
	P-value	–	**.0099****	.5613
Cleft area + other area	GOR (95% CI)	–	0.59 (0.16, 2.19)	**5 (1.04, 23.98)**
	P-value		.4283	**.0442***
Other area only	GOR (95% CI)	–	2.94 (0.65, 13.4)	5.56 (0.65, 47.83)
	P-value		.1633	.1185

[a] Logistic regression analysis was performed. CI indicates confidence interval. * $P < .05$; ** $P < .01$.

Table 6. Genotypic Distributions of *PAX9*-rs7142363 and rs2073247 According to Tooth Agenesis Type in Four Subcategories and Two Subcategories[a]

	PAX9-rs7142363					*PAX9-rs2073247*				
				P-Value					*P*-Value	
Tooth Agenesis Type	CC	CA	AA	Four Sub-categories	Two Sub-categories	CC	CT	TT	Four Sub-categories	Two Sub-categories
None	20 (44.44%)	17 (37.78%)	8 (17.78%)	**.0365***	.0845	15 (33.33%)	14 (31.11%)	16 (35.56%)	.0993	**.0092****
Cleft area only	16 (32.00%)	30 (60.00%)	4 (8.00%)			8 (16.0%)	31 (62.00%)	11 (22.00%)		
Cleft area + other area	10 (50.00%)	8 (40.00%)	2 (10.00%)		NA	6 (30.00%)	9 (45.00%)	5 (25.00%)		NA
Other area only	8 (72.73%)	1 (9.09%)	2 (18.18%)		NA	4 (36.36%)	4 (36.36%)	3 (27.27%)		NA

[a] Fisher's exact test was performed. * $P < .05$; ** $P < .01$; NA indicates not applicable.

the locus of the SNP within the *MSX1* gene or *PAX9* gene.

The results of this study might be helpful for improving our understanding of the effects of genetic variation of *MSX1* and *PAX9* genes on tooth agenesis within and outside the cleft area. However, there are several factors to consider when interpreting the results obtained in this study. First, it would be better to increase the sample size to increase the statistical power and to avoid unnecessary statistical errors. Second, it is necessary to examine the genotypic distribution in non-cleft individuals with and without tooth agenesis to verify the exact role of SNPs in tooth agenesis. Third, functional consequences of these SNPs should be verified. Fourth, further studies are needed to investigate the influence of interactions of these genes with other genes, environmental factors, or ethnic differences.

CONCLUSIONS

- Because an association between the risk of tooth agenesis and SNPs of *MSX1* and *PAX9* genes was found in nonsyndromic cleft patients, the null hypothesis was rejected.
- Genetic disturbances of *MSX1 and PAX9* genes are associated with tooth agenesis within and outside the cleft area in addition to the local tissue defect of clefting.

ACKNOWLEDGMENTS

This research was supported by the Basic Science Research Program, the National Research Foundation of Korea (NRF 2009-0069859) funded by the Ministry of Education, Science and Technology, Republic of Korea. The authors thank all participants who donated samples and acknowledge Jung Sun Cho (Hallym University College of Medicine) for assistance during this work, as well as Duk Hwan Kim, Yong Ick Ji, Eunhyun Jung, and Se Young Cho for their contributions to DNA preparation for genotyping (Center for Genome Research, Samsung Biomedical Research Institute, Seoul, Korea).

REFERENCES

1. Ranta R. A review of tooth formation in children with cleft lip/palate. *Am J Orthod Dentofacial Orthop*. 1986;90:11–18.
2. Shapira Y, Lubit E, Kuftinec MM. Hypodontia in children with various types of clefts. *Angle Orthod*. 2000;70:16–21.
3. Larmour CJ, Mossey PA, Thind BS, Forgie AH, Stirrups DR. Hypodontia—a retrospective review of prevalence and etiology. Part I. *Quintessence Int*. 2005;36:263–270.

Table 7. Genotypic Odds Ratios (GORs) of Genotypes CA and AA Compared with Genotype CC *of PAX9-rs7142363* and of Genotypes CT and TT Compared with Genotype CC *of PAX9-rs2073247* in Each Tooth Agenesis Type[a]

	PAX9-rs7142363			*PAX9-rs2073247*		
Tooth Agenesis Type	CC (Intercept)	CA	AA	CC (Intercept)	CT	TT
None	–	–	–	–	–	–
Cleft area only						
GOR (95% CI)	–	2.21 (0.91, 5.35)	0.63 (0.16, 2.46)	–	**4.15 (1.43, 12.05)**	1.29 (0.41. 4.08)
P-value		.0803	.5009		**.0088****	.6656
Cleft area + other area						
GOR (95% CI)	–	0.94 (0.3, 2.92)	0.5 (0.09, 2.81)	–	NA	NA
P-value		.9164	.4311			
Other area only						
GOR (95% CI)	–	0.15 (0.02, 1.3)	0.63 (0.11, 3.61)	–	NA	NA
P-value		.0844	.5993			

[a] Logistic regression analysis was performed. CI indicates confidence interval; NA, not applicable. ** $P < .01$.

4. Baek SH, Kim NY. Congenital missing permanent teeth in Korean unilateral cleft lip and alveolus and unilateral cleft lip and palate patients. *Angle Orthod*. 2007;77:88–93.
5. Al Jamal GA, Hazza'a AM, Rawashdeh MA. Prevalence of dental anomalies in a population of cleft lip and palate patients. *Cleft Palate Craniofac J*. 2010;47:413–420.
6. Camporesi M, Baccetti T, Marinelli A, Defraia E, Franchi L. Maxillary dental anomalies in children with cleft lip and palate: a controlled *study. Int J Paediatr Dent*. 2010;20: 442–450.
7. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human *MSX1* homeodomain missense mutation causes selective tooth agenesis. *Nat Genet*. 1996; 13:417–421.
8. Nieminen P, Arte S, Tanner D, et al. Identification of a nonsense mutation in the *PAX9* gene in molar oligodontia. *Eur J Hum Genet*. 2001;9:743–746.
9. Frazier-Bowers SA, Guo DC, Cavender A, et al. A novel mutation in human *PAX9* causes molar oligodontia. *J Dent Res*. 2002;81:129–133.
10. Hansen L, Kreiborg S, Jarlov H, Niebuhr E, Eiberg H. A novel nonsense mutation in *PAX9* is associated with marked variability in number of missing teeth. *Eur J Oral Sci*. 2007; 115:330–333.
11. Pawlowska E, Janik-Papis K, Wisniewska-Jarosinska M, Szczepanska J, Blasiak J. Mutations in the human homeobox *MSX1* gene in the congenital lack of permanent teeth. *Tohoku J Exp Med*. 2009;217:307–312.
12. Pinho T, Silva-Fernandes A, Bousbaa H, Maciel P. Mutational analysis of *MSX1* and *PAX9* genes in Portuguese families with maxillary lateral incisor agenesis. *Eur J Orthod*. 2010;32:582–588.
13. Paixão-Côrtes VR, Braga T, Salzano FM, et al. *PAX9* and *MSX1* transcription factor genes in non-syndromic dental agenesis. *Arch Oral Biol*. 2011;56:337–344.
14. Boeira BR Jr, Echeverrigaray S. Polymorphism in the *MSX1* gene in a family with upper lateral incisor agenesis. *Arch Oral Biol*. 2012;57:1423–1428.
15. Satokata I, Maas R. *Msx1* deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet*. 1994;6:348–356.
16. Peters H, Neubüser A, Kratochwil K, Balling R. *Pax9*-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Genes Dev*. 1998;12:2735–2747.
17. Jumlongras D, Lin JY, Chapra A, et al. A novel missense mutation in the paired domain of *PAX9* causes non-syndromic oligodontia. *Hum Genet*. 2004;114:242–249.
18. 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: 438–449.
19. Boeira BR Jr, Echeverrigaray S. Dentistry and molecular biology: a promising field for tooth agenesis management. *Tohoku J Exp Med*. 2012;226:243–249.
20. van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK. *MSX1* mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat Genet*. 2000;24:342–343.
21. Liang J, Zhu L, Meng L, Chen D, Bian Z. Novel nonsense mutation in *MSX1* causes tooth agenesis with cleft lip in a Chinese family. *Eur J Oral Sci*. 2012;120:278–282.
22. Modesto A, Moreno LM, Krahn K, King S, Lidral AC. *MSX1* and orofacial clefting with and without tooth agenesis. *J Dent Res*. 2006;85:542–546.
23. Slayton RL, Williams L, Murray JC, et al. Genetic association studies of cleft lip and/or palate with hypodontia outside the cleft region. *Cleft Palate Craniofac J*. 2003;40: 274–279.
24. Lai MC, King NM, Wong HM. Dental development of Chinese children with cleft lip and palate. *Cleft Palate Craniofac J*. 2008;45:289–296.
25. Suzuki A, Watanabe M, Nakano M, Takahama Y. Maxillary lateral incisors of subjects with cleft lip and/or palate: part 2. *Cleft Palate Craniofac J*. 1992;29:380–384.
26. Ribeiro LL, das Neves LT, Costa B, Gomide MR. Dental development of permanent lateral incisor in complete unilateral cleft lip and palate. *Cleft Palate Craniofac J*. 2002;39:193–196.
27. Xu Z, Taylor JA. SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic Acids Res*. 2009;37:W600–W605.
28. 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 Korean Med Sci*. 2013;28:522–526.
29. Lee JK, Park JW, Kim YH, Baek SH. Association between *PAX9* single-nucleotide polymorphisms and nonsyndromic cleft lip with or without cleft palate. *J Craniofac Surg*. 2012; 23:1262–1266.
30. Saito CPB, Bianchi FJ, Peres RCR, Line SRP. Suggestive associations between polymorphisms in *PAX9*, *MSX1* genes and third molar agenesis in humans. *Curr Genomics*. 2006;7:191–196.
31. Bianchi FJ, de Oliveira TF, Saito CB, Peres RC, Line SR. Association between polymorphism in the promoter region (G/C-915) of *PAX9* gene and third molar agenesis. *J Appl Oral Sci*. 2007;15:382–386.
32. Peres RC, Scarel-Caminaga RM, do Espírito Santo AR, Line SR. Association between *PAX-9* promoter polymorphisms and hypodontia in humans. *Arch Oral Biol*. 2005;50: 861–871.