

Is missing maxillary lateral incisor in complete cleft lip and palate a product of genetics or local environment?

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ABSTRACT

Objective: To test the null hypothesis: Subjects with isolated complete unilateral cleft lip and palate (UCLP) show no differences in overall frequency of tooth agenesis (hypodontia), comparing a subsample with cleft-side maxillary lateral incisor (MxI2) agenesis to a subsample without cleft-side MxI2 agenesis. Findings could clarify the origins of cleft-side MxI2 agenesis.

Materials and Methods: Tooth agenesis was identified from dental radiographs of 141 subjects with UCLP. The UCLP cohort was segregated into four categories according to the status and location of MxI2 in the region of the unilateral cleft: group M: subjects with one tooth, located on the mesial side of the alveolar cleft; group D: subjects with one tooth, located on the distal side of the alveolar cleft; group MD: subjects with two teeth present, one mesial and one distal to the cleft; and group ABS: subjects with lateral incisor absent (agenesis) in the cleft area.

Results: The null hypothesis was rejected. Among UCLP subjects, there was a twofold increase ($P < .0008$) in overall frequency of tooth agenesis outside the cleft region in a subsample with cleft-side MxI2 agenesis (ABS), compared to a subsample presenting with no agenesis of the cleft-side MxI2 (M+D+MD).

Conclusions: Cleft-side MxI2 agenesis in CLP subjects appears to be largely a genetically controlled anomaly associated with cleft development, rather than a collateral environmental consequence of the adjacent cleft defect, since increased hypodontia involving multiple missing teeth observed remote from a cleft clearly has a significant genetic basis. (*Angle Orthod.* 2012;82:959–963.)

KEY WORDS: Cleft palate; Cleft lip; Tooth agenesis; Hypodontia; Maxillary lateral incisor; Subphenotype

INTRODUCTION

Is the absent maxillary lateral incisor (MxI2) frequently observed around an alveolar cleft defect a collateral result of the deficient local environment or part of a genetically controlled pattern associated with

the etiology of the cleft? This is a research question that is still open.

It is widely accepted that subjects with orofacial clefting present with significantly higher rates of congenital dental abnormalities compared to the general population,¹ as do their noncleft siblings.² Certain key genetic disturbances have been implicated in both tooth agenesis and clefting, suggesting a shared genetic etiology in some cases.^{3–6} Recent research has identified tooth agenesis as a potentially useful clinical marker for defining developmental subphenotypes of isolated/nonsyndromic clefts.^{6,7} Moreover, patterns of anomalies have been proposed within individual phenotypes of clefting.^{8,9} Many studies have found MxI2 anomalies, including agenesis and microdontia, to be the most prevalent dental abnormality in these cleft populations.^{9–15}

Camporesi et al.¹⁶ concluded in a 2010 survey of maxillary dental anomalies that this high prevalence of MxI2 agenesis in cleft lip and palate (CLP) subjects is a local effect of the cleft disturbance. Others studying

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tooth agenesis outside the cleft region have postulated that the high rate of cleft-side MxI2 agenesis might be attributed to an interruption of the primordial tooth bud by the cleft defect.¹⁷ Indeed, the fusion of medial nasal and maxillary processes is an embryologic event intimately related to lateral incisor formation, both temporally and spatially.¹⁸ Thus, it has been speculated that the high rate of MxI2 agenesis in this area is due to inadequate blood supply, lack of mesenchymal support, or other local disruptions related to cleft proximity.^{15,19} So we have reason to ask: Are these MxI2 anomalies part of the same genetic disturbance that affects higher rates of overall agenesis in the remaining dentition of complete unilateral cleft lip and palate (UCLP) subjects, or are they a result of local mechanical epithelial disruption by the cleft?

Tooth agenesis, also known as hypodontia or congenital absence of teeth, is the most frequently observed developmental anomaly of the human dentition, occurring in approximately one quarter of the world population. Tooth agenesis clearly has a genetic basis, and MxI2 is among the tooth types most often affected with this abnormality.²⁰⁻²³ Hypodontia has been identified as an autosomal-dominant condition, currently mapped in single-family studies to single-gene defects. Vastardis et al.,²⁴ analyzing a kindred with agenesis of all second premolars and third molars, found a point mutation in the MSX1 gene of affected family members. A mutation of PAX9 transcription factor has been linked to familial tooth agenesis affecting all first, second, and third molars, and less frequently involving second premolars and mandibular central incisors.²⁵ Recently, other genes and transcription factors have been implicated in the development of this dental anomaly.^{20,26,27} Embryologically, tooth development and palate formation share a close relationship, as far as timing and anatomic position.²⁸ More specifically, much like the determining role genetic mutation plays in the absence of teeth, ongoing evidence suggests that genetics is a key component in the genesis of isolated orofacial clefts.²⁹⁻³⁴

Although it is known that orofacial clefting is associated with greatly increased occurrence rates of dental anomalies, the literature characterizing the type and frequency of these abnormalities among different cleft phenotypes is unclear due to high phenotypic variability.^{8,10,19} In 2007, Letra et al.⁹ showed that patients with UCLP have the highest rates of multiple dental anomalies compared with other subphenotypes of clefting. Additionally, they suggested that this group could and should be further characterized according to specific dental characteristics, such as presence and location of the cleft-side lateral incisor that may potentially indicate a particular developmental pattern.

The work of Letra et al.,⁹ indicating the need for a more sophisticated classification of clefting subphenotypes based on the occurrence of different dental developmental patterns, has prompted our novel approach now in investigating the origins of tooth agenesis outside the cleft region contiguous to the alveolar defect in UCLP.

Our objective in this study was to test the null hypothesis: among subjects with isolated UCLP, there is no difference in overall frequencies of tooth agenesis in a subsample with same-side MxI2 agenesis, compared to a subsample without MxI2 agenesis. Findings could help clarify the origins of cleft-side MxI2 agenesis.

MATERIALS AND METHODS

This retrospective study of UCLP patients utilized medical records from the Hospital for Rehabilitation of Craniofacial Anomalies (HRAC) in Bauru, São Paulo, Brazil. This project was reviewed and approved by the Institutional Review Board (IRB) and Ethical Committee at HRAC, and Harvard Medical School, Boston, Massachusetts. All records were de-identified. Clefting subjects born between 1990 and 1993 were screened for the following conditions: isolated unilateral complete clefting of the lip and palate, with longitudinal, serial records available, including panoramic dental radiographs between ages 9 and 17 years.

In this study, we identified tooth agenesis from serial panoramic radiographs of 141 nonsyndromic subjects with UCLP gathered from HRAC dental department records. The sample contained 96 boys and 45 girls. No attempts were made to identify racial/ethnic affinities of subjects in the context of the mixed multi-ethnic population of present-day Brazil.

The UCLP cohort was categorized according to the status and location of MxI2 in the region of the unilateral cleft. The 141 subjects who met the selection criteria were classified into four categories based on the distribution pattern of the cleft-side MxI2 according to the methodology established by Letra et al.⁹

- Group M: Subjects with one tooth, located on the mesial side of the alveolar cleft.
- Group D: Subjects with one tooth, located on the distal side of the alveolar cleft.
- Group MD: Subjects with two teeth, one mesial and one distal to the cleft.
- Group ABS: Subjects with no lateral incisor (congenital absence) in the area of the cleft.

Any permanent tooth on either side of the alveolar cleft, regardless of morphology, between the canine and central incisor, was considered a maxillary lateral incisor, following established research protocols.^{11,12} The aggregated data from groups M, D, and MD represent the

Table 1. Cleft-side Maxillary Lateral Incisor (MxI2) Conditions in Unilateral Complete Cleft Lip and Palate sample (n = 141, Segregated into Four phenotypic subgroups) Compared With Data From Three Earlier Studies

Phenotypic subgroups of cleft-side MxI2 condition*	Present study (n=141)	Percentage of total sample, %				
		Present study (n=141)	Tsai et al. ¹³ 1998 (n=137)	Ranta ³⁶ 1971 (n=83)	Böhn ¹⁴ 1963 (n=180)	
ABS	53	37.6%	51.8%	38.6%	41.6%	
M	19	13.5%	1.5%	7.2%	13.9%	
D	50	35.5%	46.0%	22.9%	30.6%	
MD	19	13.5%	0.7%	31.3%	13.9%	
	(88 total)	(62.4% total)	(48.2% total)	(61.4% total)	(58.4% total)	

* Phenotypic subgroups of cleft-side MxI2 condition: Subgroup ABS, subjects with no lateral incisor (congenital absence) in the area of the cleft; Subgroup M, subjects with 1 tooth, located on the mesial side of the alveolar cleft; Subgroup D, subjects with 1 tooth, located on the distal side of the alveolar cleft; Subgroup MD; subjects with 2 teeth present, one mesial and one distal to the cleft.

proportion of UCLP subjects presenting with a maxillary lateral incisor on the cleft side, according to the schema proposed by Letra et al.⁹ Tooth agenesis was determined by panoramic radiographic screening for absent permanent teeth, including third molars. Congenital absence of third molars was confirmed through radiographic examination at 14 years or older, generally considered a critical age for determining third molar development.³⁵ In our study, agenesis of MxI2 refers to congenital absence of the permanent maxillary lateral incisor on the noncleft side. Subjects with pattern ABS and no other missing teeth were not considered to have tooth agenesis in the context of this study.

Standard chi-square and Fisher exact probabilities tests were performed on all sets of comparisons with statistical significance set at $P < .05$. Odds ratio values were also calculated.

RESULTS

The distribution of UCLP subjects according to the presence and location of the permanent MxI2 is summarized in Table 1. In 141 nonsyndromic UCLP subjects, 53 (37.6%) had congenital absence (ABS) of the permanent lateral incisor in the area of the alveolar cleft. The other 88 subjects had a permanent maxillary lateral incisor mesial to the cleft (M, 35.5%), distal to the cleft (D, 13.5%), or one on each side of the cleft (MD, 13.5%). The percentage distribution of these four subphenotypes are compared to similar evaluations of UCLP samples in earlier studies: Tsai et al.¹³ (Taiwanese subjects); Ranta³⁶ (Finnish subjects); and Böhn¹⁴ (Norwegian subjects).

Table 2. Occurrence of Hypodontia (Including Third Molars, M3) Outside the Cleft Area in Unilateral Complete Cleft Lip and Palate (UCLP) Patients Without (ABS) or With (M, D, MD) a Lateral Incisor in the Region of the Cleft, Compared With the Total Group

UCLP Group	Total	Hypodontia (Including M3)	Percentage With Hypodontia
ABS	53	31	58.5%
M, D, MD	88	26	29.5%
All UCLP	141	57	40.4%

The male-female (m, f) distributions of the study sample were similar after division into the four UCLP subphenotypes: ABS, 32m, 21f; M, 12m, 7f; D, 38m, 12f; and MD, 14m, 5f.

The data from subjects with cleft-side MxI2 agenesis (ABS) were compared with the combined data from subjects in all three subphenotypes of cleft-side MxI2 presence (M, D, MD). Table 2 presents UCLP subjects with hypodontia, defined in our study as at least one congenitally absent permanent tooth, excluding the cleft-side maxillary lateral incisor. Of the 53 subjects in category ABS, 31 had hypodontia (58.5%). Of the 88 UCLP subjects with a permanent maxillary lateral incisor present in the region of the cleft (M, D, or MD), 26 expressed hypodontia (29.5%). Thus, UCLP subjects with a missing cleft-side MxI2 showed a twofold increase in the occurrence rate of other missing teeth, including third molars, compared with those UCLP patients with their cleft-side MxI2 present.

Table 3 shows the results of statistical analysis on tooth agenesis in UCLP subjects in category ABS, the subphenotype characterized by absent cleft-side MxI2. The odds ratio of 3.36 at the 95% confidence interval indicated significant strength of the connection between UCLP with missing contiguous lateral incisor and the occurrence of agenesis of other mandibular and maxillary teeth outside the cleft area. Chi-square test and Fisher exact test results indicated the nonrandomness of the strong association between cleft-side MxI2 hypodontia and the absence of other teeth in the same subjects. ABS UCLP subjects are significantly more likely ($P < .0008$) to express hypodontia outside of the cleft region, compared with hypodontia frequency in the

Table 3. Association Between ABS^a Subphenotype and Hypodontia in Unilateral Complete Cleft Lip and Palate (UCLP): Results of Relevant Statistical Testing

Hypodontia Associated With ABS Subphenotype of UCLP Subjects		
Odds ratio (95% CI)	3.36	
Pearson chi-square test	11.51	$P = .000692$
Fisher exact probability test	One tailed, $P = .000657$	Two tailed, $P = .000823$

^a ABS indicates lateral incisor absent.

combined subphenotypes with cleft-side MxI2 present. Thus, the null hypothesis was rejected: among UCLP subjects, there is a statistically significant difference in overall frequencies of tooth agenesis outside the cleft area in a subsample with cleft-side MxI2 agenesis, compared to the UCLP subsample without cleft-side MxI2 agenesis.

DISCUSSION

Comparing and testing carefully selected samples for significant occurrence differences in variables that have known genetic control mechanisms is often a strategy to comprehend and clarify how the genetic code may affect biologic processes.³⁷ Such is the case of investigating the origins for MxI2 tooth agenesis frequently noted in the affected quadrant of isolated complete UCLP. Previous studies have equivocated on the origin of cleft-side MxI2 agenesis: Is this dental anomaly physically related directly to the disruptive bony defect or its at-birth surgical revision, or is cleft-side MxI2 agenesis a genetically controlled abnormality associated with, but not causally related to, the multifactorial genetics of cleft development itself?

In this study, we used a recently proposed UCLP subphenotyping schema⁹ to give variables the needed specificity to discriminate magnitude and strength of relationships that may point to genetic control. By focusing on presence or absence of MxI2 in UCLP subjects and defining presence of MxI2 as a combination of the three subphenotypes with one or two lateral incisors expressed in the cleft area, this study reports significant differences that may have eluded other recent investigations in the field.^{15,38,39}

Finding a statistically significant increase in overall tooth agenesis in a subsample with cleft-side MxI2 agenesis supports the inference that cleft-side MxI2 agenesis in CLP subjects is largely a genetically mediated anomaly associated with the genetics of CLP development, since such tooth agenesis involving multiple missing teeth has been clearly identified as under genetic control, often in the broader context of multifactorial inheritance.^{20,26,27} Several of these important genetic controls play a shared role in the development of orofacial clefting.³⁻⁶

This insight regarding the genetic developmental basis of cleft-related MxI2 agenesis clarifies ambiguities derived from studies employing less specific sampling methods. Unlike the rationale of genome-wide screening, this study's experimental design provided inferences about genotype, the hidden variable, from evaluation of carefully defined clinical variables—subphenotypes. Our study validates the utility of the subphenotype classification of cleft samples proposed by Letra et al.⁹ to elucidate other

biologic relationships masked by the highly variable expression patterns found in CLP.

CONCLUSIONS

- Isolated UCLP subjects with agenesis of the cleft-side MxI2 showed a statistically significant increased occurrence ($P < .0008$) of tooth agenesis outside the cleft area, when compared with a UCLP subsample without cleft-side MxI2 agenesis.
- Based on this finding the inference is cleft-side MxI2 agenesis in CLP subjects is largely a genetic anomaly associated with CLP development, rather than a collateral consequence of the adjacent cleft defect, since the increased pattern of multiple missing teeth, which was found in this study outside the cleft area, clearly has a substantial genetic basis.

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