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Candidate Gene/Loci Studies in Cleft Lip/Palate and Dental Anomalies Finds Novel Susceptibility Genes for Clefts

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Abstract

We revisited 42 families with two or more cleft affected siblings that participated in previous studies and collected complete dental information. Genotypes from 1489 single nucleotide polymorphism (SNP) markers located in 150 candidate genes/loci were reanalyzed. Two sets of association analyses were carried out. First we ran the analysis solely on the cleft status. Second we assigned affection to any cleft or dental anomaly (tooth agenesis, supernumerary teeth, and microdontia), and repeated the analysis. Significant over-transmission was seen for a SNP in *ANKS6* (rs4742741, 9q22.33; p=0.0004) when a dental anomaly phenotype was included in the analysis. Significant over-transmission was also seen for a SNP in *ERBB2* (rs1810132, 17q21.1; p=0.0006). In the clefts only data, the most significant result was also for *ERBB2* (p=0.0006). Other markers with suggestive p-values included *IRF6* and 6q21-q23 loci. In contrast to the above results, suggestive over-transmission of markers in *GART*, *DPF3*, and *NRXN3* were seen only when the dental anomaly phenotype was included in the analysis. These findings support the hypothesis that some loci may contribute to both clefts and congenital dental anomalies. Thus, including dental anomalies information in the genetics analysis of cleft lip and palate will provide new opportunities to map susceptibility loci for clefts.

Introduction

Isolated or nonsyndromic cleft lip and palate (CL/P) is a complex disorder resulting from multiple genetic and environmental factors. CL/P is a common birth defect, and the source of substantial morbidity and mortality worldwide[1]. With an average birth prevalence of 1/700 live births, there is remarkable population to population variation[2]. In general, Asian populations have a higher birth prevalence of clefting (1/500 births), Caucasians are intermediate (1/1,100), and African populations have the lowest (1/2,500 births). However, the notion that Asians have a higher prevalence of clefts has been challenged based on the evidence that many published prevalence rates included all pregnancies (live and still births) and do not distinguish between syndromic and nonsyndromic clefts, or between cleft palate alone and cleft lip with or without cleft palate[3].

An examination of familial recurrence patterns in CL/P indicated that there may be anywhere from 3 to 14 interacting loci involved in clefting[4]. This analysis indicates that very large sample sizes may be necessary to detect the loci involved in CL/P. For a complex genetic disorder such as CL/P, several experimental techniques may be used. These include breakpoint mapping, deletion mapping, direct sequencing of candidate genes/loci, linkage analysis, and linkage disequilibrium analysis[5]. A number of studies on populations with clefts from the Philippines have been productive, in part because of the common occurrence of isolated clefting, large average family sizes, and a motivated public health enterprise[6]. Studies with the Filipino population included *MSX1*[7–9], *TGFA*[7–9], *TGFB2*[7], *TGFB3*[7,9], *IRF6*[10], *FGF* family of genes[11], *PVRL1*[12], genes at 19q13[13], genes at 8p11-23[14], genes at 9q21[15,16], and an additional 18 candidate genes[9]. Furthermore, a meta-analysis of seven genome scans[15] that included Filipino family data revealed significant linkage signals at 9q21 (heterogeneity LOD score 6.6) and 6q23-25 (heterogeneity LOD score 3.55) among other regions. Even though these studies included as many as 403 families (ranging from 3 to 76 individuals in each), the results were, for the most part, modest. The only exceptions are *IRF6*[10] and *MSX1*[7–9]. *IRF6* has also been consistently associated with cleft lip and palate in a number of populations[17–22]. In the same way, *MSX1* has been associated with cleft lip and palate in several independent studies[23–37]. We hypothesize that increasing the sophistication of the clinical description would allow reducing misclassification and improving ones ability to see associations that may have been otherwise masked by a larger more heterogeneous classification approach. We propose to use the presence of dental anomalies outside of the cleft area to subphenotype clefts. Preliminary analysis suggests dental anomalies are preferentially associated with clefts in some families[38] and gene expression studies show that a number of genes co-localize to the developing tooth and palate[39–42]. In order to extend these earlier studies, we proposed to revisit the subset of the initially genotyped families with two or more siblings affected by CL/P and perform a dental examination to broaden the phenotypic description of the families.

Subjects and Methods

Dental assessments

Information on dental anomalies outside the cleft area was collected from the cases and all available relatives. Aside from tooth agenesis, which is the most common congenital anomaly in humans and the one we expected to see the most, other dental anomalies included supernumerary teeth, microdontia, macrodontia, missing cusps, and supernumerary cusps. In many instances, tooth agenesis needed confirmation by an X-ray exam for which we used a portable X-ray system (MinXray P200D MarkIII; Toshiba, Tokyo, Japan). In addition, missing teeth due to tooth decay (caries) needed to be distinguished from congenitally missing teeth. We conducted careful exams and collected comprehensive caries data (data not shown) to aid in the differential diagnosis.

The University of Iowa IRB (approval # 200507743) and University of Pittsburgh IRB (approval # 0511198) gave approval for the study in conjunction with local approval in the Philippines.

Despite local political issues, geographic locations, and weather conditions (thirteen typhoons and severe tropical storms hit the Philippines between May 23rd and December 19th, 2006), we were able to re-contact 46 families with two or more siblings affected with cleft lip with or without cleft palate (CL/P) out of the 70 families that we attempted to contact. Forty-two of the 46 families had available genotyping data. All 42 families had additional affected relatives beyond the two or more affected siblings. We collected data on approximately 500 individuals, including 100 unrelated control families that were used to calculate dental anomalies frequency in the general population for our power studies.

Candidate Gene Association Analysis

Forty-two families for which clinical dental information was available were genotypes for 1489 SNP polymorphisms. These SNPs included 727 SNPs in 150 candidate genes, 431 spanning 6q23-25, and 331 9q21, respectively. The complete list of the markers is presented in the appendix. Genotypes were performed by CIDR (Center for Inherited Disease Research) using the Illumina bead system. The design of using families with multiple affected individuals (and with additional sib cases of dental anomalies only added in by our study) allowed us to increase the statistical power of the linkage disequilibrium approaches. The candidate genes we have been studying (*MSX1, IRF6, PAX9*, and *FGFR1*) are represented in this collection of 500 markers, as well as other interesting regions. Among the 150 candidate genes are *BMP2, BMP4, EGF* and its receptor, *DLX* family members, *FGF1, FGF8, FGF10, MSX2, PVR, PVRL* family members, *TGFA, TGFB* family members and their receptors, *SKI, SHH, PTCH, WNT* family members, *TBX* family members, *PITX2*, and *RARA*.

The data for all SNPs were consistent with Hardy-Weinberg equilibrium in both the affected and unaffected individuals, as well as in a group of unrelated individuals. Alleles at each marker were tested for association twice under an additive model: (1) first only those individuals with CL/P were considered affected, (2) second, the affection status was broadened to include individuals with dental anomalies who were also assigned as affected. The Family Based Association Test implemented in the FBAT software package[43,44] was used in these analyses.

Results

In the 42 families, there were 519 individuals total. One hundred and twenty-eight (128) people were born with CL/P, and genotyping data was available for 125 of them. The remaining 391 family members were not affected by CL/P, and genotyping data was available for 215 of them. Among the 391 unaffected relatives, 48 individuals had dental anomalies (and genotyping data was available for 43 of them).

Tooth agenesis was the most prevalent dental anomaly found in this study. Third molars were the most frequently affected tooth, followed by second premolars. Although other dental anomalies such as supernumerary teeth, microdontia, and supernumerary cusps were found in the families, the affected individuals usually had tooth agenesis as well, or these families always had other family members with tooth agenesis. Only nine probands did not have any relatives with dental anomalies (the other 33 probands had relatives with dental anomalies). However, four probands out of the nine did have dental anomalies outside the cleft area themselves. A total of 23 probands had concomitant dental anomalies outside the cleft area.

Table 1 presents all markers with p-values 0.05 or below (before multiple test correction) in each of the analyses. A SNP in *ANKS6* (rs4742741, 9q22.33; p=0.0004) was significantly over-transmitted when the dental anomalies were added to the analysis. Another significantly over-transmitted SNP was seen in *ERBB2* (rs1810132, 17q21.1; p=0.0006). In the clefts only analysis, a SNP in *ERBB2* was significantly over-transmitted (p=0.0006). Other markers with interesting p-values included *IRF6, CDH2*, and 6q21-q23 loci (Table 1). Table 2 highlights the differences found between the two analyses performed. In summary, many of the over-transmitted SNPs were seen under both analysis (cleft only versus cleft plus dental anomalies), but notably the loci 14q24.3-q31.1 (*DPF3* and *NRXN3*) and 21q22.11 (*GART*) showed evidence for over-transmission only with the addition of dental anomaly phenotypes in the analysis.

Discussion

Our results from the candidate gene data suggest that dental anomalies are part of an extended cleft phenotype. In addition, some genes may contribute to clefts in association with dental anomalies. However, there are obvious limitations in our study. Although the Filipino families included in our study tend to have large sibships, it was not always possible to examine all potential subjects in all families. A number of reasons account for that, such as having a job in another city and not being available at the time of data collection, or choosing not to participate in the study. Another limitation is that this family dataset is probably not representative of the Filipino population. Although it is possible that this group of families may be representative of the Cebu province or even the Central Visayas region, the lack of official population-based records of birth defects in the Philippines does not allow us to make any assumptions regarding the Filipino population as a whole.

The association we found between families with clefts and *IRF6* confirms our previous work[10] with this same population. It is remarkable that the association is still evident with only 42 families, which corroborates that *IRF6* is a major contributor to clefts in Filipinos. While concerned about multiple testing, we did not apply the strict Bonferroni correction as it would increase type II errors and a major focus of this study was to identify putative associations with the combined dental anomaly/cleft phenotype for further studies. For example, under the Bonferroni correction, we would have lowered the alpha to 0.00003 (0.05/1489) and the known association with *IRF6* (interferon regulatory factor 6; p = 0.001) would have been missed. Therefore we report here all results with p-values below 0.05. However, our data must be carefully interpreted since it is expected that some of the p-values below 0.05 can be due to chance.

Analyses under both the narrow and broad affection statuses resulted in significant evidence of over-transmission for markers in 6q21-q23.2, 9q21, and 17q12. The 6q21-q23.2 and 9q21 regions previously showed linkage to clefts in a meta-analysis of genome wide scan data from seven populations[15]. In the current study, markers in 6q21-q23.2 yielded p-values between 0.009 and 0.003, and those in 9q21 yielded p-values between 0.009 and 0.0004. The most significantly over-transmitted marker in 9q21 was rs4742741 in *ANKS6* (ankyrin repeat and sterile alpha motif domain containing 6) located at 9q22.33 (p = 0.001 for clefts only, and p = 0.0004 for clefts and dental anomalies). Adrenomedulin, a vasodilator peptide, prevents the suppression of the inhibitory SMAD6 (SMAD, mother against DPP homolog 6) protein by *TGFB1* (transforming growth factor beta 1) and restores *SMAD2-ANKS6* complex formation in human renal tubular epithelial cell lines[45]. TGFB/BMP (bone morphogenetic protein) signals rely on SMAD-dependent pathways in the ectomesenchyme to mediate epithelial-mesenchymal interactions that control the first branchial arch patterning and tooth development[46].

The rs1810132 marker in *ERBB2* (receptor tyrosine-protein kinase erbB-2, precursor), located in 17q12, yielded p-values of 0.0006. Previous work has suggested that *RARA* (retinoic acid receptor alpha), located at 17q21.1, is associated with isolated cleft lip and palate[47,48]. *ERBB2* is 642,088 base pairs upstream from *RARA*. Since they are relatively near to each other, the previous association suggested for *RARA* could actually be due to variation in *ERBB2*. *ERBB2* is an essential component of a neuregulin-receptor complex but it is not activated by *EGF* (ectodermal growth factor) or *TGFA* (transforming growth factor alpha). *Erbb2*-deficient mice die at birth and display defects in pre-synaptic development[49]. Ethanol consumption during pregnancy affects the expression of *Erbb2* and induces a delay in murine fetal dental morphogenesis[50]. *ERBB2* has not been previously considered as a candidate gene for clefts.

In contrast to the above results, suggestive over-transmission of markers in *GART* (phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase), *DPF3* (D4, zinc and double PHD fingers, family 3), and *NRXN3* (neurexin 3) were seen only when the dental anomaly phenotype was included in the analysis. These genes have not been shown to be expressed during tooth development and their function is still largely unknown. According to the Entrez database, *GART* is required for de novo purine biosynthesis, *NRXN3* functions in the vertebrate nervous system as cell adhesion molecules and receptors, and *DPF3* is probably involved in RNA transcription.

In summary, our results support the hypothesis that increasing the complexity of the clinical description by adding dental anomalies information will provide new opportunities to map susceptibility loci for clefts. Here we report, for the first time, an extensive candidate gene analysis for cleft susceptibility loci using dental anomalies to subphenotype clefts. This approach appears to be a promising one and may help in the identification of genetic variants that increase cleft susceptibility, which would be a crucial step that may allow better estimates of recurrence risks for individual families.

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Literature Cited

1. Murray JC. Gene/environment causes of cleft lip and/or palate. Clin Genet 2002;61:248–256. [PubMed: 12030886]
2. Mossey, PA.; Little, J. Epidemiology of oral clefts: an international perspective. In: Wyszynski, DF., editor. Cleft lip & palate. From origin to treatment. New York: Oxford University Press; 2002. p. 127-158.
3. Cooper ME, Ratay JS, Marazita ML. Asian oral-facial cleft birth prevalence. Cleft Palate Craniofac J 2006;43:580–589. [PubMed: 16986997]
4. Schliekelman P, Slatkin M. Multiplex relative risk and estimation of the number of loci underlying an inherited disease. Am J Hum Genet 2002;71:1369–1385. [PubMed: 12454800]
5. Lidral AC, Murray JC. Genetic approaches to identify disease genes for birth defects with cleft lip/palate as a model. Birth Defects Res A Clin Mol Teratol 2004 Dec;70(12):893–901. [PubMed: 15578714]
6. Murray JC, Daack-Hirsch S, Buetow KH, Munger R, Espina L, Paglinawan N, Villanueva E, Rary J, Magee K, Magee W. Clinical and epidemiologic studies of cleft lip and palate in the Philippines. Cleft Palate Craniofac J 1997;34:7–10. [PubMed: 9003905]
7. Lidral AC, Murray JC, Buetow KH, Basart AM, Schearer H, Shiang R, Naval A, Layda E, Magee K, Magee W. Studies of the candidates 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]
8. Jezewski PA, Vieira AR, Nishimura C, Ludwig B, Johnson M, O'Brien SE, Daack-Hirsch S, Schultz RE, Weber A, Nepomucena B, Romitti PA, Christensen K, Orioli IM, Castilla EE, Machida J, Natsume N, Murray JC. Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. J Med Genet 2003;40:399–407. [PubMed: 12807959]
9. Vieira AR, Avila JR, Daack-Hirsch S, Dragan E, Felix TM, Rahimov F, Harrington J, Schultz RR, Watanabe Y, Johnson M, Fang J, O'Brien SE, Orioli IM, Castilla EE, Fitzpatrick DR, Jiang R, Marazita ML, Murray JC. Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. PLoS Genet 2005a;1:e64. [PubMed: 16327884]
10. Zucchero TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, Caprau D, Christensen K, Suzuki Y, Machida J, Natsume N, Yoshiura K, Vieira AR, Orioli IM, Castilla EE, Moreno L, Arcos-Burgos M, Lidral AC, Field LL, Liu YE, Ray A, Goldstein TH, Schultz RE, Shi

M, Johnson MK, Kondo S, Schutte BC, Marazita ML, Murray JC. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip and palate. N Engl J Med 2004;351:769–780. [PubMed: 15317890]

11. Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, Russo ET, Vieira AR, Dodé C, Mohammadi M, Marazita ML, Murray JC. Impaired FGF signaling contributes to cleft lip and palate. Proc Natl Acad Sci USA 2007a;104:4512–4517. [PubMed: 17360555]
12. Avila JR, Jezewski PA, Vieira AR, Orioli IM, Castilla EE, Christensen K, Daack-Hirsch S, Romitti PA, Murray JC. PVRL1 variants contribute to nonsyndromic cleft lip and palate in multiple populations. Am J Med Genet 2006A;140:2562–2570. [PubMed: 17089422]
13. Warrington A, Vieira AR, Christensen K, Orioli IM, Castilla EE, Romitti PA, Murray JC. Genetic evidence for the role of loci at 19q13 in cleft lip and palate. J Med Genet 2006;43:e26. [PubMed: 16740910]
14. Riley BM, Schultz RE, Cooper ME, Goldstein-McHenry T, Daack-Hirsch S, Lee KT, Dragan E, Vieira AR, Lidral AC, Marazita ML, Murray JC. A genome-wide linkage scan for cleft lip and cleft palate identifies a novel locus on 8p11-23. Am J Med Genet 2007b;143A:846–852. [PubMed: 17366557]
15. Marazita ML, Murray JC, Lidral AC, Arcos-Burgos M, Cooper ME, Goldstein T, Maher BS, Daack-Hirsch S, Schultz R, Field LL, Liu YE, Prescott N, Malcolm S, Winter R, Ray A, Moreno L, Valencia C, Neiswanger K, Wyszynski DF, Bailey-Wilson JE, Albacha-Hejazi H, Beaty TH, McIntosh I, Hetmanski JB, Tuncbilek G, Edwards M, Harkin L, Scott R, Roddick LG. Meta-analysis of 13 genome scans reveals multiple cleft lip/palate genes with novel loci on 9q21 and 2q32-35. Am J Hum Genet 2004;75:161–173. [PubMed: 15185170]
16. Mansilla MA, Cooper ME, Goldstein T, Castilla EE, Lopez-Camelo JS, Marazita ML, Murray JC. Contributions of PTCH gene variants to isolated cleft lip and palate. Cleft Palate Craniofac J 2006;43:21–29. [PubMed: 16405370]
17. Blanton SH, Cortez A, Stal S, Mulliken JB, Finnell RH, Hecht JT. Variation in IRF6 contributes to nonsyndromic cleft lip and palate. Am J Med Genet 2005;137A:259–262. [PubMed: 16096995]
18. Ghassibé M, Bayet B, Revencu N, Verellen-Dumoulin C, Gillerot Y, Vanwijck R, Vikkula M. Interferon regulatory factor-6: a gene predisposing to isolated cleft lip with or without cleft palate in the Belgian population. Eur J Hum Genet 2005;13:1239–1242. [PubMed: 16132054]
19. Scapoli L, Palmieri A, Martinelli M, Pezetti F, Carinci P, Tognon M, Carinci F. Strong evidence of linkage disequilibrium between polymorphisms at the IRF6 locus and nonsyndromic cleft lip with or without cleft palate, in an Italian population. Am J Hum Genet 2005;76:180–183. [PubMed: 15558496]
20. Srichomthong C, Siriwan P, Shotelersuk V. Significant association between IRF6 820G->A and non-syndromic cleft lip with or without cleft palate in the Thai population. J Med Genet 2005;42:e46. [PubMed: 15994871]
21. Park JW, McIntosh I, Hetmanski JB, Jabs EW, Vander Kolk CA, Wu-Chou YH, Chen PK, Chong SS, Yeow V, Jee SH, Park BY, Fallin MD, Ingersoll R, Scott AF, Beaty TH. Association between IRF6 and nonsyndromic cleft lip with or without cleft palate in four populations. Genet Med 2007;9:219–227. [PubMed: 17438386]
22. Vieira AR, Cooper ME, Marazita ML, Orioli IM, Castilla EE. Interferon regulatory factor 6 (IRF6) is associated with oral-facial cleft in individuals that originate in South America. Am J Med Genet 2007a;143A:2075–2078. [PubMed: 17702008]
23. Lidral AC, Romitti PA, Basart AM, Doetschman T, Leysens NJ, Daack-Hirsch S, Semina EV, Johnson LR, Machida J, Burds A, Parnell TJ, Rubenstein JLR, Murray JC. Association of MSX1 and TGFB3 with nonyndromic clefting in humans. Am J Hum Genet 1998;63:557–568. [PubMed: 9683588]
24. 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]
25. Beaty TH, Wang H, Hetmanski Jb, Zeiger JS, Liang KY, Chiu YF, Vander Kolk CA, Seifert KC, Wulfsberg EA, Raymond G, Panny SR, McIntosh I. A case-control study of nonsyndromic oral clefts in Maryland. Ann Epidemiol 2001;11:434–442. [PubMed: 11454503]

26. Blanco R, Chakraborty R, Barton SA, Carreno H, Paredes M, Jara L, Palomino H, Schull WJ. Evidence of a sex-dependent association between the MSX1 locus and nonsyndromic cleft lip with or without cleft palate in the Chilean population. Hum Biol 2001;73:81–89. [PubMed: 11332647]
27. Beaty TH, Hetmanski JB, Zeiger JS, Fan YT, Liang KY, Vander Kolk CA, McIntosh I. Testing candidate genes for non-syndromic oral clefts using a case-parent trio design. Genet Epidemiol 2002;22:1–11. [PubMed: 11754469]
28. Fallin MD, Hetmanski JB, Park J, Scott AF, Ingersoll R, Fuemkranz HA, McIntosh I, Beaty TH. Family-based analysis of MSX1 haplotypes for association with oral clefts. Genet Epidemiol 2003;25:168–175. [PubMed: 12916025]
29. Jugessur A, Lie RT, Wilcox AJ, Murray JC, Taylor JA, Saugstad OD, Vindenes HA, Abyholm F. Variants of developmental genes (TGFA, TGFB3, and MSX1) and their associations with orofacial clefts: a case-parent triad analysis. Genet Epidemiol 2003;24:230–239. [PubMed: 12652527]
30. Slayton RL, Williams L, Murray JC, Wheeler JJ, Lidral AC, Nishimura CJ. Genetic association studies of cleft lip and/or palate with hypodontia outside the cleft region. Cleft Palate-Craniofac J 2003;40:274–279. [PubMed: 12733956]
31. Vieira AR, Orioli IM, Castilla EE, Cooper ME, Marazita ML, Murray JC. MSX1 and TGFB3 contribute to clefting in South America. J Dent Res 2003;82:289–292. [PubMed: 12651933]
32. Suazo J, Santos JL, Carreño H, Jara L, Blanco R. Linkage disequilibrium between MSX1 and non-syndromic cleft lip/palate in the Chilean population. J Dent Res 2004;83:782–785. [PubMed: 15381719]
33. Suzuki Y, Jezewski PA, Machida J, Watanabe Y, Shi M, Cooper ME, Vietle T, Nguyen TD, Hai H, natsume N, Shimozato K, Marazita ML, Murray JC. In a Vietnamese population, MSX1 variants contribute to cleft lip and palate. Genet Med 2004;6:117–125. [PubMed: 15354328]
34. 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. [PubMed: 16723652]
35. Tongkobpetch S, Siriwan P, Shotelersuk V. MSX1 mutations contribute to nonsyndromic cleft lip in a Thai population. J Hum Genet 2006;51:671–676. [PubMed: 16868654]
36. Park J, Park BY, Kim HS, Lee JE, Suh I, Nam CM, Kang DR, Kim S, Yun JE, Go EN, Jee SH, Beaty TH. MSX1 polymorphism associated with risk of oral cleft in Korea: evidence form case-parent trio and case-control studies. Yonsei Med J 2007;48:101–108. [PubMed: 17326252]
37. Wu PA, Li YL, Fan GZ, Wang K. Association study on microsatellite polymorphisms of MSX1 gene and nonsyndromic cleft lip and palate. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2007;24:325–327. [PubMed: 17557248]
38. Letra A, Menezes R, Granjeiro JM, Vieira AR. Defining subphenotypes for oral clefts based on dental development. J Dent Res 2007;86:986–991. [PubMed: 17890676]
39. Peters H, Neubüser A, Kratochwil K, Bailing R. Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. Genes Dev 1998;12:2735–2747. [PubMed: 9732271]
40. Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Waranabe Y, Howard E, Lima RL, Daack-Hirsch S, Sander A, McDonald-McGinn DM, Zackai EH, Lammer EJ, Aylsworth AS, Ardinger HH, Lidral AC, Pober BR, Moreno L, Arcos-Burgos M, Valencia C, Houdayer C, Bahuau M, Moretti-Ferreira D, Richieri-Costa A, Dixon MJ, Murray JC. Mutations in interferon regulatory factor 6 cause Van der Woude and popliteal pterygium syndromes. Nat Genet 2002;32:285–289. [PubMed: 12219090]
41. Zhang Z, Song Y, Zhao X, Zhang X, Fermin C, Chen Y. Rescue of cleft palate in Msx1-deficinet mice by transgenic Bmp4 reveals a network of BMP and Shh signaling in the regulation of mammalian palatogenesis. Development 2002;129:4135–4146. [PubMed: 12163415]
42. Rice R, Thesleff I, Rice DP. Regulation of Twist, Snail, and Id1 is conserved between the developing murine palate and tooth. Dev Dyn 2005;234:28–35. [PubMed: 16028273]
43. Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype-phenotype associations. Eur J Hum Genet 2001;9:301–306. [PubMed: 11313775]
44. Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. Genet Epidemiol 2004;26:61–69. [PubMed: 14691957]

45. Huang H, Ma C, Yang M, Tang C, Wang H. Adrenomedullin impairs the profibrotic effects of transforming growth factor-beta 1 through recruiting Smad6 protein in human renal tubular cells. Cell Physiol Biochem 2005;15:117–124. [PubMed: 15665522]

46. Ko SO, Chung IH, Xu X, Oka S, Deng C, Chai Y. Smad-dependent TGFB/BMP signaling in regulating first branchial arch patterning and tooth development. Eur Cells Material 2007;14:23.

47. Chenevix-Trench G, Jones K, Green AC, Duffy DL, Martin NG. Cleft lip with or without cleft palate: associations with transforming growth factor alpha and retinoic acid receptor loci. Am J Hum Genet 1992;51:1377–1385. [PubMed: 1361101]

48. Peanchitlertkajorn S, Cooper ME, Liu YE, Field LL, Marazita ML. Chromosome 17: gene mapping studies of cleft lip with or without cleft palate in Chinese families. Cleft Palate Craniofac J 2003;40:71–79. [PubMed: 12498608]

49. Lin W, Sanchez HB, Deerinck T, Morris JK, Ellisman M, Lee. KF. Aberrant development of motor axons and neuromuscular synapses in erbB2-deficient mice. Proc Natl Acad Sci USA 2000;97:1299–1304. [PubMed: 10655525]

50. Jiménez-Farfán D, Guevara J, Zenteno E, Malagón H, Hernández-Guerrero JC. EGF-R and erbB-2 in murine tooth development after ethanol exposure. Birth Defects Res A Clin Mol Teratol 2005;73:65–71. [PubMed: 15690356]

Table 1

Most significant linkage disequilibrium results in the cleft lip and palate families with and without dental anomalies as an additional affection status.

Cleft Lip and Palate + Dental Anomalies					
chromosome	**snp**	**cM**	**p-value**	**Gene**	
1	rs2013162	206.3571	0.002	IRF6	
1	rs2279455	91.89721	0.008	TGFBR3	
1	rs674433	206.3533	0.01	IRF6	
1	rs3738480	147.8039	0.02	PRUNE	
1	rs786908	88.96538	0.04	PKN2	
2	rs377122	70.67868	0.03	TGFA	
2	rs7583130	202.7306	0.04	SUMO1	close to
3	rs9849690	185.8107	0.02	EPHB3	close to
3	rs1515490	191.0796	0.04	p63/TP73L	
4	rs6841268	139.5262	0.04	SLC7A11	
4	rs7677751	54.96539	0.04	PDGFRA	
5	rs4559013	170.7842	0.04	FGF18	
5	rs3934591	170.8002	0.05	FGF18	
6	rs9320231	108.1769	0.005	SCML4	
6	rs6921044	140.3791	0.006	BC039503	
6	rs969282	134.2468	0.008	TCF21	
6	rs971402	112.5946	0.008	LAMA4	
6	rs2503791	153.7521	0.009	MTRF1L	
6	rs7772821	132.9342	0.01	TAAR6	
6	rs9206	151.7713	0.01	MTHFD1L	
6	rs3757316	151.8665	0.01	Corf211	
6	rs1555091	127.4783	0.02	AK127472	
6	rs9491385	125.6747	0.02	IBRDC1	close to
6	rs1546943	116.6082	0.02	NT5DC1	
6	rs485640	125.4073	0.03	IBRDC1	
6	rs2503322	127.499	0.03	RSPO3	
6	rs2811674	134.3728	0.03	SLC2A12	
6	rs3800223	108.6792	0.03	SNX3	

Cleft Lip and Palate + Dental Anomalies					
chromosome	**snp**	**cM**	**p-value**	**Gene**	
6	rs238590	115.4712	0.03	HS3ST5	
6	rs6570847	148.7266	0.04	SASH1	
6	rs1741820	122.7632	0.04	HSF2	
6	rs1267948	122.8153	0.04	SERINC1	
6	rs576247	122.7886	0.05	HSF2	
6	rs2802288	109.0029	0.05	FOXO3A	
6	rs6913898	151.474	0.05	MTHFD1L	
6	rs911477	109.3696	0.05	ARMC2	
9	rs4742741	98.61916	0.0004	ANKS6	
9	rs843258	102.6709	0.007	CYLC2	
9	rs1930135	98.49069	0.009	GABBR2	
9	rs1020884	97.23761	0.01	FOXE1	
9	rs4743088	97.23837	0.01	FOXE1	
9	rs2636879	114.1241	0.01	COL27A1	
9	rs4443717	107.0198	0.01	ZNF462	
9	rs418919	99.11206	0.02	TGFBR1	close to
9	rs4129220	94.46965	0.03	FBP1	
9	rs337572	98.5662	0.03	ANKS6	
9	rs1555573	99.20898	0.03	DQ673940	
9	rs773515	91.06249	0.03	AUH	
9	rs3747496	97.1669	0.03	KIAA1529	
9	rs4743077	97.17104	0.03	KIAA1529	
9	rs2416682	118.5309	0.03	TLR4	
9	rs3794486	105.5419	0.04	TMEM38B	
9	rs1979993	105.6141	0.04	TMEM38B	
9	rs3793524	109.299	0.04	PTPN3	
9	rs1059273	97.92906	0.04	TRIM14	
9	rs4743348	99.25561	0.05	TGFBR1	close to
9	rs2281732	97.92456	0.05	TRIM14	
14	rs2536143	72.25479	0.03	DPF3	
14	rs221430	79.13787	0.04	NRXN3	

Cleft Lip and Palate + Dental Anomalies

chromosome	snp	cM	p-value	Gene	
14	rs1018466	36.193	0.04	PAX9	close to
15	rs2879515	32.65859	0.01	SLC12A5	close to
15	rs878960	24.48003	0.02	GABRB3	
15	rs690	56.62203	0.03	LIPC	
15	rs1426223	24.50339	0.05	GABRB3	
17	rs1810132	35.11953	0.0005	ERBB2	
17	rs2015729	42.70949	0.002	ITGB3	
17	rs2292699	42.71729	0.005	ITGB3	
17	rs890397	45.45893	0.01	DLX3/DLX4	
17	rs1905339	37.83582	0.01	STAT3	close to
17	rs8071740	22.54986	0.02	WSB1	close to
17	rs744166	37.76773	0.02	STAT3	
17	rs2313430	35.18334	0.04	IKZF3	
17	rs9906933	37.66357	0.04	STAT5B	
18	rs2215502	24.03802	0.02	CDH2	close to
20	rs819133	32.33398	0.02	AHCY	
20	rs6123674	55.19635	0.04	BMP7	
21	rs4817579	33.83209	0.02	GART	

Cleft Lip and Palate

chromosome	snp	cM	p-value	Gene	
1	rs674433	206.3533	0.001	IRF6	
1	rs2013162	206.3571	0.001	IRF6	
1	rs513287	167.3959	0.003	PRRX1	
1	rs2279455	91.89721	0.01	TGFBR3	
1	rs4245660	78.25275	0.02	GIPC2	
1	rs1051740	222.3264	0.03	EPHX1	
1	rs786908	88.96538	0.03	PKN2	
1	rs1007512	75.2837	0.05	LHX8	close to
2	rs7583130	202.7306	0.01	SUMO1	close to

Cleft Lip and Palate + Dental Anomalies

chromosome	snp	cM	p-value	Gene	
2	rs4328603	9.611782	0.01	ADAM17	
2	rs2280509	202.7229	0.03	FZD7	
2	rs6705408	9.580829	0.03	ADAM17	
2	rs2276338	9.596387	0.03	ADAM17	
2	rs377122	70.67868	0.04	TGFA	
2	rs512535	21.17943	0.04	OSR1	close to
3	rs9849690	185.8107	0.02	EPHB3	close to
5	rs7715062	7.959907	0.04	MTRR	close to
6	rs2503791	153.7521	0.003	MTRF1L	close to
6	rs971402	112.5946	0.003	LAMA4	
6	rs7772821	132.9342	0.005	TAAR6	
6	rs9320231	108.1769	0.01	SCML4	
6	rs6921044	140.3791	0.01	BC039503	
6	rs969282	134.2468	0.02	TCF21	close to
6	rs1546943	116.6082	0.02	NT5DC1	
6	rs1983721	117.0122	0.02	RWDD1	
6	rs485640	125.4073	0.02	IBRDC1	
6	rs911477	109.3696	0.03	ARMC2	
6	rs718174	108.4799	0.03	OSTM1	
6	rs3734679	107.6221	0.03	PDSS2	
6	rs2503322	127.499	0.03	RSPO3	
6	rs3800229	109.1037	0.03	FOXO3A	
6	rs2811674	134.3728	0.03	SLC2A12	
6	rs3127657	107.2111	0.04	QRSL1	
6	rs549332	116.5613	0.04	NT5DC1	
6	rs1555091	127.4783	0.04	AK127472	
6	rs9400504	112.213	0.04	FYN	
8	rs6987534	38.41887	0.02	FGFR1	
8	rs3925	38.40082	0.03	FGFR1	
9	rs4742741	98.61916	0.001	ANKS6	
9	rs418919	99.11206	0.005	TGFBR1	close to

Cleft Lip and Palate + Dental Anomalies					
chromosome	**snp**	**cM**	**p-value**	**Gene**	
9	rs1020884	97.23761	0.01	FOXE1	close to
9	rs4743088	97.23837	0.01	FOXE1	close to
9	rs2636879	114.1241	0.01	COL27A1	
9	rs1930135	98.49069	0.01	GABBR2	
9	rs4129220	94.46965	0.01	FBP1	
9	rs2281732	97.92456	0.02	TRIM14	
9	rs1059273	97.92906	0.02	TRIM14	
9	rs843258	102.6709	0.02	CYLC2	
9	rs1555573	99.20898	0.02	DQ673940	
9	rs995294	109.8189	0.03	PALM2-AKAP2	
9	rs3794486	105.5419	0.03	TMEM38B	
9	rs3750396	88.85173	0.03	AK127258	
9	rs4743348	99.25561	0.03	TGFBR1	close to
9	rs1320547	93.79928	0.04	BARX1	close to
9	rs1462090	97.24908	0.05	FOXE1	close to
9	rs773515	91.06249	0.05	AUH	
11	rs10790332	119.0589	0.02	PVRL1	
12	rs11065374	119.8629	0.01	TCF1	close to
12	rs1039302	119.699	0.02	UNQ1887	
14	rs1018466	36.193	0.01	PAX9	close to
15	rs690	56.62203	0.02	LIPC	
17	rs1810132	35.11953	0.0006	ERBB2	
17	rs2015729	42.70949	0.001	ITGB3	
17	rs2292699	42.71729	0.01	ITGB3	
17	rs890397	45.45893	0.01	DLX3/DLX4	close to
17	rs2056131	42.68874	0.01	ITGB3	
17	rs8071740	22.54986	0.01	WSB1	close to
17	rs4461115	43.15458	0.03	ITGB3	close to
18	rs2215502	24.03802	0.003	CDH2	close to

Table 2

Contrasting results between the two candidate genes/association analyses.

Locus	Gene	SNP	CLP Data (p-value)	CLP + Dental Anomalies Data (p-value)
Loci where association is present in both clefts and clefts + dental anomalies data				
9q22.33	ANKS6	rs4742741	0.001	0.0004
	CYLC2	rs843258	0.02	0.008
	GABRB2	rs1930135	0.01	0.009
	TGFBR1	rs418919	0.005	0.02
	FOXE1	rs1020884	0.01	0.01
	FOXE1	rs4743088	0.01	0.01
17q21.1	ERBB2	rs1810132	0.0006	0.0006
	ITGB3	rs2015729	0.002	0.003
	ITGB3	rs2292699	0.01	0.005
1q32.3-q41	IRF6	rs2013162	0.002	0.002
	IRF6	rs674433	0.001	0.01
6q21-q23	SCML4	rs9320231	0.01	0.006
	BC039503	rs6921044	0.01	0.006
	TCF21	rs969282	0.02	0.008
	LAMA4	rs971402	0.003	0.008
	MTRFL1	rs2503791	0.003	0.009
	TAAR6	rs7772821	0.005	0.01
2p13	TGFA	rs377122	0.04	0.03
Loci where association is stronger in the clefts + dental anomalies than that in the clefts data				
5q34	FGF18	rs4559013	0.09	0.05
	FGF18	rs3934591	0.1	0.05
14q24.3-q31.1	DPF3	rs2536143	0.18	0.03
	NRXN3	rs221430	0.32	0.04
15q11.2-q12	SLC12A5	rs2879515	0.06	0.01
	GABRB3	rs878960	0.06	0.02
	GABRB3	rs1426223	0.06	0.05
	LIPC	rs690	0.02	0.04
20q13	AHCY	rs819133	0.06	0.02
	BMP7	rs6123674	0.07	0.04
21q22.11	GART	rs4817579	0.16	0.02