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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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Purpose: We revisited 42 families with two or more cleft-affected siblings who participated in previous studies. Complete dental information was collected to test the hypothesis that dental anomalies are part of the cleft phenotype spectrum, and can provide new opportunities for identification of cleft susceptibility genes. **Methods:** Genotypes from 1489 single nucleotide polymorphism 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. **Results:** Significant over-transmission was seen for a single nucleotide polymorphism in ankyrin repeat and sterile alpha motif domain containing 6 (rs4742741, 9q22.33; $P = 0.0004$) when a dental anomaly phenotype was included in the analysis. Significant over-transmission was also seen for a single nucleotide polymorphism 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 interferon regulatory factor 6 and 6q21–q23 loci. In contrast to the above results, suggestive over-transmission of markers in *GART*, *DPF3*, and neurexin 3 were seen only when the dental anomaly phenotype was included in the analysis. **Conclusions:** 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. ***Genet Med* 2008:10(9):668–674.**

Key Words: *cleft lip and palate, tooth agenesis, dental anomalies, oral clefts, ANKS6, SAMD6, ERBB2, IRF6*

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), whites are intermediate (1/1100), and African populations have the lowest (1/2500 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 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] transforming growth factor alpha (*TGFA*),[7–9] transforming growth factor beta 2 (*TGFB2*),[7] *TGFB3*,[7,9] interferon regulatory factor 6 (*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]

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that included Filipino family data revealed significant linkage signals at 9q21 (heterogeneity logarithm of odds [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 CL/P in a number of populations.[17–22] In the same way, *MSX1* has been associated with CL/P 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 that dental anomalies are preferentially associated with clefts in some families,[38] and gene expression studies show that a number of genes colocalize to the developing tooth and palate.[39–42] 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.

PATIENTS 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 examination 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 examinations and collected comprehensive caries data (data not shown) to aid in the differential diagnosis.

The University of Iowa Institutional Review Board (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 (13 typhoons and severe tropical storms hit the Philippines between May 23rd and December 19th, 2006), we were able to recontact 46 families with two or more siblings affected with cleft lip with or without cleft palate (CL/P) of the 70 families who 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 genotyped for 1489 single nucleotide polymorphisms (SNP). These SNPs included 727 SNPs in 150 candidate genes, 431 spanning 6q23–25, and 331 9q21. The complete list of the markers is presented in the appendix. Genotypes were performed by the 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, and other interesting regions. Among the 150 candidate genes are bone morphogenetic protein 2 (*BMP2*), *BMP4*, ectodermal growth factor (*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 retinoic acid receptor alpha (*RARA*).

The data for all SNPs were consistent with Hardy-Weinberg equilibrium in both the affected and unaffected individuals, and 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 twenty-eight people were born with CL/P and genotyping data were available for 125 of them. The remaining 391 family members were not affected by CL/P and genotyping data were available for 215 of them. Among the 391 unaffected relatives, 48 individuals had dental anomalies (and genotyping data were 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 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. An SNP in ankyrin repeat and sterile alpha motif domain containing 6 (*ANKS6*) (rs4742741, 9q22.33; $P = 0.0004$) was signifi-

Table 1
Most significant linkage disequilibrium results in the cleft lip and palate families with and without dental anomalies as an additional affection status

Chromosome	SNP	cM	*P*	Gene	
Cleft lip and palate + dental anomalies					
1	rs2013162	206.3571	0.002	*IRF6*	
	rs2279455	91.89721	0.008	*TGFBR3*	
	rs674433	206.3533	0.01	*IRF6*	
	rs3738480	147.8039	0.02	*PRUNE*	
	rs786908	88.96538	0.04	*PKN2*	
2	rs377122	70.67868	0.03	*TGFA*	
	rs7583130	202.7306	0.04	*SUMO1*	Close to
3	rs9849690	185.8107	0.02	*EPHB3*	Close to
	rs1515490	191.0796	0.04	*p63/TP73L*	
4	rs6841268	139.5262	0.04	*SLC7A11*	
	rs7677751	54.96539	0.04	*PDGFRA*	
5	rs4559013	170.7842	0.04	*FGF18*	
	rs3934591	170.8002	0.05	*FGF18*	
6	rs9320231	108.1769	0.005	*SCML4*	
	rs6921044	140.3791	0.006	*BC039503*	
	rs969282	134.2468	0.008	*TCF21*	
	rs971402	112.5946	0.008	*LAMA4*	
	rs2503791	153.7521	0.009	*MTRF1L*	
	rs7772821	132.9342	0.01	*TAAR6*	
	rs9206	151.7713	0.01	*MTHFD1L*	
	rs3757316	151.8665	0.01	*Corf211*	
	rs1555091	127.4783	0.02	*AK127472*	
	rs9491385	125.6747	0.02	*IBRDC1*	Close to
	rs1546943	116.6082	0.02	*NT5DC1*	
	rs485640	125.4073	0.03	*IBRDC1*	
	rs2503322	127.499	0.03	*RSPO3*	
	rs2811674	134.3728	0.03	*SLC2A12*	
	rs3800223	108.6792	0.03	*SNX3*	
	rs238590	115.4712	0.03	*HS3ST5*	
	rs6570847	148.7266	0.04	*SASH1*	
	rs1741820	122.7632	0.04	*HSF2*	
	rs1267948	122.8153	0.04	*SERINC1*	
	rs576247	122.7886	0.05	*HSF2*	
	rs2802288	109.0029	0.05	*FOXO3A*	
	rs6913898	151.474	0.05	*MTHFD1L*	
	rs911477	109.3696	0.05	*ARMC2*	
9	Rs4742741	98.61916	0.0004	*ANKS6*	
	rs843258	102.6709	0.007	*CYLC2*	
	rs1930135	98.49069	0.009	*GABBR2*	
	rs1020884	97.23761	0.01	*FOXE1*	
	rs4743088	97.23837	0.01	*FOXE1*	
	rs2636879	114.1241	0.01	*COL27A1*	
	rs4443717	107.0198	0.01	*ZNF462*	
	rs418919	99.11206	0.02	*TGFBR1*	Close to
	rs4129220	94.46965	0.03	*FBP1*	
	rs337572	98.5662	0.03	*ANKS6*	
	rs1555573	99.20898	0.03	*DQ673940*	
	rs773515	91.06249	0.03	*AUH*	
	rs3747496	97.1669	0.03	*KIAA1529*	
	rs4743077	97.17104	0.03	*KIAA1529*	
	rs2416682	118.5309	0.03	*TLR4*	
	rs3794486	105.5419	0.04	*TMEM38B*	
	rs1979993	105.6141	0.04	*TMEM38B*	
	rs3793524	109.299	0.04	*PTPN3*	
	rs1059273	97.92906	0.04	*TRIM14*	
	rs4743348	99.25561	0.05	*TGFBR1*	Close to
	rs2281732	97.92456	0.05	*TRIM14*	
14	rs2536143	72.25479	0.03	*DPF3*	
	rs221430	79.13787	0.04	*NRXN3*	
	rs1018466	36.193	0.04	*PAX9*	Close to
15	rs2879515	32.65859	0.01	*SLC12A5*	Close to
	rs878960	24.48003	0.02	*GABRB3*	
	rs690	56.62203	0.03	*LIPC*	
	rs1426223	24.50339	0.05	*GABRB3*	
17	rs1810132	35.11953	0.0005	*ERBB2*	
	rs2015729	42.70949	0.002	*ITGB3*	
	rs2292699	42.71729	0.005	*ITGB3*	
	rs890397	45.45893	0.01	*DLX3/DLX4*	
	rs1905339	37.83582	0.01	*STAT3*	Close to
	rs8071740	22.54986	0.02	*WSB1*	Close to
	rs744166	37.76773	0.02	*STAT3*	
	rs2313430	35.18334	0.04	*IKZF3*	
	rs9906933	37.66357	0.04	*STAT5B*	
18	rs2215502	24.03802	0.02	*CDH2*	Close to
20	rs819133	32.33398	0.02	*AHCY*	
	rs6123674	55.19635	0.04	*BMP7*	
21	rs4817579	33.83209	0.02	*GART*	
Cleft lip and palate					
1	rs674433	206.3533	0.001	*IRF6*	
	rs2013162	206.3571	0.001	*IRF6*	
	rs513287	167.3959	0.003	*PRRX1*	
	rs2279455	91.89721	0.01	*TGFBR3*	

(*Continued*)

Table 1
Continued

Chromosome	SNP	cM	*P*	Gene	
	rs4245660	78.25275	0.02	*GIPC2*	
	rs1051740	222.3264	0.03	*EPHX1*	
	rs786908	88.96538	0.03	*PKN2*	
	rs1007512	75.2837	0.05	*LHX8*	Close to
2	rs7583130	202.7306	0.01	*SUMO1*	Close to
	rs4328603	9.611782	0.01	*ADAM17*	
	rs2280509	202.7229	0.03	*FZD7*	
	rs6705408	9.580829	0.03	*ADAM17*	
	rs2276338	9.596387	0.03	*ADAM17*	
	rs377122	70.67868	0.04	*TGFA*	
	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
	rs971402	112.5946	0.003	*LAMA4*	
	rs7772821	132.9342	0.005	*TAAR6*	
	rs9320231	108.1769	0.01	*SCML4*	
	rs6921044	140.3791	0.01	*BC039503*	
	rs969282	134.2468	0.02	*TCF21*	Close to
	rs1546943	116.6082	0.02	*NT5DC1*	
	rs1983721	117.0122	0.02	*RWDD1*	
	rs485640	125.4073	0.02	*IBRDC1*	
	rs911477	109.3696	0.03	*ARMC2*	
	rs718174	108.4799	0.03	*OSTM1*	
	rs3734679	107.6221	0.03	*PDSS2*	
	rs2503322	127.499	0.03	*RSPO3*	
	rs3800229	109.1037	0.03	*FOXO3A*	
	rs2811674	134.3728	0.03	*SLC2A12*	
	rs3127657	107.2111	0.04	*QRSL1*	
	rs549332	116.5613	0.04	*NT5DC1*	
	rs1555091	127.4783	0.04	*AK127472*	
	rs9400504	112.213	0.04	*FYN*	
8	rs6987534	38.41887	0.02	*FGFR1*	
	rs3925	38.40082	0.03	*FGFR1*	
9	rs4742741	98.61916	0.001	*ANKS6*	
	rs418919	99.11206	0.005	*TGFBR1*	Close to
	rs1020884	97.23761	0.01	*FOXE1*	Close to
	rs4743088	97.23837	0.01	*FOXE1*	Close to
	rs2636879	114.1241	0.01	*COL27A1*	
	rs1930135	98.49069	0.01	*GABBR2*	
	rs4129220	94.46965	0.01	*FBP1*	
	rs2281732	97.92456	0.02	*TRIM14*	
	rs1059273	97.92906	0.02	*TRIM14*	
	rs843258	102.6709	0.02	*CYLC2*	
	rs1555573	99.20898	0.02	*DQ673940*	
	rs995294	109.8189	0.03	*PALM2-AKAP2*	
	rs3794486	105.5419	0.03	*TMEM38B*	
	rs3750396	88.85173	0.03	*AK127258*	
	rs4743348	99.25561	0.03	*TGFBR1*	Close to
	rs1320547	93.79928	0.04	*BARX1*	Close to
	rs1462090	97.24908	0.05	*FOXE1*	Close to
	rs773515	91.06249	0.05	*AUH*	
11	rs10790332	119.0589	0.02	*PVRL1*	
12	rs11065374	119.8629	0.01	*TCF1*	Close to
	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*	
	rs2015729	42.70949	0.001	*ITGB3*	
	rs2292699	42.71729	0.01	*ITGB3*	
	rs890397	45.45893	0.01	*DLX3/DLX4*	Close to
	rs2056131	42.68874	0.01	*ITGB3*	
	rs8071740	22.54986	0.01	*WSB1*	Close to
	rs4461115	43.15458	0.03	*ITGB3*	Close to
18	rs2215502	24.03802	0.003	*CDH2*	Close to

cantly 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, an 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 neurexin 3[*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

Table 2
Contrasting results between the two candidate genes/association analyses

Locus	Gene	SNP	CLP Data (*P*)	CLP + dental anomalies data (*P*)
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

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. Although 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* ($P = 0.001$) would have been missed. Therefore, here we report all results with *P* values below 0.05. However, our data must be carefully interpreted because 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* 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 (mothers against decapentaplegic [SMAD], mother against decapentaplegic, homolog 6) protein by *TGFB1* and restores *SMAD2-ANKS6* complex formation in human renal tubular epithelial cell lines.[45] TGFB/BMP 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*, located at 17q21.1, is associated with isolated CL/P.[47,48] *ERBB2* is 642,088 base pairs upstream from *RARA*. Because 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* or *TGFA*. *Erbb2*-deficient mice die at birth and display defects in presynaptic 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, and phosphoribosylaminoimidazole synthetase), *DPF3* (D4, zinc and double plant homeo domain fingers, family 3), and *NRXN3* 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 seems 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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