Published in final edited form as:
Cleft Palate Craniofac J. 2003 May ; 40(3): 274–279.

Genetic Association Studies of Cleft Lip and/or Palate With Hypodontia Outside the Cleft Region

Rebecca L. Slayton, D.D.S., Ph.D., **Laura Williams, M.P.H.**, **Jeffrey C. Murray, M.D.**, **James J. Wheeler, D.D.S., M.S.**, **Andrew C. Lidral, D.D.S., Ph.D.**, and **Carla J. Nishimura, B.S.**
Dr. Slayton is with the Department of Pediatric Dentistry, Oregon Health and Science University School of Dentistry, Portland, Oregon. Dr. Murray and Ms. Nishimura are with the Department of Pediatrics, University of Iowa College of Medicine. Dr. Wheeler is with the Departments of Hospital Dentistry and Orthodontics, and Dr. Lidral is with the Department of Orthodontics at the University of Iowa College of Dentistry, Iowa City, Iowa. Ms. Williams is with the National Center on Birth Defects and Developmental Disabilities at the Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract

Objective—The purpose of this study was to determine whether the candidate genes previously studied in subjects with cleft lip, cleft palate, or both are associated with hypodontia outside the region of the cleft.

Subjects—One hundred twenty subjects from the Iowa Craniofacial Anomalies Research Center were selected based on the availability of both dental records and genotype information.

Method—The type of orofacial clefting and type and location of dental anomalies (missing teeth, supernumerary teeth, or peg laterals) were assessed by dental chart review and radiographic examination. Genotype analysis of candidate genes was performed using polymerase chain reaction/single-strand conformation polymorphism analysis.

Results—The prevalence of hypodontia in this sample was 47.5%, with 30.0% of subjects having missing teeth outside the cleft. There was a positive association between subjects with cleft lip or cleft lip and palate who had hypodontia outside the cleft region (compared with noncleft controls) and both muscle segment homeo box homolog 1 (MSX1) ($p = .029$) and transforming growth factor beta 3 (TGFB3) ($p = .024$). It was not possible in this analysis to determine whether this association was specifically associated with orofacial clefting combined with hypodontia or whether it was due primarily to the clefting phenotype.

Conclusions—In this sample, there was a significantly greater incidence of hypodontia outside the cleft region in subjects with cleft lip and palate, compared with cleft lip only or cleft palate only. Cleft lip and/or palate with hypodontia outside the cleft region was positively associated with both TGFB3 and MSX1, compared with noncleft controls.

Keywords

cleft lip and palate; genetics; hypodontia

The prevalence of cleft lip with or without cleft palate (CL/P) varies depending on racial and ethnic backgrounds (Croen et al., 1998), geographic origin (Vanderas, 1987), and

Address correspondence to: Dr. Rebecca Slayton, Department of Pediatric Dentistry, Oregon Health and Science University, SD 182, 611 SW Campus Drive, Portland, OR 97239. E-mail slaytonr@ohsu.edu.

socioeconomic status (Lidral et al., 1997; Schutte and Murray, 1999). In some populations, the prevalence is as frequent as 1:500 births and in others it may be as low as 1:2500 births (Schutte and Murray, 1999). The etiology of clefting is complex and involves both genetic and environmental factors. Recent evidence confirms that gene-environment interactions contribute significantly to the risk for CL/P (Shaw et al., 1996; Romitti et al., 1999).

Numerous studies have reported the presence of dental anomalies in association with various forms of cleft lip, cleft palate, or both. The anomalies consist of variations in number, size, and position of developing teeth (Ranta, 1982, 1988; Ranta et al., 1983; Tsai et al., 1998; Shapira et al., 2000). Evidence that individuals with CL/P have a greater prevalence of hypodontia in areas outside the cleft, compared with control individuals, is less conclusive. In the general population, the prevalence of hypodontia (excluding third molars) has been reported to range from 3% to 10% (Dolder, 1936; Brown, 1957; Eidelman et al., 1973; Graber, 1978; Ranta, 1988; Nieminen et al., 1995). Studies of subjects with clefts have found the prevalence of premolar agenesis to range from 18% to 27.8% (Olin, 1964; Tsai et al., 1998; Shapira et al., 1999; Eerens et al., 2001). In contrast, when Lekkas et al. (2000) examined unoperated adult patients with cleft, they found no absence of permanent teeth in the maxillary arch outside the cleft (distal to the canines), suggesting that the surgical procedure done to close palatal clefts disrupts the formation of the developing tooth buds. Interestingly, their subjects had a lower prevalence of hypodontia than that cited in other studies of subjects without cleft.

Evidence for a similar genetic etiology for phenotypes including the simultaneous occurrence of both orofacial clefting and hypodontia comes from a variety of sources. First, mouse knockout models for genes including muscle segment homeo box homolog 1 (MSX1) and paired box gene 9 (PAX9) result in a phenotype that includes cleft palate and hypodontia (Satokata and Maas, 1994; Peters et al., 1998). Second, a number of single-gene disorders such as Van der Woude syndrome, ectrodactyly-ectodermal dysplasia-clefting syndrome, and Kallmann's syndrome have both clefting and hypodontia as typical phenotypic findings (Ranta and Rintala, 1983; King et al., 1994; Molsted et al., 1997).

Previous studies found an association between CL/P and the genes MSX1, transforming growth factor beta 3 (TGFB3) and transforming growth factor alpha (TGFA) and between cleft palate only (CPO) and MSX1 (Ardinger et al., 1989; Lidral et al., 1998). In addition, MSX1 and PAX9 mutations have been identified in families with specific patterns of hypodontia (Vastardis et al., 1996; Stockton et al., 2000; Jumlongras et al., 2001; Lidral and Reising, 2002) and in an extended family with both clefting and hypodontia (van den Boogaard et al., 2000).

The purpose of this study was to determine whether the candidate genes previously studied in subjects with CL/P are associated with hypodontia outside the cleft in these subjects. In addition, the prevalence of various dental anomalies was assessed relative to the type of cleft [cleft lip only (CLO), CL/P, or CPO] and the location of the anomaly (outside the cleft or within the cleft area).

Materials and Methods

Subject Ascertainment and Chart Review

Subjects were originally ascertained as a population-based case-control study within the University of Iowa Craniofacial Anomalies Research Center (CARC; Lidral et al., 1998). This study had University of Iowa institutional review board approval, and informed consent was obtained from all subjects. For the current study, a subset of the CARC subjects were used on the basis of the availability of dental records, genotyping information, and a DNA sample. Dental radiographs are made routinely on all children seen in the Craniofacial Anomalies Clinic

for diagnostic purposes. Subjects with clefting that was associated with a syndrome were excluded. There were 120 subjects who met all criteria. One hundred ninety subjects were excluded because of lack of dental examination findings. Of these, 46 subjects had CLO, 94 subjects had cleft lip and palate, and 50 subjects had CPO. The subset of subjects who met all criteria had been previously genotyped for variants in the candidate genes MSX1 and TGFA and TGFB3 (Lidral et al., 1998). As part of the current study, subjects were genotyped for a newly identified PAX9 variant located in the 3′ untranslated region (UTR) of the gene. A chart review of all eligible subjects was conducted to verify the type of cleft present and identify dental anomalies including missing teeth, supernumerary teeth, or malformed teeth (primarily peg lateral incisors). Findings from the chart review were confirmed by evaluation of available radiographs.

Genetic Markers

Markers used included MSX1 CA, a dinucleotide repeat (Padanilam et al., 1992); MSX1 1.3, a single nucleotide polymorphism (Lidral et al., 1998); TGFB3 CA, a dinucleotide repeat (Lidral et al., 1997); PAX9 3′UTR, a single nucleotide polymorphism; and TGFA *TaqI* (Basart et al., 1994). The method of genotyping the MSX1, TGFB3, and TGFA markers was described in detail elsewhere (Lidral et al., 1997). The PAX9 variant is a single nucleotide change (T to C) located 818 nucleotides downstream from the translation stop codon. Polymerase chain reaction was performed in 10-μL reactions containing 2 ng DNA; 200 μM deoxynucleotide triphosphate (dNTP); 1.5 mM $MgCl_2$; 10 mM Tris/HCl, pH 8.3; 50 mM KCl; 20 μM of each primer; and 0.01 U *Taq* polymerase. Thermocycle settings consisted of a denaturation step at 94 degrees for 3 minutes followed by 35 cycles of 94 degrees for 30 seconds, 55 degrees for 30 seconds, and 72 degrees for 30 seconds. PAX9 primers 5′-ACTTTCTGGCAACGTCTTTG-3′ and 5′-CACTGCATACACCAAATTTG-3′ were used to amplify an 89-bp fragment. Four microliters of sample were combined with 4 μL of loading dye, denatured at 94 degrees for 5 minutes, and electrophoresed on a single-strand conformation polymorphism (SSCP), mutation detection enhancement (MDE) gel for 3 hours at 20 W. Samples with known genotypes were loaded on each gel as controls. Gels, bonded to glass plates, were silver stained, air dried, and scored independently by two investigators.

Genetic and Phenotypic Analysis

Subjects with cleft were divided into two groups based on the location of the missing teeth. Group 1 had missing or extra teeth associated with the cleft only, and group 2 had missing or extra teeth outside the cleft in addition to anomalies within the cleft region. The regions defined as “outside the cleft” included the entire mandibular arch and maxillary arch distal to the canines on the side of the cleft(s). Dental anomalies were classified as missing teeth, supernumerary teeth, and small teeth (peg laterals). One additional group of control subjects was included in the analysis of allele frequencies. Group 3 consisted of previously genotyped noncleft control samples, referred to as CARC controls (Lidral et al., 1998). Allele frequencies were calculated for each group and each variant of the four candidate genes. Chi square analysis using Fisher's exact test was used to compare frequencies. A p value of .05 was considered to be statistically significant. A number of case-control comparisons were made. In the first analysis, subjects with clefting but no hypodontia outside the clefting region (cleft controls) were used as controls. In the second analysis, subjects without clefting whose hypodontia status was unknown (CARC controls) were used as controls. Using the most stringent criteria, the Bonferroni correction for 40 comparisons would yield an $\alpha = 0.00125$.

Results

A chart review was completed on 120 subjects who are seen regularly in the University of Iowa Craniofacial Anomalies Clinic to confirm the type of clefting present and determine the type

and location of any dental anomalies. A summary of the types of clefts and prevalence of hypodontia outside the cleft is shown in Table 1.

Of the subjects studied, 31 (25.8%) had bilateral CL/P; 42 (35.0%) had left CL/P; 26 (21.7%) had right CL/P; and 21 (17.5%) had CPO. Sixty-three (52.5%) of the subjects had no evidence of hypodontia, and the remainder were missing teeth either in the region of the cleft or outside the cleft. Thirty-six (30.0%) of the subjects had missing teeth outside the cleft region and 21 (17.5%) had missing teeth that were limited to the region of the cleft.

Genotype analysis of case and control samples for the selected candidate genes are summarized in Tables 2 and 3. Allele frequencies were determined for each marker and chi square analysis was used to determine whether there were significant differences among the groups. When subjects with clefting but without hypodontia were used as controls, there were no significant differences among allele frequencies for any of the markers studied (Table 2). When noncleft (CARC) subjects were used as controls, there was a statistically significant difference between allele frequencies for the marker MSX1 1.3 ($p = .026$) when control subjects and subjects with clefting and hypodontia outside the cleft were compared (Table 3).

To determine whether the association with MSX1 1.3 was related to the clefting phenotype separate from the hypodontia, the chi square analysis was done using noncleft (CARC) controls with cases defined as those subjects with any type of clefting regardless of their hypodontia status. There was a significant association ($p = .003$) for this group of subjects at this marker as well (Table 4), suggesting that it was the clefting phenotype and not the hypodontia that was in linkage disequilibrium.

Frequencies of dental anomalies were determined by cleft type and location of the anomaly. Cleft types were divided into three groups based on the extent of clefting. The first group was composed of CLO (either bilateral or unilateral); the second group included clefts of both the lip and palate (bilateral or unilateral cleft lip and palate); the third group included subjects with CPO. There were significantly more subjects with missing teeth outside the cleft region in subjects with either CLO or cleft lip and palate, compared with those with CPO ($p < .01$) (Table 5). This pattern was also true for supernumerary teeth. No supernumerary teeth were detected in subjects with CPO, and 58% of patients with CLO and 26.7% of subjects with cleft lip and palate had supernumerary teeth in the region of the cleft. Chi square analysis of these two subgroups demonstrated a statistically significant difference between the occurrence of supernumerary teeth in subjects with CLO, compared with those with cleft lip and palate ($p < .01$) (Table 5). Peg-shaped lateral incisors are often seen as part of the spectrum of missing teeth and have frequently been reported in subjects with CL/P (Brook, 1984;Nieminen et al., 1995). There was no significant association between the occurrence of peg laterals and any of the clefting types ($p = .094$). Finally, the occurrence of any of the three dental anomalies described above in association with different cleft types was assessed. There were significantly more dental anomalies associated with cleft lip and cleft lip and palate than with CPO ($p < .01$) (Table 5).

Based on the evidence that the frequency of hypodontia varied significantly by cleft type, allele frequencies for each candidate gene were calculated for each cleft type with or without hypodontia, and these frequencies were compared with CARC controls. This analysis yielded some surprising results. When subjects with CLO were compared with CARC controls, there was a statistically significant association in subjects without hypodontia for the MSX1 1.3 marker ($p = .011$) and for subjects with hypodontia for the TGFB3 marker ($p = .009$) (Table 6). When subjects with CLO were combined with subjects with cleft lip and palate, there was a positive association with the MSX1 1.3 marker ($p = .029$) and the TGFB3 marker ($p = .024$) for subjects with hypodontia and for PAX9 and MSX1 1.3 in subjects without hypodontia (p

= .045 and p = .037, respectively). When subjects with cleft lip and palate were considered separately, there were no significant associations in subjects without hypodontia but the significant association with the MSX1 1.3 marker in subjects with hypodontia remained (p = .026). In subjects with CPO, there was a significant association with the MSX1 1.3 marker for subjects without hypodontia (p = .028).

Discussion

The purpose of this study was to compare patterns of hypodontia in children with CL/P and determine whether hypodontia outside the area of the cleft is associated with one or more of the candidate genes for clefting. The frequency of hypodontia outside the cleft region found in this study (30.0%) was comparable with that reported in a number of previous studies (18% to 27.8%; Olin, 1964; Tsai et al., 1998; Shapira et al., 1999; Eerens et al., 2001) but in conflict with the findings of one study of the prevalence of hypodontia in adults with unoperated clefts (Lekkas et al., 2000). It is not clear why there is such a discrepancy in the findings of these authors, but because the prevalence of hypodontia in both the unoperated cleft subjects and controls in this study were lower than what has been reported in the general population, it is possible that this particular group of subjects has an inherently lower prevalence of hypodontia.

Supernumerary teeth frequently have been reported in children with cleft lip or cleft lip and palate (Fishman, 1970; Ranta, 1988; Tsai et al., 1998) but infrequently reported in children with CPO (Fishman, 1970; Larson et al., 1998). This is consistent with findings in the current study in which there were no supernumerary teeth seen in children with CPO, and the frequency of supernumerary teeth in children with CLO or with cleft lip and palate was 58% and 27%, respectively. The frequency of extra teeth in CPO would be expected to be similar to that seen in noncleft controls, which has been reported to range from 1.3% to 2% (Larson et al., 1998). The relatively small number of subjects with CPO in this study (n = 22) may explain why no supernumerary teeth were observed.

Genetic association studies have been done for clefting and hypodontia but not for the combined phenotype of clefting with hypodontia outside the cleft region. In the current study, candidate genes that are known to be associated with both phenotypes were analyzed. The selection of controls in such a study is crucial to the final outcome. By using subjects with clefting but without hypodontia outside the cleft region as controls, we have focused on the genetic association of hypodontia. A secondary analysis using control subjects without clefts and with unknown patterns of hypodontia allowed us to focus on the combined phenotype.

There is good evidence to support the use of the candidate genes analyzed in this study, both from human studies and mouse knockout models. Therefore, it was surprising that no significant associations were found between subjects with hypodontia outside the cleft region, compared with cleft controls without hypodontia. One possible explanation for this is that the genes that are responsible for clefting with or without hypodontia are essentially the same and that one subgroup cannot serve as the control for another. There is also evidence that different genes are responsible for specific patterns of hypodontia. It may be inaccurate to group subjects with any type of hypodontia outside the cleft region into one group.

To address the first concern, a secondary analysis was performed using control (CARC) subjects without clefting. Although the hypodontia status of these subjects was not known, it was assumed to be similar to the prevalence seen in the general (Caucasian) population. Interestingly, when this group of control subjects was used for the analysis, a significant association was found with the MSX1 1.3 marker. Lidral et al. (1998) reported a similar finding for subjects with CL/P and CPO. When the allele frequencies for all the subjects with cleft were combined (those with and without hypodontia outside the cleft region), this significant

association was maintained, suggesting that it is the clefting phenotype rather than the hypodontia that is associated with the MSX1 marker. Further analysis of this marker in subjects sorted by cleft type showed that the association was maintained for subjects with CLO (without hypodontia) and subjects with cleft lip and palate who had hypodontia outside the cleft region. These data lend additional support to previous studies showing that MSX1 plays an important role in both craniofacial and dental development. Functional and mutation studies that identify key genes that interact with MSX1 are currently underway and are expected to provide additional insights into the role this important gene plays in development.

The association of TGFB3 with subjects with clefting and hypodontia outside the cleft was intriguing. Although this gene has been shown to be associated with nonsyndromic clefting by a number of investigators (Lidral et al., 1998; Scapoli et al., 2002), its role in hypodontia is less well understood. Expression studies of TGFB3 suggest that it is involved in the differentiation of a number of tissues including tooth and palate (Pelton et al., 1990). The expression pattern of TGFB3 in the developing mouse tooth specifically occurs in the stellate reticulum during the cap and bell stage and in the dental papilla and preodontoblasts during the late bell stage (University of Helsinki, 1996). Although this expression pattern does not suggest a role for TGFB3 in hypodontia, it is conceivable that interactions between this gene and other craniofacial developmental genes could affect both tooth and palate development.

Because PAX9 has consistently been shown to be associated with specific patterns of hypodontia, it was interesting to note that in this study, there was a positive association with clefting in subjects without hypodontia outside the cleft region. Studies in mice have suggested a role for PAX9 in palatal development and a PAX9 knockout mouse was shown to have a cleft secondary palate (Peters et al., 1998). To date there is only one documented case of a child with a microdeletion that included the PAX9 gene who had bilateral CL/P (Schuffenhauer et al., 1999). Because this child was examined at 3 years of age, there was no report of his permanent tooth development.

Although a number of associations were significant using a p value of .05, when the most conservative Bonferroni correction is applied, none of the associations remain significant. In studies of this type, it is important to repeat these analyses with different samples to confirm the significance of any findings.

Conclusions

The overall prevalence of hypodontia in this sample of children with CL/P or CPO was 47.5%, with 30.0% of subjects having missing teeth outside the cleft. Dental anomalies were more frequently associated with CL/P than with CPO ($p < .01$). In addition, hypodontia outside the cleft region was more likely to occur in subjects with cleft lip and palate than with CLO or CPO ($p < .01$). Analysis of candidate genes found no significant association between the subjects with hypodontia outside the cleft region, compared with controls with clefting but no hypodontia outside the cleft region. However, significant associations were found with the MSX1 1.3 marker and the TGFB3 CA marker in subjects with clefting who had hypodontia outside the cleft region when noncleft (CARC) subjects were used as controls.

Using the most stringent statistical analysis, the findings of this study could be interpreted as being caused by chance. However, previous data supporting this association combined with biological evidence of a role for these genes in both hypodontia and orofacial clefting suggest a significant influence from both MSX1 and TGFB3. Future studies will focus on identification of mutations within the MSX1 and TGFB3 genes in subjects with clefting and/or hypodontia and functional studies of both MSX1 and TGFB3.

Acknowledgments

We would like to thank Erin Grimm for her technical assistance on this project. This study was supported by National Institute of Health grants DE 08559 and DE 13076.

The study was supported by NIH grants P60 DE 13076 and DE 08559.

References

Ardinger HH, Buetow KH, Bell GI, Bardach J, VanDemark DR, Murray JC. Association of genetic variation of the transforming growth factor-alpha gene with cleft lip and palate. Am J Hum Genet 1989;45:348–353. [PubMed: 2570526]

Basart AM, Qian JF, May E, Murray JC. A PCR method for detecting polymorphism in the TGFA gene. Hum Mol Genet 1994;3:678. [PubMed: 8069321]

Brook AH. A unifying aetiological explanation for anomalies of human tooth number and size. Arch Oral Biol 1984;29:373–378. [PubMed: 6611147]

Brown RV. The pattern and frequency of congenital absence of teeth. Iowa Dent J 1957;43:60–61.

Croen LA, Shaw GM, Wasserman CR, Tolarova MM. Racial and ethnic variations in the prevalence of orofacial clefts in California, 1983–1992. Am J Med Genet 1998;79:42–47. [PubMed: 9738868]

Dolder AA. A statistical survey of deficient dentition. Dent Rec 1936;57:56.

Eerens K, Vlietinck R, Heidbuchel K, Van Olmen A, Derom C, Willems G, Carels C. Hypodontia and tooth formation in groups of children with cleft, siblings without cleft, and nonrelated controls. Cleft Palate Craniofac J 2001;38:374–378. [PubMed: 11420017]

Eidelman E, Chosack A, Rosenzweig KA. Hypodontia: prevalence amongst Jewish population of different origin. Am J Phys Anthropol 1973;39:129–134. [PubMed: 4713560]

Fishman LS. Factors related to tooth number, eruption time, and tooth position in cleft palate individuals. ASDC J Dent Child 1970;37:303–306. [PubMed: 4913173]

Graber LW. Congenital absence of teeth: a review with emphasis on inheritance patterns. J Am Dent Assoc 1978;96:266–275. [PubMed: 342579]

Jumlongras D, Bei M, Stimson JM, Wang WF, DePalma SR, Seidman CE, Felbor U, Maas R, Seidman JG, Olsen BR. A nonsense mutation in MSX1 causes Witkop syndrome. Am J Hum Genet 2001;69:67–74. [PubMed: 11369996]

King NM, Tong MC, Ling JY. The ectrodactyly-ectodermal dysplasia-clefting syndrome: a literature review and case report. Quintessence Int 1994;25:731–736. [PubMed: 9161252]

Larson M, Hellquist R, Jakobsson OP. Dental abnormalities and ectopic eruption in patients with isolated cleft palate. Scand J Plast Reconstr Surg Hand Surg 1998;32:203–212. [PubMed: 9646370]

Lekkas C, Latief BS, ter Rahe SP, Kuijpers-Jagtman AM. The adult unoperated cleft patient: absence of maxillary teeth outside the cleft area. Cleft Palate Craniofac J 2000;37:17–20. [PubMed: 10670884]

Lidral AC, Murray JC, Buetow KH, Basart AM, Schearer H, Shiang R, Naval A, Layda E, Magee K, Magee W. Studies of the candidate genes TGFB2, MSX1, TGFA, and TGFB3 in the etiology of cleft lip and palate in the Philippines. Cleft Palate Craniofac J 1997;34:1–6. [PubMed: 9003904]

Lidral AC, Reising BC. The role of MSX1 in human tooth agenesis. J Dent Res 2002;81:271–278.

Lidral AC, Romitti PA, Basart AM, Doetschman T, Leysens NJ, Daack-Hirsch S, Semina EV, Johnson LR, Machida J, Burds A, Parnell TJ, Rubenstein JL, Murray JC. Association of MSX1 and TGFB3 with nonsyndromic clefting in humans. Am J Hum Genet 1998;63:557–568. [PubMed: 9683588]

Molsted K, Kjaer I, Giwercman A, Vesterhauge S, Skakkebaek NE. Craniofacial morphology in patients with Kallmann's syndrome with and without cleft lip and palate. Cleft Palate Craniofac J 1997;34:417–424. [PubMed: 9345610]

Nieminen P, Arte S, Pirinen S, Peltonen L, Thesleff I. Gene defect in hypodontia: exclusion of MSX1 and MSX2 as candidate genes. Hum Genet 1995;96:305–308. [PubMed: 7649547]

Olin WH. Dental anomalies in cleft lip and cleft palate patients. Angle Orthod 1964;34:119–123.

Padanilam BJ, Stadler HS, Mills KA, McLeod LB, Solursh M, Lee B, Ramirez F, Buetow KH, Murray JC. Characterization of the human HOX 7 cDNA and identification of polymorphic markers. Hum Mol Genet 1992;1:407–410. [PubMed: 1284527]

Pelton RW, Dickinson ME, Moses HL, Hogan BL. In situ hybridization analysis of TGF beta 3 RNA expression during mouse development: comparative studies with TGF beta 1 and beta 2. Development 1990;110:609–620. [PubMed: 1723948]

Peters H, Neubuser 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. [PubMed: 9732271]

Ranta R. Comparison of tooth formation in noncleft and cleft-affected children with and without hypodontia. ASDC J Dent Child 1982;49:197–199. [PubMed: 6953081]

Ranta R. Numeric anomalies of teeth in concomitant hypodontia and hyperdontia. J Craniofac Genet Dev Biol 1988;8:245–251. [PubMed: 3209686]

Ranta R, Rintala AE. Correlations between microforms of the van der Woude syndrome and cleft palate. Cleft Palate J 1983;20:158–162. [PubMed: 6573981]

Ranta R, Stegars T, Rintala AE. Correlations of hypodontia in children with isolated cleft palate. Cleft Palate J 1983;20:163–165. [PubMed: 6573982]

Romitti PA, Lidral AC, Munger RG, Daack-Hirsch S, Burns TL, Murray JC. Candidate genes for nonsyndromic cleft lip and palate and maternal cigarette smoking and alcohol consumption: evaluation of genotype-environment interactions from a population-based case-control study of orofacial clefts. Teratology 1999;59:39–50. [PubMed: 9988882]

Satokata I, Maas R. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development [comments]. Nat Genet 1994;6:348–356. [PubMed: 7914451]

Scapoli L, Martinelli M, Pezzetti F, Carinci F, Bodo M, Tognon M, Carinci P. Linkage disequilibrium between GABRB3 gene and nonsyndromic familial cleft lip with or without cleft palate. Hum Genet 2002;110:15–20. [PubMed: 11810291]

Schuffenhauer S, Leifheit HJ, Lichtner P, Peters H, Murken J, Emmerich P. De novo deletion (14) (q11.2q13) including PAX9: clinical and molecular findings [In Process Citation]. J Med Genet 1999;36:233–236. [PubMed: 10204852]

Schutte BC, Murray JC. The many faces and factors of orofacial clefts. Hum Mol Genet 1999;8:1853–1859. [PubMed: 10469837]

Shapira Y, Lubit E, Kuftinec MM. Congenitally missing second premolars in cleft lip and cleft palate children. Am J Orthod Dentofacial Orthop 1999;115:396–400. [PubMed: 10194283]

Shapira Y, Lubit E, Kuftinec MM. Hypodontia in children with various types of clefts. Angle Orthod 2000;70:16–21. [PubMed: 10730671]

Shaw GM, Wasserman CR, Lammer EJ, O'Malley CD, Murray JC, Basart AM, Tolarova MM. Orofacial clefts, parental cigarette smoking, and transforming growth factor-alpha gene variants. Am J Hum Genet 1996;58:551–561. [PubMed: 8644715]

Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of PAX9 is associated with oligodontia. Nat Genet 2000;24:18–19. [PubMed: 10615120]

Tsai TP, Huang CS, Huang CC, See LC. Distribution patterns of primary and permanent dentition in children with unilateral complete cleft lip and palate. Cleft Palate Craniofac J 1998;35:154–160. [PubMed: 9527312]

University of Helsinki, Developmental Biology Programme. Gene expression in tooth. 1996 [March 12, 2002]. Available at: http://bite-it.helsinki.fi/

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. [PubMed: 10742093]

Vanderas AP. Incidence of cleft lip, cleft palate, and cleft lip and palate among races: a review. Cleft Palate J 1987;24:216–225. [PubMed: 3308178]

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. [PubMed: 8696335]

TABLE 1

Frequency by Cleft Type and Occurrence of Hypodontia Outside the Cleft Region

Type of Cleft*	Frequency (%)	Hypodontia Outside the Cleft (%)
CL&P	75 (62.5)	32 (26.7)
CLO	24 (20.0)	1 (0.8)
CPO	21 (17.5)	3 (2.5)
Total	120 (100)	36 (30)

*CL&P = cleft lip and palate; CLO = cleft lip only; CPO = cleft palate only.

TABLE 2

Genotype Analysis of Candidate Genes With Cleft Controls

Marker and Study Group*	Allele 1	2	3	4	p Value
MSX1 CA					
Clefting without hypodontia	11	37	7	89	
Clefting with hypodontia	6	9	4	37	.463
MXS1 1.3					
Clefting without hypodontia	9	133			
Clefting with hypodontia	4	48			.478
TGFA *Taq*I					
Clefting without hypodontia	135	15			
Clefting with hypodontia	56	6			.582
TGFB3 CA					
Clefting without hypodontia	80	44	10		
Clefting with hypodontia	21	16	5		.467
PAX9 3′UTR					
Clefting without hypodontia	39	122			
Clefting with hypodontia	14	50			.426

*MSX = muscle segment homeo box homolog 1; TGFA = transforming growth factor alpha; TGFB3 = transforming growth factor beta 3; PAX9 = paired box gene 9; UTR = untranslated region.

TABLE 3

Genotype Analysis of Candidate Genes in Subjects With Clefting and Hypodontia Using Noncleft (CARC) Controls

Marker and Study Group†	Allele 1	2	3	4	p Value
MSX1 CA					
CARC control	64	139	37	310	
Clefting with hypodontia	6	9	4	37	.449
MSX1 1.3					
CARC control	5	313			
Clefting with hypodontia	4	48			.026*
TGFA					
CARC control	449	53			
Clefting with hypodontia	56	6			.521
TGFB3 CA					
CARC control	303	161	22		
Clefting with hypodontia	21	16	5		.069
PAX9 3′UTR					
CARC control	7	53			
Clefting with hypodontia	14	50			.101

†MSX = muscle segment homeo box homolog 1; TGFA = transforming growth factor alpha; TGFB3 = transforming growth factor beta 3; PAX9 = paired box gene 9; UTR = untranslated region.

*Statistically significant.

TABLE 4
Genotype Analysis of MSX1 1.3 for Clefting With or Without Hypodontia

	MSX1 1.3 Alleles		
Study Group	**1**	**2**	**p Value**
CARC control	5	313	
All clefts	13	181	.003*
CARC control	5	313	
Clefting without hypodontia	9	133	.009*
CARC control	5	313	
Clefting with hypodontia	4	48	.026*

*Statistically significant.

TABLE 5

Frequency of Dental Anomalies Associated With Cleft Types

	Hypodontia Outside Cleft Region		Supernumerary Teeth		Peg-Shaped Lateral Incisors		Any Dental Anomalies	
Cleft Type*	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
CLO	23	1	10	14	19	5	5	24
CL&P	45	32	55	20	66	9	10	75
CPO	18	3	21	0	21	0	18	3
p value		<.01		<.01		.094		<.01

* CLO = cleft lip only; CL&P = cleft lip and palate; CPO = cleft palate only.

TABLE 6
Genotype Analysis of Candidate Genes by Cleft Type Without (−) or With (+) Hypodontia

Marker and Study Group[†]	Allele 1	Allele 2	Allele 3	p Value
MSX1 1.3				
CARC control	5	313		
CLO − hypodontia	4	36		.011*
CLO + hypodontia	0	2		.759
CL/CL&P[‡] − hypodontia	6	104		.037*
CL/CL&P + hypodontia	4	50		.029*
CL&P − hypodontia	2	68		.369
CL&P + hypodontia	4	48		.026*
CPO − hypodontia	3	29		.028*
CPO + hypodontia	0	4		.939
TGFB3 CA				
CARC control	303	161	22	
CLO − hypodontia	25	11	2	.862
CLO + hypodontia	1	0	1	.009*
CL/CL&P − hypodontia	69	31	6	.689
CL/CL&P + hypodontia	22	16	6	.024*
CL&P − hypodontia	44	20	4	.764
CL&P + hypodontia	21	16	5	.069
CPO − hypodontia	13	13	2	.239
CPO + hypodontia	1	1	0	.856
PAX9 3′UTR				
CARC control	7	53		
CLO − hypodontia	10	34		.108
CLO + hypodontia	1	1		.243
CL/CL&P − hypodontia	28	92		.045*
CL/CL&P + hypodontia	13	49		.126
CL&P − hypodontia	18	58		.056
CL&P + hypodontia	12	48		.159
CPO − hypodontia	9	27		.080
CPO + hypodontia	2	4		.186

[†] CL&P = cleft lip and palate; CLO = cleft lip only; CPO = cleft palate only; CARC = no cleft.

[‡] Cleft lip only combined with cleft lip and palate.

* Statistically significant.