Published in final edited form as:

Birth Defects Res A Clin Mol Teratol. 2009 February ; 85(2): 169–173. doi:10.1002/bdra.20489.

AXIN2 and CDH1 Polymorphisms, Tooth Agenesis and Oral Clefts

Ariadne Letra^{1,*}, Renato Menezes^{1,*}, Jose M. Granjeiro², and Alexandre R. Vieira^{1,3,§}

¹Department of Oral Biology, Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh

²Department of Cell and Molecular Biology, Fluminense Federal University

³Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh

Abstract

Background—*AXIN2* and *CDH1* genes play important roles during craniofacial morphogenesis. Mutations in these genes have been described in families presenting colorectal cancer and tooth agenesis, and gastric cancer and cleft lip/palate (CL/P). Oral clefts have been associated with tooth agenesis. We investigated if *AXIN2* and *CDH1* polymorphisms were associated with clefts or with any associated dental subphenotypes.

Methods—Markers in *AXIN2* and *CDH1* were genotyped using Taqman chemistry in a sample cohort comprised of 500 cleft individuals and 500 unrelated controls.

Results—Comparison between cleft and control groups showed a trend for association for *AXIN2* with incomplete cleft palate ($P=0.006$) and *CDH1* with unilateral cleft lip/palate ($P=0.03$ for left CL/P and $P=0.04$ for right CL/P). Comparison of cleft subphenotypes with tooth agenesis and controls revealed borderline associations for *CDH1* ($P=0.008$) and *AXIN2* ($P=0.01$) with unilateral right cleft lip/palate with tooth agenesis.

Conclusions—We observed only borderline results for the association of *AXIN2* and *CDH1* with CL/P with and without tooth agenesis. Nevertheless, implication of these genes in the simultaneous occurrence of CL/P and cancer, and in tooth agenesis and cancer, is rather intriguing and warrants further investigations with other geographic and ethnic populations.

Keywords

oral clefts; tooth agenesis; *AXIN2*; *CDH1*; polymorphisms

INTRODUCTION

Nonsyndromic oral clefts are among the most common congenital anomalies among live births and are composed of two separate entities: cleft lip with or without cleft palate (CL/P) and cleft palate alone (CP) (Gorlin, 2001). It is generally accepted that CL/P and CP have different etiologies. CP is less common, with a prevalence of approximately 1 in 1500 births in most racial backgrounds. In contrast, CL/P occurs more commonly (approximately 1–2/1000 births) and the prevalence varies considerably by ancestral origin, with the highest incidence in populations of Asian ancestry followed by European and African populations (Mossey and Little, 2002). Most cases of CL/P are non-syndromic and the involvement of genetic factors is

§Corresponding author: Alexandre R. Vieira, DDS, MS, PhD, Assistant Professor, 614 Salk Hall, Department of Oral Biology, Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, 3501 Terrace Street, Pittsburgh, PA 15261, Phone (412) 383-8972, FAX (412) 624-3080, E-mail: arv11@dental.pitt.edu.

*These authors have contributed equally to this work.

well established (Lidral and Moreno, 2005). Previous evidences indicate that genetic variation in a handful of genes may account for ~15–20% of all cleft cases (Zuccherro et al., 2004; Vieira et al., 2005; Riley et al., 2007).

Recent genetic studies of CL/P have shown the association of genes that are also involved in cancer, raising a hypothesis that the same genes may be involved in embryonic development and later in cancer development. *CDH1* (E-cadherin) and *AXIN2* (AXIS inhibition protein 2) genes play important roles during craniofacial morphogenesis (Lüning et al., 1994; Kerrigan et al., 2000; Giles et al., 2003) and mutations in these genes have been described in families presenting gastric cancer and CL/P (Frebourg et al., 2006), and colorectal cancer and tooth agenesis (Lammi et al., 2004), respectively.

Tooth agenesis is a dental anomaly characterized by the congenital absence of one or more permanent teeth and affects approximately 20% of the population worldwide (Vastardis, 2000). It is often seen in individuals with clefts and has been suggested as an extended phenotype for oral clefts (Vieira, 2003). We have recently shown that dental anomalies in general, and particularly tooth agenesis, are disturbances of dental development associated with oral clefts. Segregation of different cleft types with specific dental anomalies revealed interesting patterns of association that, when significantly different from controls, were proposed as new cleft subphenotypes to be included in future genetic studies (Letra et al., 2007).

The most critical events of formation of teeth, lip and palate occur almost concurrently. Cell proliferation and differentiation as well as apoptosis define these structures, while disruption of these processes by environmental and/or genetic abnormalities may predispose to dental anomalies and clefts (Tonge, 1967). Therefore, since many of the genes involved in the formation of these processes are likely the same, and considering that oral clefts and tooth agenesis may lie within the same phenotypic spectrum, we investigated if *CDH1* and *AXIN2* polymorphisms were associated with clefts or with its associated dental subphenotypes.

MATERIALS & METHODS

Subjects

The subjects of this study have been previously described in detail (Letra et al., 2007). Briefly, 1000 individuals, 500 cases with non-syndromic clefts and 500 control individuals without clefts or family history of clefting were ascertained at the Dental Clinics of the Hospital of Rehabilitation and Craniofacial Anomalies (HRAC) and Bauru Dental School, both of the University of São Paulo, Bauru, SP, Brazil. Cleft cases were patients currently in treatment at HRAC and had been previously examined by the hospital genetics team and classified as non-syndromic. Subjects were clinically examined regarding cleft type (when applicable) and the presence of dental anomalies. Genomic DNA of all individuals was isolated from buccal epithelial cells following established protocols. The study was conducted with the consent of the participants and approved by the Research and Ethics Committee of the aforementioned institution. In the case of children under 15 years of age, authorization was also requested from their parents or from the individual legally in charge of the child. The State of São Paulo, located in the southeast region of Brazil, is characterized by a predominantly Caucasian population. However, individuals of Japanese ancestry are very frequent, since most of the Japanese immigrants established themselves within the state of São Paulo. Individuals were ascertained as Caucasians when there was no history of African or Japanese ascendant. Details of the studied population are presented in Table 1.

Single Nucleotide Polymorphism (SNP) Selection and Genotyping

Three SNPs in *CDH1* and two in *AXIN2* were selected from the HapMap electronically (<http://www.hapmap.org>) based on: 1) classification of being potentially functional (for altering amino acid sequence of the protein product or for occurring in the proximal promoter of the gene and potentially influence gene expression), or 2) haplotype tagging SNPs selected to act as surrogates for the linkage disequilibrium blocks of the region surrounding the candidate gene.

Genotyping of the selected polymorphisms was carried out by real-time PCR using the Taqman method (Ranade et al., 2001) in an ABI 7900 automatic instrument (Applied Biosystems, Foster City, CA). Assays and reagents were supplied by Applied Biosystems (Applied Biosystems, Foster City, CA). Details of the studied polymorphisms are shown in Table 1.

All five SNPs were first tested in a set of CEPH (Center d'Etude du Polymorphisme Humain) control population, which are European descents. Genotypes and alleles were scored by two investigators (AL and RM) independently. The Taqman method relies on using extremely sensitive allele-specific probes, with great reliability, in a way that all samples were scored for the respective SNP. In case of discrepancy, a third investigator was asked to score the sample genotype. A negative control template was included in every reaction plate.

Linkage Disequilibrium Analyses

We tested for linkage disequilibrium between the markers using the software GOLD (Graphical Overview of Linkage Disequilibrium) (Abecasis and Cookson, 2001) since no information is available in the current databases (e.g. HapMap) regarding the SNPs tested in the population studied. LD results were generated separately for cases and controls, and also pooled.

Statistical Analysis

chi-square and Fisher exact tests were used for statistical analysis and determination of fit to Hardy-Weinberg equilibrium. Differences in the frequencies of each polymorphism between cleft subphenotypes and controls were considered statistically significant if $P \leq 0.001$ (0.05/45) under Bonferroni correction. For comparison of cleft subphenotypes with tooth agenesis and controls, $P \leq 0.0016$ (0.05/30) was considered significant under Bonferroni correction.

RESULTS

Genotype and allele distributions were within Hardy-Weinberg equilibrium (data not shown). Nevertheless, because differences in ethnic background between cases and controls could account for spurious results due to problems of population stratification, we performed statistical analyses including all individuals, and in addition, we also performed analyses including only Caucasian individuals (herein interpreted as not presenting any contribution of African or Japanese ascendant). The associations found for both sets of analyses did not significantly differ (data not shown) and the results presented reflect the results for Caucasians only. Raw genotypes for each of the SNPs in cases and controls are available as supplemental material (Appendix).

Results of Association with Cleft Lip/Palate Phenotypes

Cleft cases were divided into CL/P bilateral, unilateral, complete or incomplete, and cleft palate complete or incomplete before comparing to controls. A complete cleft meant involvement of primary and secondary palates entirely.

After correction for multiple testing, we observed a trend for association with a marker located at the 5' untranslated region of the *AXIN2* gene with incomplete cleft palate ($P=0.006$). If we

consider a nominal threshold of 0.05 however, an intronic marker in *AXIN2* showed a trend for allelic association with bilateral CL/P ($P=0.02$ and $P=0.03$). Both markers in *CDH1* showed a trend for association with unilateral CL/P: SNP rs11642413 showed association with unilateral right CL/P ($P=0.04$) while SNP rs9929218 showed association with unilateral left CL/P ($P=0.03$) (Table 2).

Results of Association with Cleft Lip/Palate Subphenotypes with Tooth Agenesis

We used cleft subphenotypes with tooth agenesis to test for association with *CDH1* and *AXIN2*. We found borderline association between markers in *CDH1* ($P=0.008$ for rs11642413 and $P=0.04$ for rs9929218) and *AXIN2* ($P=0.01$ for rs7591) with unilateral right CL/P with tooth agenesis (Table 2).

DISCUSSION

Mutations in *CDH1* and *AXIN2* have been implicated in the simultaneous occurrence of CL/P and cancer, and tooth agenesis and cancer, respectively. We speculated that they might also contribute to CL/P itself or any agenesis-related subphenotypes in a previously described population (Letra et al., 2007). These individuals were all from Brazil and represent a tri-hybrid ethnic mixture of Latin-European whites, Afro-Brazilian blacks, and Amerindians, which in turn makes it very difficult to match perfectly the ethnicity of control and cases. Therefore, we anticipate that some confounding effects of undetectable population stratification could cause unreliability in the interpretation of our case-control study. In order to overcome the problem of stratification, we performed statistical analyses including all individuals, and in addition, we also performed analyses including only individuals of Caucasian background, herein interpreted as not presenting any contribution of African or Japanese ascendant. The associations found for both sets of analyses did not significantly differ (data not shown).

Our study shows only borderline associations between *CDH1* and *AXIN2* and CL/P with or without tooth agenesis, under the strictness of the Bonferroni correction. These results for *AXIN2* and incomplete cleft palate ($p=0.006$) and for *CDH1* and unilateral right CL/P with tooth agenesis ($p=0.008$) would be significant if a traditional alpha of 0.05 was considered. It would be interesting to see any correlation between these results and history of cancer; however cancer data was not available for the studied cohort. Nevertheless, the relationship between CL/P and additional congenital anomalies such as tooth agenesis is interesting as it might reflect interactions between or similarities in their etiologic mechanisms and may be able to provide insights for additional studies as well as aid in genetic counseling procedures.

E-Cadherin (*CDH1*) is a most reasonable candidate gene for CL/P. It is expressed in the epithelium of the palate prior to and after shelf fusion, as well as in the tongue and dental lamina (Lüning et al., 1994). It plays a key role in cell adhesion, which is vital to normal development, including craniofacial morphogenesis and palatal fusion (Kerrigan et al., 2000). The transformation of epithelial cells into mesenchymal cells in a phenomenon known as epithelial-mesenchymal transformation (EMT) is suggested to occur during the process of palatogenesis to remove epithelial cells from the palatal medial edge epithelia and allow for mesenchymal continuity and palatal fusion. At an early stage of transformation the epithelial cell down regulates its expression of E-cadherin which frees the attachment of the cells from one another. The close relationship between EMT and E-cadherin has led some investigators to consider the *CDH1* gene as the 'master gene' which switches EMT on and off (Hay, 1995). Hence, defects in *CDH1* are involved in the dysfunction of the cell-cell adhesion system, and could contribute to the complex events that lead to human clefting. Mutations in another cell adhesion molecule, Nectin-1, encoded by the *PVRL1* gene, have been shown to cause syndromic CL/P (Suzuki et al., 2000). Interestingly, Nectin-1 interacts with E-cadherin, and previous evidence

has shown the segregation of CL/P and *CDH1* mutations in families with hereditary diffuse gastric cancer (Frebourg et al., 2006).

The *AXIN2* gene is known as a negative feedback regulator of *WNT*-signaling which regulates embryonic development and organogenesis (Giles et al., 2003). There are a number of observations that may link *AXIN2* with CL/P, mainly through its effects on WNT signaling. Studies with mouse models have shown that *Wnt* mutations result in CL/P with penetrance as high as 90% in some strains (Juriloff and Harris, 2008). In humans, some of these WNT genes are located at 17q, a region implicated by numerous human linkage and association studies on nonsyndromic CL/P (Juriloff and Harris, 2008), and also where *AXIN2* is located. Mutations in *AXIN2* have been associated with familial and sporadic tooth agenesis (Lammi et al., 2004; Mostowska et al., 2006), and tooth agenesis itself has been reported in association with CL/P by our group and other investigators (Letra et al., 2007; Vieira et al., 2004, 2007). Lammi et al. (2004) reported mutations in *AXIN2* segregating with oligodontia (the more severe type of tooth agenesis where six or more permanent teeth are absent) and colorectal cancer in a Finnish four-generation family. We reported in a previous study the preferential associations of certain dental anomalies with specific cleft subtypes (particularly tooth agenesis including hypodontia and oligodontia) to conclude that tooth agenesis can be considered an extended phenotype for CL/P; thus candidate genes for CL/P or tooth agenesis could provide clues in the search for genes for either condition. Hence our reason to consider *AXIN2* as a potential candidate gene for CL/P. Other CL/P genes (*MSX1*, *TGFA*, *FGFR1*, and *IRF6*) have also been associated with tooth agenesis to further support our hypothesis (Vieira et al., 2004, 2007). Variations in *AXIN2* have also been reported in association with certain types of cancer (Giles et al., 2003) and it has been suggested (although not proven) that children with congenital malformations, including CL/P, present an increased incidence of cancer (Bille et al., 2005).

In summary, little is known about the connection between *CDH1* and *AXIN2* genes and congenital malformations in humans. In our study, we observed only borderline results for the association of *CDH1* and *AXIN2* with CL/P with and without tooth agenesis. Nevertheless, implication of these genes in the simultaneous occurrence of CL/P and cancer, and tooth agenesis and cancer, is rather intriguing and warrants further investigations with other geographic and ethnic populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors are indebted to the participants of the study. This work was supported by CAPES/BEX Grant 3413/05-5 (Brazil) and NIH R21-DE016718.

Grant Information and Numbers: Ariadne Letra was supported by CAPES/BEX Grant 3413/05-5 (Brazil), Alexandre R. Vieira was supported by NIH R21-DE016718.

LITERATURE CITED

1. Abecasis GR, Cookson WOC. GOLD - Graphical Overview of Linkage Disequilibrium. *Bioinformatics* 2001;16:182–183. [PubMed: 10842743]
2. Bille C, Winther JF, Bautz A, Murray JC, Olsen J, Christensen K. Cancer risk in persons with oral cleft – a population-based study of 8,093 cases. *Am J Epidemiol* 2005;161:1047–1055. [PubMed: 15901625]
3. Frebourg T, Oliveira C, Hochain P, Karam R, Manouvrier S, Graziadio C, Vekemans M, Hartmann A, Baert-Desurmont S, Alexandre C, Lejeune Dumoulin S, Marroni C, Martin C, Castedo S, Lovett M, Winston J, Machado JC, Attié T, Jabs EW, Cai J, Pellerin P, Triboulet JP, Scotte M, Le Pessot F,

- Hedouin A, Carneiro F, Blayau M, Seruca R. Cleft lip/palate and *CDH1*/E-cadherin mutations in families with hereditary diffuse gastric cancer. *J Med Genet* 2006;43:138–142. [PubMed: 15831593]
4. Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta* 2003;1653:1–24. [PubMed: 12781368]
5. Gorlin RJ.; Cohen, MM. Syndromes of the Head and Neck. New York: Oxford University Press; 2001.
6. Hay ED. An overview of epithelio-mesenchymal transformation. *Acta Anat (Basel)* 1995;154:8–20. [PubMed: 8714286]
7. Juriloff DM, Harris MJ. Mouse genetic models of cleft lip with or without cleft palate. *Birth Defects Res A Clin Mol Teratol* 2008;82:63–77. [PubMed: 18181213]
8. Kerrigan JJ, Mansell JP, Sengupta A, Brown N, Sandy JR. Palatogenesis and potential mechanisms for clefting. *J R Coll Surg Edinb* 2000;45:351–358. [PubMed: 11153422]
9. Lammi L, Arte S, Somer M, Jarvinen H, Lahermo P, Thesleff I, Pirinen S, Nieminen P. Mutations in *AXIN2* cause familial tooth agenesis and predispose to colorectal cancer. *Am J Hum Genet* 2004;74:1043–1050. [PubMed: 15042511]
10. Letra A, Menezes R, Granjeiro JM, Vieira AR. Defining cleft subphenotypes based on dental development. *J Dent Res* 2007;86:986–991. [PubMed: 17890676]
11. Lidral AC, Moreno LM. Progress toward discerning the genetics of cleft lip. *Curr Opin Pediatr* 2005;17:731–739. [PubMed: 16282779]
12. Luning C, Rass A, Rozell B, Wroblewski J, Obrink B. Expression of E-cadherin during craniofacial development. *J Craniofac Genet Dev Biol* 1994;14:207–216. [PubMed: 7883867]
13. Mossey, PA.; Little, J. Epidemiology of oral clefts: an international perspective. In: Wyszynski, DF., editor. *Cleft lip and Palate. From origin to treatment*. New York: Oxford; 2002. p. 127–158.
14. Mostowska A, Biedziak B, Jagodzinski PP. Axis inhibition protein 2 (*AXIN2*) polymorphisms may be a risk factor for selective tooth agenesis. *J Hum Genet* 2006;51:262–266. [PubMed: 16432638]
15. Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, Pesich R, Hebert J, Chen YD, Dzau VJ, Curb D, Olshen R, Risch N, Cox DR, Botstein D. High-throughput genotyping with single nucleotide polymorphisms. *Genome Res* 2001;11:1262–1268. [PubMed: 11435409]
16. 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* 2007;104:4512–4517. [PubMed: 17360555]
17. Suzuki K, Hu D, Bustos T, Zlotogora J, Richieri-Costa A, Helms JA, Spritz RA. Mutations of *PVRL1*, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. *Nat Genet* 2000;25:427–430. [PubMed: 10932188]
18. Tonge CH. Identification of cell patterns in human tooth differentiation. *J Dent Res* 1967;46:876–878. [PubMed: 5234028]
19. Vastardis H. The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. *Am J Orthod Dentofacial Orthop* 2000;117:650–656. [PubMed: 10842107]
20. Vieira AR. Oral clefts and syndromic forms of tooth agenesis models for genetics of isolated tooth agenesis. *J Dent Res* 2003;82:162–165. [PubMed: 12598542]
21. Vieira AR, Meira R, Modesto A, Murray JC. *MSX1*, *PAX9*, and *TGFA* contribute to tooth agenesis in humans. *J Dent Res* 2004;83:723–727. [PubMed: 15329380]
22. Vieira AR, Avila JR, Daack-Hirsch S, Dragan E, Félix 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* 2005;1:e64. [PubMed: 16327884]
23. Vieira AR, Modesto A, Meira R, Barbosa Ar, Lidral AC, Murray JC. Interferon regulatory factor 6 (*IRF6*) and fibroblast growth factor receptor 1 (*FGFR1*) contribute to human tooth agenesis. *Am J Med Genet* 2007;143A:538–545. [PubMed: 17318851]
24. Zuccherro TM, Cooper ME, Maher Bs, Daack-Hirsch S, Nepomuceno B, Ribeiro L, Caprau D, Christensen K, Suzuki Y, Machida J, Natsume N, Yoshiura KI, Vieira AR, Orioli IM, Castilla EE, Moreno L, Arcos-Burgos M, Lidral AC, Field LL, Liu Y, Ray A, Goldstein T, 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 or palate. *New Engl J Med* 2004;351:769–780. [PubMed: 15317890]

Table 1

Details of the population, genes and polymorphisms studied.

Variables	Cleft		Control	
	N	%	N	%
Age				
Range	4–59	---	4–94	---
Mean	17.32	---	36.8	---
Gender				
Males	302	60	165	33
Females	198	40	335	67
Race				
Caucasian	406	82	285	58.2
African	79	16	38	7.8
Asian	9	2	167	34
Unknown	6	16	10	18
Total	500	100	500	100

Genes	Polymorphism	Gene region	Base position ^a	Base change
AXIN2	rs7591	5' untranslated region	60,955,294	A/T
	rs11867417	intron	60,968,110	C/T
	rs2240308	exon	60,984,803	A/G
CDH1	rs11642413	intron	67,347,645	A/G
	rs9929218	intron	67,378,197	A/G

Linkage Disequilibrium between Markers ^b				
AXIN2	rs7591	rs11867417	rs2240308	
rs7591	-----	0.636	0.193	
rs11867417	0.855	-----	0.223	
rs2240308	0.548	0.635	-----	
CDH1	rs11642413	rs9929218		
rs11642413	----	0.343		
rs9929218	0.830	----		

^a According to the USCS Genome Browser Human March 2006 Assembly.^b r² is above the diagonal; D' is below the diagonal.

Table 2

Results for association of *AXIN2* and *CDH1* genes with cleft subphenotypes with and without tooth agenesis^a. Numbers represent comparison between subphenotypes and controls.

Gene	Markers	Cleft Lip/Palate						Cleft Palate		
		Bilatera			Unilateral			Complete (n=15)	Incomplete (n=38)	Complete + Incomplete (n=53)
		Complete (n=101)	Incomplete (n=22)	Complete + incomplete (n=123)	Right (n=60)	Left (n=140)	Right + Left (n=200)			
AXIN2	rs7591									
	P genotype	0.83	0.66	0.93	0.57	0.88	0.71	0.26	0.006	0.13
	P allele	0.63	0.37	0.91	0.38	0.92	0.62	0.36	0.79	0.50
	rs11867417									
	P genotype	0.09	0.52	0.10	0.72	0.85	0.71	0.62	0.06	0.08
	P allele	0.02	0.68	0.03	0.43	0.57	0.42	0.31	0.20	0.11
	rs2240308									
	P genotype	0.88	0.77	0.88	0.24	0.28	0.12	0.38	0.09	0.33
	P allele	0.61	0.69	0.69	0.15	0.22	0.10	0.99	0.09	0.16
CDH1	rs11642413									
	P genotype	0.74	0.47	0.65	0.04	0.09	0.67	0.33	0.58	0.79
	P allele	0.56	0.16	0.37	0.93	0.05	0.40	0.14	0.46	0.87
	rs9929218									
	P genotype	0.37	0.38	0.64	0.08	0.08	0.37	0.78	0.42	0.68
	P allele	0.19	0.52	0.36	0.35	0.03	0.50	0.71	0.75	0.66

Gene	Markers	Cleft Subphenotypes with Tooth Agenesis					
		All Clefts (n=106)	CP (n=12)	CL/P (n=94)	CL/P right (n=24)	CL/P left (n=36)	CL/P bilateral (n=34)
AXIN2	rs7591						
	P genotype	0.28	0.48	0.15	0.01	0.66	0.62
	P allele	0.41	0.23	0.20	0.03	0.66	1.00
	rs11867417						
	P genotype	0.58	0.05	0.88	0.50	0.63	0.18
	P allele	0.94	0.87	0.90	0.23	0.35	0.08
CDH1	rs2240308						
	P genotype	0.32	0.62	0.17	0.10	0.28	0.36
	P allele	0.14	0.33	0.06	0.04	0.66	0.18
	rs11642413						
	P genotype	0.70	0.32	0.56	0.008	0.63	0.49
	P allele	0.63	0.95	0.60	0.46	0.88	0.57
CDH1	rs9929218						
	P genotype	0.52	0.86	0.56	0.04	0.80	0.93
	P allele	0.69	0.79	0.73	0.26	0.72	0.88

^aFor details, see Letra et al. (10)

* P-values, for genotype (top) and allele (bottom). Cleft subphenotypes without tooth agenesis were compared with controls (n=285). Cleft subphenotypes with tooth agenesis were compared with controls without tooth agenesis (n=252).