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Follow-Up Association Studies of Chromosome Region 9q and Nonsyndromic Cleft Lip/Palate

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Abstract

Cleft lip/palate comprises a large fraction of all human birth defects, and is notable for its significant lifelong morbidity and complex etiology. Several studies have shown that genetic factors appear to play a significant role in the etiology of cleft lip/palate. Human chromosomal region 9q21 has been suggested in previous reports to contain putative cleft loci. Moreover, a specific region (9q22.3-34.1) was suggested to present a ~45% probability of harboring a cleft susceptibility gene. Fine mapping of fifty SNPs across the 9q22.3-34.11 region was performed to test for association with cleft lip/palate in families from United States, Spain, Turkey, Guatemala, and China. We performed family-based analysis and found evidence of association of cleft lip/palate with *STOM* (rs306796) in Guatemalan families ($P=0.004$) and in all multiplex families pooled together ($P=0.002$). This same SNP also showed borderline association in the US families ($P=0.04$). Under a nominal value of 0.05, other SNPs also showed association with cleft lip/palate and cleft subgroups. SNPs in *STOM* and *PTCH* genes and nearby *FOXE1* were further associated with cleft phenotypes in Guatemalan and Chinese families. Gene prioritization analysis revealed *PTCH* and *STOM* ranking among the top

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Web Resources: Accession numbers and URLs for data presented herein are as follows:

Applied Biosystems, <http://www.appliedbiosystems.com/index.cfm>

Family Based Association Test, <http://www.biostat.harvard.edu/~fbat>

International HapMap Project, <http://hapmap.org>

Endeavour, <http://www.esat.kuleuven.be/endeavour>

Supplementary information is available at the American Journal of Medical Genetics website.

fourteen candidates for cleft lip/palate among 339 genes present in the region. Our results support the hypothesis that the 9q22.32-34.1 region harbors cleft susceptibility genes. Additional studies with other populations should focus on these loci to further investigate the participation of these genes in human clefting.

Keywords

cleft lip and palate; chromosome 9q; fine mapping; craniofacial defect

Introduction

Oral-facial clefts arise as a failure of facial embryonic processes to completely merge and/or fuse. They comprise a large fraction of all human birth defects, and may occur as part of single-gene Mendelian syndromes, as part of chromosomal abnormalities, or due to teratogen exposure [Murray, 2002]. Clefts of the lip and clefts of the palate are notable for their significant lifelong morbidity and complex etiology, and are considered different entities: cleft lip can occur with or without cleft palate (cleft lip/palate) while cleft palate may occur as an isolated defect [Fogh-Andersen, 1942]. Cleft lip/palate affects about 1/700 births with wide variability related to geographic origin and socioeconomic status. In general, Native American and Asian populations present the highest frequencies, sometimes at 1/500 or higher, followed by Caucasian, and African-derived populations showing the lowest frequencies around 1/2500 births [Mossey and Little, 2002].

Several studies have shown that genetic factors appear to play a significant role in the etiology of cleft lip/palate. In recent years, it has become evident that cleft lip/palate is heterogeneous, and different chromosome regions such as 1q, 2p, 4q, 6p, 14q, 17q, and 19q have been claimed to contain a clefting locus [Vieira, 2008]. Association of specific genes such as *MSX1* [Lidral et al., 1998; Beatty et al., 2001; Jezewski et al., 2003; Vieira et al., 2003], *IRF6* [Zuccherro et al., 2004; Ghassibe et al., 2005; Park et al., 2007; Vieira et al., 2007; Jia et al., 2009; Jugessur et al., 2009], *PVRL1* [Sozen et al., 2001; Avila et al., 2006], and genes of the *FGF* signaling pathway [Riley et al., 2007; Menezes et al., 2008] have been reported as well. Recently, genome-wide association studies with German, American and European populations have revealed a new major susceptibility locus for cleft lip/palate located in a gene desert region on chromosome 8q24 [Birnbaum et al., 2009; Grant et al., 2009; Nikopensus et al., 2009].

Human chromosomal region 9q21 has also been suggested in previous reports [Marazita et al., 2002; 2004; 2009], and in a mouse model [Juriloff et al., 2004] to contain putative cleft loci. A marker (D9S1122) located at 9q21.13 showed positive association with cleft lip/palate in Chinese multiplex families [Marazita et al., 2002]. Furthermore, a meta-analysis of a 10-cM genome scan of 388 extended multiplex families with cleft lip/palate from multiple populations revealed potential candidate genes in six chromosomal regions, including 9q21 (heterogeneity LOD score [HLOD] = 6.6). Positive association results (indicating close proximity to a clefting locus) were also found with four of the five selected candidate genes in the region [Marazita et al., 2004]. Moreover, the PPL (posterior probability of linkage) statistical test pointed towards a nearby region, from bands 9q22.3 to q34.1 (between 95Mb and 128Mb), as presenting ~45% probability of harboring a causative gene for clefting (Govil et al., unpublished observations). The *FOXE1* (forkhead box E1) gene, located in this region and implicated in syndromic cleft palate (Bamforth-Lazarus syndrome, OMIM 241850) has recently been reported to be associated with both isolated cleft lip with or without cleft palate and isolated cleft palate, and suggested as a key player in primary palatogenesis [Moreno et al., 2009].

To follow up on this data, we performed association studies with densely spaced markers spanning the 9q22.3-34.1 region to test for association with cleft lip/palate.

Materials and Methods

Subjects and Samples

Details of the population data sets are presented in Table I. Briefly, we assessed 291 multiplex cleft families (one or more members affected with an oral cleft) from the United States, Spain, Turkey, Guatemala and China. All cases had nonsyndromic cleft lip with or without cleft palate. Families were ascertained through probands, and additional relatives were recruited. Individuals presenting syndromic clefts, cleft palate only, or unknown cleft types, as well as control individuals presenting positive family history of clefting were excluded. This study was approved by local and the University of Pittsburgh institutional review boards. A signed informed consent sheet was obtained from all study participants after being explained the objectives and procedures of the study.

Blood or saliva samples were collected to obtain genomic DNA. DNA samples from the populations from China, Turkey, and United States recruited until 2005 were obtained from blood. DNA samples from the population from Guatemala, Spain, and United States recruited after 2005 were obtained from saliva. DNA extraction was performed according to previously published protocols [Trevillato and Line, 2000]. There was no difference in the performance of the samples obtained from blood or saliva in regards to our genotyping approach described below.

SNP Selection and Genotyping

We used the International HapMap Project Database to guide our selection of single nucleotide polymorphisms (SNPs) to be genotyped. We based our selection using the approach devised by Carlson et al. [Carlson et al., 2004], selecting from a set of SNPs which maximally represented the linkage disequilibrium structure of the 9q22.3-34.1 region. Fifty SNPs spanning the 9q22.3-34.1 region were selected (Figure I). Preference was given to tag SNPs with a minor allele frequency of at least 10%, taking into consideration the various ethnicities represented in this study.

Genotypes were generated using Taqman chemistry [Ranade et al., 2001] on an automatic sequence-detection instrument (ABI Prism 7900HT, Applied Biosystems, Foster City, CA). Assays and reagents were supplied by Applied Biosystems (Applied Biosystems, Foster City, CA). Details of the studied polymorphisms are presented in Table II.

Statistical Analyses

The transmission disequilibrium test was used to assess association of alleles at each marker with cleft lip with or without cleft palate with the use of the Family Based Association Test (FBAT) software [Horvath et al., 2001]. We also performed haplotype analyses using the “hbat” function of the FBAT software to identify if sets of markers were in linkage disequilibrium with cleft lip/palate. We also analyzed the entire dataset according to the following cleft subgroups: CLO + CLCLP (families where all affecteds have cleft lip only plus families where at least one affected has cleft lip only and one affected has cleft lip and palate, excluding any family where an affected has cleft palate only); and CLO + CLP + CLCLP (families where all affecteds have cleft lip only plus families where all affecteds have cleft lip and palate plus families where at least one affected has cleft lip only and one affected has cleft lip and palate, excluding any family where an affected has cleft palate only). Populations were analyzed individually and as a pooled dataset. P-values below 0.001 (0.05 divided by 50 markers) were considered statistically significant.

Gene Prioritization

We attempted to identify candidate genes present in the 9q22.3-34.1 region using the gene prioritization software Endeavour [Aerts et al., 2006; Tranchevent et al., 2008]. Endeavour is a web server that allows users to prioritize candidate genes with respect to their biological processes or diseases of interest. It relies on the similarity between candidate genes and models built with a set of manually inputted reference genes. We used 10 genes to compose the reference set: *IRF6*, *MSX1*, *PVRL1*, *TGFB3*, *CLPTM1*, *TGFA*, *FGFR1*, *RARA*, *BCL3*, and *TBX22*. We did not use *PTCH* or *FOXE1* as reference genes in the target region as they would be automatically removed by the program due to redundancy between reference and test sets of genes.

Results

Fine Mapping Association Studies

We genotyped fifty SNPs spanning 339 genes present in chromosomal region 9q22.3-q34.1. Detailed information on allele frequencies per population and additional replication analyses of specific markers are available as Supplemental Material. We performed family-based analysis to assess genotype and allelic associations between the investigated SNPs and cleft lip/palate. After correcting for multiple testing, we found borderline association of cleft lip/palate with a SNP in *STOM* (rs306796, 9q33.2) in Guatemalan families ($P=0.004$) and all families pooled together ($P=0.002$). This same SNP (rs306796) also showed a trend for association in the US population ($P=0.04$) (Table III). Of note, SNPs in or nearby *STOM* also showed modest association with cleft lip/palate in four of the five populations studied ($P=0.04$). Under a nominal value of 0.05, other SNPs also showed association with cleft lip/palate and cleft subgroups (Tables III and IV).

When analyzing families according to cleft subgroups, we found association of markers in *PTCH* (rs2234607, $P=0.004$; rs2297088, $P=0.01$; and rs10512248, $P=0.01$) and in/nearby *STOM* (rs306796, $P=0.01$; and rs2300934, $P=0.03$) with the CLO+CLCLP subgroup in Guatemalan families. We also found borderline association of markers nearby *FOXE1* in Chinese (rs2900463; $P=0.03$) and Guatemalan (rs1031111; $P=0.04$) families (Table IV). Results for the CLO +CLP +CLCLP groups did not differ from the association results in individual populations (data not shown).

We also performed haplotype analysis of the investigated SNPs by population (Table V) and by population according to cleft subgroup (Table VI). Altered transmission of haplotypes involving markers in/nearby *STOM* was evident in families from the US (rs1928623-rs2251495-rs2808416; $P=0.02$), Turkey (rs1928623-rs2251495-rs2808416-rs1928482; $P=0.02$), Guatemala (rs2300934-rs306796; $P=0.02$ and rs306796-rs10739600; $P=0.03$), and China (rs2300934-rs306796; $P=0.04$) (Table V). Altered transmission was also observed for *PTCH* rs357654-rs2236407-rs2297088 haplotype ($P=0.03$) and for were *PTCH* rs357654-rs2236407-rs2297088-rs10512248 haplotype ($P=0.02$) in Guatemalan families (Table V). When analyzed by cleft subgroup, interesting results were observed for haplotype markers involving *PTCH* (rs357654-rs2236407; $P=0.01$) and *PTCH* and *FOXE1* (rs10512248-rs894673) in families from Guatemala ($P=0.01$) and China ($P=0.03$) presenting CLO + CLCLP (Table VI).

Gene Prioritization

We found 339 genes within the 9q22.3-34.1 region with Endeavour (Supplementary Table I). The algorithm prioritized all genes within the 9q22.3-34.1 region in relation to cleft lip/palate. The highest priority gene according to Endeavour was *TGFBRI*. The associated genes,

PTCH and *STOM*, ranked in tenth and fourteenth places in the priority output, respectively. *FOXE1* ranked in 39th place. Detailed results are available as Supplemental Material.

Discussion

We investigated a region on chromosome 9q identified in previous studies as to harbor cleft susceptibility loci. Among the genes present in the region are *PTCH* and *FOXE1*, in which missense mutations and polymorphic variants have been described as having a role in cleft lip/palate [Marazita et al., 2004; 2009; Mansilla et al., 2006; Vieira et al., 2005; Moreno et al., 2009].

We performed fine mapping association studies with fifty SNPs across a 32.65 Mb region and multiple populations. Although using 50 SNPs to investigate 339 genes in a region may not seem a dense enough strategy, we used information on linkage disequilibrium of the markers to avoid choosing redundant markers. We found association of a polymorphism in *STOM* (rs306796) in the Guatemalan families ($P=0.004$) and in all families pooled together ($P=0.002$). *STOM* encodes stomatin, a membrane protein that was first isolated from human red blood cells [Zhang et al., 1999]. In mouse, the *STOM* homolog presents a wide pattern of expression, with high levels of mRNA in heart, liver, skeletal muscle, and testis but low levels in lung, brain, and spleen [Gallagher et al., 1995b]. Despite being associated with a variety of diseases such as cancer, kidney failure and anemia, precise functions of this protein remain unclear. In humans, the absence of stomatin is associated with a form of hemolytic anemia known as hereditary stomatocytosis [Yokoyama et al., 2006]. Lower expression of stomatin results in an increase in the basal rate of glucose transport [Zhang et al., 2001]. Interestingly, diabetic pregnant women are at an increased risk for having offspring with neural tube defects and oral-facial clefts [Spilson et al., 2001] and excessive exposure to glucose has been postulated to play a role in the pathogenesis of nonsyndromic cleft lip/palate [Krapels et al., 2004].

Suggestive evidence of association was also observed for several other markers among the populations implicating that the chromosome 9q region might contain cleft susceptibility genes ($P<0.05$). When analyzing the dataset according to cleft subgroups, we observed borderline association of *PTCH* variants (rs2236407, $P=0.004$; rs2297088, $P=0.01$; and rs10512248, $P=0.01$) in Guatemalan families with CLO + CLCLP (families where all affecteds have cleft lip only plus families where at least one affected has cleft lip only and one affected has cleft lip and palate). *PTCH* was initially suggested as a candidate for human clefting because of its mouse homolog located in the candidate region *clf2* [Juriloff et al., 2001]. Although no causal mutation in *Ptch* coding sequence was found in mice, alterations in the regulatory sequence are not ruled out. Given the “normal” phenotype of noncleft A/~ strain mice, a mild regulatory defect seems likely if *Ptch* is *clf2* [Juriloff et al., 2004]. In humans, mutations in *PTCH* cause Gorlin syndrome with diverse developmental anomalies, often including rib and craniofacial abnormalities (and cleft palate) and a mixture of tumor types [Cohen, 1999]. In their study with *PTCH* and isolated cleft lip/palate, Mansilla et al. sequenced all 23 exons of the gene and found seven new normal variants spread along the entire gene and three missense mutations in cases with cleft lip/palate, one of which was not found in 1,188 control samples [Mansilla et al., 2006]. Although the authors did not find statistically significant evidence of linkage (multipoint HLOD peak=2.36), they reported over-transmission of the *PTCH* rs2297088–rs2236407 haplotype with borderline statistical significance ($P=0.08$) in Filipino families with two or more affected members. They further concluded that missense mutations in *PTCH* may be rare causes of isolated cleft lip/palate and yet unidentified variants near *PTCH* may act as modifiers of the cleft phenotype. Corroborating with these findings, we observed significant evidence of transmission distortion for the *PTCH* rs357654–rs2236407–rs2297088 ($P=0.03$) and rs357654–rs2236407–rs2297088–rs10512248 ($P=0.02$) haplotypes in Guatemalan families.

It is unknown whether the associated *PTCH* polymorphisms (rs2236407 and rs2297088) located in introns and therefore not in coding regions or splice sites might cause a functional change in the final protein. Notwithstanding, intronic polymorphisms have been demonstrated in association with other complex diseases, including the association of *IRF6* with cleft lip/palate [Zuccherro et al., 2004; Ghassibe et al., 2005; Park et al., 2007; Vieira et al., 2007; Jia et al., 2009; Jugessur et al., 2009] and thus should not be disregarded as potentially damaging. Of note, in a recent association study with genes in chromosome 9q, *PTCH* SNP rs2297088 showed the strongest signal for Filipino families, based on the lowest p-value (P-value=6.49E-03), indicating that *PTCH* may have a possible role in the etiology of cleft lip with or without cleft palate in some populations.

A previous study from our group has shown that point mutations in *FOXE1* may be rare causes for isolated cleft lip/palate [Vieira et al., 2005]. Following studies have shown positive association and linkage between *FOXE1* and cleft lip/palate [Marazita et al., 2004; 2009], including a recent publication which identified *FOXE1* in strong association with both isolated cleft lip with or without cleft palate and isolated cleft palate [Moreno et al., 2009]. We did not find significant association of *FOXE1* with cleft phenotypes in the populations tested although suggestive association was observed for variants in/nearby *FOXE1* in Chinese and Guatemalan families with CLO + CLCLP (P=-0.03 and P=0.04, respectively). *FOXE1* belongs to a family of fork-head domain-genes involved in embryonic pattern formation, which have been identified as factors that bind to regulatory elements in mammalian genes expressed in terminally differentiated cells [Kaestner et al., 1993]. *Foxe1* null mice exhibit cleft palate and either a sublingual or completely absent thyroid gland [De Felice et al., 1998]. In humans, mutations in *FOXE1* result in the Bamforth syndrome, characterized by thyroid agenesis, cleft palate, spiky hair, and choanal atresia [Clifton-Bligh et al., 1998]. It is possible that variants in this gene may be in linkage disequilibrium with variants in other genes that jointly increase the susceptibility to cleft lip/palate. For example, we found overtransmission of *PTCH-FOXE1* haplotypes in families Guatemalan and Chinese families presenting CLO + CLCLP (P=0.01 and P=0.03, respectively).

Using gene prioritization software, we identified 339 genes in the 9q22.3-34.11 candidate region, which were ranked based on similar roles or participation in similar biological processes with genes known to be associated with cleft lip/palate. Other investigators have used this gene prioritization approach as well, in studies of obesity and type II diabetes genes [Elbers et al., 2007; Sookoian et al., 2009], idiopathic pulmonary fibrosis [Tzoulevekis et al., 2007] and even cleft lip/palate [Osoegawa et al., 2008]. This approach however has the limitation of not allowing any control on the quality of gene annotation and quantity and quality of information across genes in different databases. It is difficult to predict how much these aspects influenced our results and the results of others but at minimal, this information adds to the bulk of the results and help interpreting the association data. In comparison with a reference set of 11 established candidate genes for human clefting, *PTCH* and *STOM* ranked in tenth and fourteenth places, respectively, among the 339 genes present in the investigated region, thus at the top five percent ranking as candidates for cleft lip/palate. These observations taken together with the association findings, suggest that those genes may be considered plausible candidates for cleft lip/palate. Meanwhile, we checked for conservation of the associated SNPs, and found that the wild-type nucleotide is conserved in the following species: rhesus monkey, dog and horse for rs2297088 in *PTCH*, and dog and horse for rs306796 in *STOM*.

In summary, we performed fine mapping analysis of chromosome 9q22.32-34.1 region previously suggested to harbor cleft susceptibility genes. Our association results support a role for *PTCH* as contributor for cleft lip/palate and suggest *STOM* as a possible new candidate gene. Haplotype and gene prioritization analyses confirmed the individual association findings with *PTCH* and *STOM*, ranking in the top five percent of the highest priority candidate genes

for cleft lip/palate. We failed to replicate previous findings suggesting *FOXE1* contributes to nonsyndromic cleft lip and palate. Additional studies with other populations should focus on these loci to further investigate the participation of these genes in human clefting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure I.

Overview of chromosome 9. The lighter box in the detail indicates the investigated region (9q22.32-34.11), spanning from 95Mb to 128Mb (Obtained from HapMap International Project, available at <http://www.hapmap.org>). Solid black bars below chromosome structure represent genes in region. Outlined gray bars below black bars indicate number of single nucleotide polymorphisms in the region.

Table 1

Summary of families and individuals ascertained for the study.

Group	Ancestral Origin	No. of Families	No. of Individuals	Affected Individuals	Unaffected Individuals	Affected Individuals by Phenotype	
						CL ¹	CL/P ²
Families							
North America	US	89	529	145	384	39	106
Central America	Guatemala	77	514	93	421	20	73
Europe	Spain	36	136	43	93	10	33
	Turkey	29	288	38	250	17	21
East Asia	China	60	180	60	120	14	46
TOTAL		291	1647	379	1268	100	279

¹ CL, cleft lip;

² CL/P, cleft lip with or without cleft palate.

Table II

Details of the SNPs investigated in the study.

SNP No.	SNP name	Chromosome	Region	Base Position ¹	Mapping Gene (SNP Location)	Mapping Gene	Function	Base Change
1	rs357564	9q22.32		97249415	<i>PTCH</i>	<i>PTCH</i>	coding-nonsynonymous	AG
2	rs2236407	9q22.32		97277617	<i>PTCH</i>	<i>PTCH</i>	intron/next to exon	AG
3	rs2297088	9q22.32		97282805	<i>PTCH</i>	<i>PTCH</i>	intron/next to exon	AG
4	rs10512248	9q22.32		97299524	<i>PTCH</i>	<i>PTCH</i>	intron	AC
5	rs894673	9q22.33		99652091	<i>FOXEl</i>	<i>FOXEl</i>	near gene 5' UTR	AT
6	rs3758249	9q22.33		99653961	<i>FOXEl</i>	<i>FOXEl</i>	near gene 5' UTR	AG
7	rs10984103	9q22.33		99679096	intergenic	C9orf156	unknown	AC
8	rs2900463	9q22.33		100017278	<i>TBC1D2</i>	<i>TBC1D2</i>	intron	CT
9	rs2808557	9q22.33		100283185	<i>GPR51</i>	<i>GPR51</i>	intron/next to exon	GT
10	rs1031111	9q22.33		101201619	intergenic	intergenic	unknown	AG
11	rs1526267	9q31.1		101629566	<i>NR4A3</i>	<i>NR4A3</i>	5' UTR	AG
12	rs1226588	9q31.1		102380835	<i>TMEFF1</i>	<i>TMEFF1</i>	intron	CT
13	rs1448576	9q31.1		105023498	<i>340511</i>	<i>340511</i>	unknown	AG
14	rs1992917	9q31.1		105029464	<i>340511</i>	<i>340511</i>	unknown	AG
15	rs1993434	9q31.1		105074129	intergenic	intergenic	unknown	AG
16	rs2014343	9q31.3		112899132	intergenic	intergenic	unknown	CG
17	rs4979032	9q31.3		113466796	intergenic	intergenic	intron	AG
18	rs2762475	9q32		113928337	<i>SUSD1</i>	<i>SUSD1</i>	intron	CT
19	rs1999263	9q32		114249494	<i>HSDL2</i>	<i>HSDL2</i>	intron	AC
20	rs10739367	9q32		114406899	intergenic	intergenic	intron	CT
21	rs1539339	9q32		114633550	<i>SNX30</i>	<i>SNX30</i>	intron/next to exon	AC
22	rs818714	9q32		115223122	<i>C9orf43</i>	<i>C9orf43</i>	intron/next to exon	CT
23	rs1008138	9q32		115849291	<i>ZNF618</i>	<i>ZNF618</i>	intron/next to exon	GT
24	rs942519	9q32		116208854	<i>DFNB31</i>	<i>DFNB31</i>	coding-nonsynonymous	AG
25	rs2992147	9q33.1		116889135	<i>TNC</i>	<i>TNC</i>	coding-synonymous	CT
26	rs971037	9q33.1		117706363	<i>C9orf27</i>	<i>C9orf27</i>	intron/next to exon	AT
27	rs1888636	9q33.1		117979155	<i>PAPPA</i>	<i>PAPPA</i>	intron	AT
28	rs1418495	9q33.1		120130612	intergenic	intergenic	unknown	AG
29	rs1335256	9q33.1		120366850	intergenic	intergenic	unknown	CT

SNP No.	SNP name	Chromosome Region	Base Position ^I	Mapping Gene (SNP Location)	Mapping Gene	Function	Base Change
30	rs7859743	9q33.2	122196412	intergenic	intergenic	intron/next to exon	AG
31	rs2300934	9q33.2	122848784	intergenic	intergenic	intron/next to exon	AC
32	rs306796	9q33.2	123167156	<i>STOM</i>	<i>STOM</i>	intron	CT
33	rs10739600	9q33.2	123663694	<i>TTLL11</i>	<i>TTLL11</i>	unknown	AG
34	rs7853089	9q33.2	123972807	<i>C9orf18</i>	<i>C9orf18</i>	intron	GT
35	rs1928623	9q33.2	124348830	<i>ORIN2</i>	<i>ORIN2</i>	unknown	AC
36	rs2251495	9q33.2	124682508	<i>MNAB</i>	<i>MNAB</i>	intron/next to exon	CT
37	rs2808416	9q33.2	125159980	<i>CRB2</i>	<i>CRB2</i>	intron	AG
38	rs1928482	9q33.2	125472269	<i>DENND1A</i>	<i>DENND1A</i>	intron	CT
39	rs2274782	9q33.3	126123895	intergenic	intergenic	intron/next to exon	CT
40	rs10739650	9q33.3	126363597	<i>NR6A1</i>	<i>NR6A1</i>	intron	CT
41	rs646527	9q33.3	126717053	<i>GOLGA1</i>	<i>GOLGA1</i>	intron	CT
42	rs7853181	9q33.3	127262845	<i>MAPKAP1</i>	<i>MAPKAP1</i>	intron	AG
43	rs10760403	9q33.3	127648316	<i>PBX3</i>	<i>PBX3</i>	intron	GT
44	rs10987185	9q33.3	127981942	intergenic	intergenic	unknown	CG
45	rs887659	9q33.3	128224130	<i>C9orf28</i>	<i>C9orf28</i>	intron/next to exon	AG
46	rs3850585	9q33.3	128476093	<i>LMX1B</i>	<i>LMX1B</i>	intron	CT
47	rs1890546	9q33.3	128991962	intergenic	intergenic	intron	CT
48	rs7869023	9q33.3	129241499	<i>RPL12</i>	<i>RPL12</i>	intron	AG
49	rs913989	9q34.1	129572809	<i>SH2D3C</i>	<i>SH2D3C</i>	intron/next to exon	AC
50	rs10819354	9q34.1	129893311	<i>SLC25</i>	<i>SLC25</i>	intron, near 5' UTR	AG

^I According to the UCSC Genome Browser March 2006 Assembly.

Table III

Summary of results for association tests with markers in the chromosome 9q region and cleft lip/palate in the studied populations.

SNP Name	UCSC base position ¹	Mapping Gene (SNP Location)	Family-Based Analyses (P-values ²)						
			Individual Populations				Pooled Populations		
			USA	Guatemala	Spain	Turkey	China		
rs357564	95,289,149	<i>PTCH</i>	0.40	0.19	0.49	0.74	0.50	0.09	
rs2236407	95,317,351	<i>PTCH</i>	0.49	0.16	0.40	0.64	1.00	0.52	
rs2297088	95,322,539	<i>PTCH</i>	0.66	0.64	0.71	0.64	0.89	0.87	
rs10512248	95,339,258	<i>PTCH</i>	0.75	0.16	1.00	0.64	0.58	0.90	
rs894673	97,691,825	<i>FOXE1</i>	0.85	0.93	0.48	0.82	0.19	0.58	
rs3758249	97,693,695	<i>FOXE1</i>	0.60	0.93	0.43	0.65	0.26	0.71	
rs10984103	97,718,830	C9orf156	0.89	0.86	0.27	0.67	0.24	0.52	
rs2900463	98,057,012	<i>TBC1D2</i>	0.63	0.52	0.87	0.06	0.18	0.11	
rs2808557	98,322,919	<i>GPR51</i>	0.93	0.47	0.78	0.53	0.18	0.62	
rs1031111	99,241,353	intergenic	0.66	0.85	0.55	1.00	0.71	0.99	
rs1526267	99,669,300	<i>NR4A3</i>	0.49	0.65	0.05	0.12	0.48	0.79	
rs1226588	100,420,569	<i>TMEFF1</i>	0.06	0.88	0.74	0.37	0.66	0.25	
rs1448576	103,063,232	<i>340511</i>	0.56	0.65	1.00	0.68	0.04	0.13	
rs1992917	103,069,198	<i>340511</i>	0.68	0.89	0.86	0.70	0.28	0.84	
rs1993434	103,113,863	intergenic	0.92	0.66	0.42	0.84	0.76	0.59	
rs2014343	110,938,866	intergenic	0.31	0.01	0.49	0.03	0.28	0.01	
rs4979032	111,506,520	intergenic	0.89	0.39	0.74	0.39	0.58	0.50	
rs2762475	111,968,071	<i>SUSD1</i>	0.47	0.84	0.49	1.00	0.79	0.89	
rs1999263	112,289,228	<i>HSDL2</i>	0.71	0.20	1.00	0.25	0.15	0.97	
rs10739367	112,446,633	intergenic	0.97	0.26	0.59	0.67	0.65	0.87	
rs1539339	112,673,284	<i>SNX30</i>	0.94	0.83	0.44	1.00	0.09	0.25	
rs818714	113,262,855	<i>C9orf43</i>	0.12	0.53	0.39	0.64	0.86	0.16	
rs1008138	113,889,024	<i>ZNF618</i>	0.46	0.14	0.12	0.80	0.63	0.25	
rs942519	114,248,587	<i>DFNB31</i>	0.80	0.18	0.45	0.47	0.53	0.52	
rs2992147	114,928,868	<i>TNC</i>	0.33	0.78	0.85	0.37	0.88	0.37	

SNP Name	UCSC base position ¹	Mapping Gene (SNP Location)	Family-Based Analyses (P-values ²)						
			Individual Populations				Pooled Populations		
			USA	Guatemala	Spain	Turkey	China		
rs971037	115,746,096	<i>C9orf27</i>	0.81	0.78	0.52	0.09	0.56	0.56	
rs1888636	116,018,888	<i>PAPPA</i>	0.06	0.61	0.43	0.81	0.89	0.39	
rs1418495	118,170,345	intergenic	0.04	0.91	0.42	0.68	0.53	0.27	
rs1335256	118,406,583	intergenic	0.69	0.63	0.71	0.82	0.39	0.65	
rs7859743	120,236,145	intergenic	0.75	0.18	0.04	0.37	0.79	0.19	
rs2300934	120,888,517	intergenic	0.52	0.04	0.89	0.39	0.04	0.91	
rs306796	121,206,889	<i>STOM</i>	0.04	0.004	0.26	0.41	0.06	0.002	
rs10739600	121,703,427	<i>TTL11</i>	0.92	0.93	0.49	0.82	0.09	0.82	
rs7853089	122,012,540	<i>C9orf18</i>	0.44	0.45	0.87	0.22	0.81	0.73	
rs1928623	122,388,563	<i>ORIN2</i>	0.71	0.17	0.41	0.25	0.16	0.06	
rs2251495	122,722,241	<i>MNAB</i>	0.18	0.92	1.00	0.32	0.89	0.73	
rs2808416	123,199,713	<i>CRB2</i>	0.51	0.25	0.21	0.83	0.74	0.55	
rs1928482	123,512,002	<i>DENND1A</i>	0.03	0.41	0.39	0.24	0.65	0.24	
rs2274782	124,163,628	intergenic	0.32	0.44	0.39	0.13	0.28	0.95	
rs10739650	124,403,330	<i>NR6A1</i>	0.21	0.15	0.56	0.20	0.37	0.94	
rs646527	124,756,786	<i>GOLGA1</i>	0.67	0.40	0.95	0.32	0.22	0.78	
rs7853181	125,302,578	<i>MAPKAP1</i>	0.56	0.32	0.49	0.58	0.69	0.95	
rs10760403	125,688,049	<i>PBX3</i>	0.52	0.90	0.65	0.41	0.54	0.90	
rs10987185	126,021,675	intergenic	0.42	0.42	0.71	0.81	0.34	0.64	
rs887659	126,263,863	<i>C9orf28</i>	0.85	0.63	0.83	0.49	0.47	0.82	
rs3850585	126,515,826	<i>LMX1B</i>	0.95	0.73	0.21	0.62	0.79	0.64	
rs1890546	127,031,695	intergenic	0.47	0.22	0.20	0.43	0.22	0.64	
rs7869023	127,281,232	<i>RPL12</i>	0.58	0.65	0.05	0.65	0.17	0.09	
rs913989	127,612,542	<i>SH2D3C</i>	0.52	0.22	0.20	0.67	0.41	0.68	
rs10819354	127,933,044	<i>SLC25</i>	0.27	0.37	0.13	0.49	0.05	0.87	

¹ According to the UCSC Genome Browser March 2006 Assembly.

² Family-Based Association Test.

Table IV

Summary of results for association tests with markers in the chromosome 9q region and families with cleft lip only plus cleft lip and palate in Guatemala and China.

CLO + CL,CLP^I			
		P-values	
SNP Name	SNP Location	Guatemala	China
rs357564	<i>PTCH</i>	0.41	--
rs2236407	<i>PTCH</i>	0.004	0.11
rs2297088	<i>PTCH</i>	0.01	0.11
rs10512248	<i>PTCH</i>	0.01	0.07
rs894673	<i>FOXE1</i>	0.07	0.05
rs3758249	<i>FOXE1</i>	0.13	0.05
rs10984103	<i>C9orf156</i>	0.07	0.16
rs2900463	<i>TBC1D2</i>	0.78	0.03
rs2808557	<i>GPR51</i>	0.16	0.16
rs1031111	intergenic	0.04	0.11
rs1526267	<i>NR4A3</i>	0.87	0.76
rs1226588	<i>TMEFF1</i>	0.05	0.25
rs1448576	<i>340511</i>	0.28	0.32
rs1992917	<i>340511</i>	0.94	0.05
rs1993434	intergenic	0.80	0.76
rs2014343	intergenic	0.05	0.81
rs4979032	intergenic	0.84	0.32
rs2762475	<i>SUSD1</i>	0.18	0.76
rs1999263	<i>HSDL2</i>	0.28	0.64
rs10739367	intergenic	0.32	0.44
rs1539339	<i>SNX30</i>	0.34	0.03
rs818714	<i>C9orf43</i>	0.26	0.56
rs1008138	<i>ZNF618</i>	0.13	0.29
rs942519	<i>DFNB31</i>	0.43	0.48
rs2992147	<i>TNC</i>	1.00	0.71
rs971037	<i>C9orf27</i>	0.41	0.83
rs1888636	<i>PAPPA</i>	0.49	0.71
rs1418495	intergenic	0.55	0.11
rs1335256	intergenic	0.83	0.37
rs7859743	intergenic	0.64	0.76
rs2300934	intergenic	0.03	0.53
rs306796	<i>STOM</i>	0.01	0.32
rs10739600	<i>TLL11</i>	1.00	1.00
rs7853089	<i>C9orf18</i>	0.49	0.65

CLO + CL,CLP ¹			
SNP Name	SNP Location	P-values	
		Guatemala	China
rs1928623	<i>OR1N2</i>	0.49	--
rs2251495	<i>MNAB</i>	0.41	0.48
rs2808416	<i>CRB2</i>	0.07	0.74
rs1928482	<i>DENND1A</i>	0.20	1.00
rs2274782	intergenic	0.74	0.76
rs10739650	<i>NR6A1</i>	0.65	1.00
rs646527	<i>GOLGA1</i>	1.00	0.29
rs7853181	<i>MAPKAP1</i>	0.94	0.74
rs10760403	<i>PBX3</i>	0.18	0.08
rs10987185	intergenic	0.65	0.48
rs887659	<i>C9orf28</i>	0.13	0.10
rs3850585	<i>LMX1B</i>	0.20	0.17
rs1890546	intergenic	0.22	1.00
rs7869023	<i>RPL12</i>	0.83	0.37
rs913989	<i>SH2D3C</i>	1.00	0.32
rs10819354	<i>SLC25</i>	0.40	0.74

¹ CLO + CL,CLP, families where all affecteds have cleft lip only plus families where at least one affected has cleft lip only and one affected has cleft lip and palate. Excludes any family where an affected has cleft palate only.

Table V

Results of haplotype transmission analysis in families with cleft lip with or without cleft palate by population.

Marker Haplotype	Genes in Haplotype	Population	HBAT P-value ²
rs1928623-rs2251495-rs2808416	Intergenic-MNAB (~1Mb of STOM)- CRB2	US	0.02
rs1888636-rs1418495-rs1335256-rs7859473	PAPPA-intergenic-intergenic-CDK5RAP2	US	0.02
rs7869023-rs913989	ZNF79-SH2D3C	Spain	0.03
rs1928623-rs2251495-rs2808416-rs1928482	Intergenic-MNAB (~1Mb of STOM)- CRB2-KIAA1608	Turkey	0.02
rs2014343-rs4979032	Intergenic-GNG10	Guatemala	0.04
rs357654-rs2236407-rs2297088	PTCH-PTCH-PTCH	Guatemala	0.03
rs357654-rs2236407-rs2297088-rs10512248	PTCH-PTCH-PTCH-PTCH	Guatemala	0.02
rs2300934-rs306796	C5-STOM	Guatemala	0.03
rs306796-rs10739600	STOM-TTLL11 (~500kb of STOM)	Guatemala	0.03
rs306796-rs10739600-rs7853089-rs1928623	STOM-TTLL11 (~500kb of STOM)-C9orf18 (~800kb of STOM)	Guatemala	0.02
rs2300934-rs306796	C5-STOM	China	0.04
rs306796-rs10739600-rs7853089-rs1928623	STOM-TTLL11 (~500kb of STOM)-C9orf18 (~800kb of STOM)	China	0.04

¹ Comprises analyses of US, Spain, and Turkey families pooled together.

² Transmission distortion significant when $p < 0.05$.

Table VI

Results of haplotype transmission analysis according to cleft subgroup by population.

Marker Haplotype	Genes in Haplotype	Cleft Group	Population	HBAT P-value ²
rs357654-rs2236407	PTCH-PTCH	CLO + CLCLP ¹	Guatemala	0.01
rs10512248-rs894673	PTCH-FOXE1	CLO + CLCLP	Guatemala	0.01
rs2297088-rs10512248-rs894673	PTCH-PTCH-FOXE1	CLO + CLCLP	Guatemala	0.01
rs2236407-rs2297088-rs10512248-rs894673	PTCH-PTCH-PTCH-FOXE1	CLO + CLCLP	Guatemala	0.01
rs10512248-rs894673	PTCH-FOXE1	CLO + CLCLP	China	0.03
rs10512248-rs894673-rs3758249	PTCH-FOXE1-FOXE1	CLO + CLCLP	China	0.03
rs10512248-rs894673-rs3758249-rs10984103	PTCH-FOXE1-FOXE1-C9orf156	CLO + CLCLP	China	0.03

¹ CLO + CLCLP, families where all affecteds have cleft lip only plus families where at least one affected has cleft lip only and one affected has cleft lip and palate. Excludes any family where an affected has cleft palate only.

² Transmission distortion significant when $p < 0.05$.