Published in final edited form as:
*Birth Defects Res A Clin Mol Teratol*. 2010 February ; 88(2): 84–93. doi:10.1002/bdra.20639.

# Testing reported associations of genetic risk factors for oral clefts in a large Irish study population

**Tonia C. Carter**[1], **Anne M. Molloy**[2], **Faith Pangilinan**[3], **James F. Troendle**[1], **Peadar N. Kirke**[4], **Mary R. Conley**[1], **David J. A. Orr**[5], **Michael Earley**[5], **Eamon McKiernan**[5], **Ena C. Lynn**[4], **Anne Doyle**[4], **John M. Scott**[2], **Lawrence C. Brody**[3], and **James L. Mills**[1]

[1]Division of Epidemiology, Statistics, and Prevention Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland [2]School of Immunology and Biochemistry, Trinity College, Dublin, Ireland [3]Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland [4]Child Health Epidemiology Unit, Health Research Board of Ireland, Dublin, Ireland [5]Dublin Cleft Centre, St. James's Hospital and Our Lady's Children's Hospital, Dublin, Ireland

## Abstract

**BACKGROUND—**Suggestive, but not conclusive, studies implicate many genetic variants in oral cleft etiology. We used a large, ethnically homogenous study population to test whether reported associations between nonsyndromic oral clefts and 12 genes (*CLPTM1*, *CRISPLD2*, *FGFR2*, *GABRB3*, *GLI2*, *IRF6*, *PTCH1*, *RARA*, *RYK*, *SATB2*, *SUMO1*, *TGFA*) could be confirmed.

**METHODS—**Thirty-one single nucleotide polymorphisms (SNPs) in exons, splice sites, and conserved non-coding regions were studied in 509 patients with cleft lip with or without cleft palate (CLP), 383 with cleft palate only (CP), 838 mothers and 719 fathers of patients with oral clefts, and 902 controls from Ireland. Case-control and family-based statistical tests were performed using isolated oral clefts for the main analyses.

**RESULTS—**In case-control comparisons, the minor allele of *PTCH1* A562A (rs2066836) was associated with reduced odds of CLP (OR: 0.29, 95% CI: 0.13–0.64 for homozygotes) whereas the minor allele of *PTCH1* L1315P (rs357564) was associated with increased odds of CLP (OR: 1.36, 95% CI: 1.07–1.74 for heterozygotes and OR: 1.56, 95% CI: 1.09–2.24 for homozygotes). The minor allele of one *SUMO1* SNP, rs3769817 located in intron 2, was associated with increased odds of CP (OR: 1.45, 95% CI: 1.06–1.99 for heterozygotes). Transmission disequilibrium was observed for the minor allele of *TGFA* V159V (rs2166975) which was over-transmitted to CP cases ($P$=0.041).

**CONCLUSIONS—**For 10 of the 12 genes, this is the largest candidate gene study of nonsyndromic oral clefts to date. The findings provide further evidence that *PTCH1*, *SUMO1*, and *TGFA* contribute to nonsyndromic oral clefts.

Tonia Carter, PhD, Division of Epidemiology, Statistics, and Prevention Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, 6100 Executive Blvd, Room 7B03C MSC 7510, Bethesda, MD 20892-7510, Tel: (301) 435–7597, Fax: (301) 402–2084, carterto@mail.nih.gov.

Presented in poster form at the 22nd annual meeting of the Society for Pediatric and Perinatal Epidemiologic Research, June 22–23, 2009, Anaheim, California.

### Keywords

cleft lip; cleft palate; congenital abnormalities

## INTRODUCTION

Oral clefts are among the most common birth defects. Population-based estimates of the prevalence of nonsyndromic cleft lip with or without cleft palate (CLP) and cleft palate only (CP) are 4–13/10,000 births and 2–5/10,000 births, respectively, with worldwide estimates varying by ethnicity and geographic region (Hashmi et al., 2005). Research suggests that multiple genes are involved in oral clefts etiology (Schliekelman and Slatkin, 2002) and that various signaling pathways are important in lip and palate development (Marazita and Mooney, 2004), but the specific genes in these pathways that play a role in causing oral clefts in humans remain unknown. There have been consistent findings of an association with nonsyndromic oral clefts for only one gene, interferon regulatory factor 6 (*IRF6*), however this gene does not account for the majority of the genetic contribution to nonsyndromic oral clefts and more genes remain to be identified. Studies using a variety of approaches have produced inconclusive or conflicting results, possibly because of inadequate power and population diversity. To address these limitations we have assembled a large, ethnically homogenous study population in Ireland to examine genetic risk factors for oral clefts. The intent of the current study was to follow-up on previously published reports of genes examined for an association with oral clefts in humans and to confirm whether an association was present in an independent study population. Therefore, we performed case-control and trio-based analyses to examine whether selected SNPs in genes that were previously suggested to be risk factors for oral clefts were associated with nonsyndromic CLP and CP.

## MATERIALS AND METHODS

### Cases

Study subject enrollment and data collection have been described in detail previously (Mills et al., 2008). Subjects with CLP or CP were identified by one of two methods. First, patients at the Dublin Cleft Centre which treats most patients with oral clefts (80%) in Ireland, were recruited. The attending surgeons examined all patients, reported a diagnosis of CLP or CP, and confirmed the presence or absence of other birth defects. Second, patients were identified through membership in the Cleft Lip and Palate Association of Ireland, a patient support group. Medical record data on the diagnosis of oral clefts were available for 87% of all patients. Recruited case-parent trios gave either blood or buccal samples. Study participants, or in the case of minors, their parents, provided written informed consent. The Research Ethics Committees of the Health Research Board of Ireland, the participating hospitals, and the Institutional Review Board of the National Human Genome Research Institute gave ethical approval for the study.

### Controls

Blood samples had previously been collected from 56,049 pregnant women who had attended any of the three main maternity hospitals in Dublin between 1986 and 1990. These hospitals deliver approximately 90% of Dublin births and about one-third of all Irish births occur in Dublin. Controls were a random sample (N=926) from this group. Women were not eligible to be controls if they had delivered a child with a known birth defect or if they had previously had a child with a neural tube defect. All the genes selected for this study are located on autosomes and, among the controls, allele frequencies for genes on the autosomes

reflect those of the underlying Irish population (Parle-McDermott et al., 2003; Kirke et al., 2004).

## Genes and SNPs

We selected 11 candidate genes that have been implicated in previous studies that produced suggestive, but not conclusive, evidence that they were associated with nonsyndromic oral clefts. We attempted to focus on genes that did not have a large body of published literature concerning their association with oral clefts as well as genes with previous controversial findings related to oral clefts. Genes were chosen if there were a) any reports of mutations or chromosomal breakage within the gene in patients with oral clefts; b) expression or gene-knockout data from animal studies linking the gene to oral clefts; or c) previous conflicting reports concerning their associations with oral clefts. Also, either the gene or the chromosomal region in which it is located had to have been examined in at least one human study. The selected genes were cleft lip and palate associated transmembrane protein 1 (*CLPTM1*), cysteine-rich secretory protein LCCL domain containing 2 (*CRISPLD2*), fibroblast growth factor receptor 2 (*FGFR2*), gamma-aminobutyric acid A receptor, beta 3 (*GABRB3*), GLI-Kruppel family member GLI2 (*GLI2*), patched homolog 1 (*Drosophila*) (*PTCH1*), retinoic acid receptor alpha (*RARA*), RYK receptor-like tyrosine kinase (*RYK*), SATB homeobox 2 (*SATB2*), SMT3 suppressor of mif two 3 homolog 1 (*Saccharomyces cerevisiae*) (*SUMO1*), and transforming growth factor alpha (*TGFA*). In addition to the 11 genes, *IRF6* was also included in this study to investigate gene-gene interactions and because the gene has not been examined in the Irish population.

SNPs were selected for study based on published reports and/or their locations in the genes. Our aim was to target SNPs that might affect function rather than to SNP-tag or examine all the SNPs within these genes. Thirty-one SNPs were selected located in exons, splice sites, or conserved non-coding regions, locations in which nucleotide changes could have functional consequences. SNPs in conserved regions were identified from multi-species alignments of DNA sequences using the University of California at Santa Cruz Genome Browser (http://genome.ucsc.edu/).

## Genotyping

Genomic DNA was extracted from blood or buccal samples using a QIAamp DNA Blood Mini Kit (Qiagen, Sussex, UK). KBiosciences (Herts, United Kingdom) performed SNP genotyping using a competitive allele-specific PCR genotyping system. Quality control measures included the use of blank wells, the repetition of one human DNA sample across multiple plates, and repeat genotyping for approximately 4% of study subjects. Fifteen of the 31 SNPs were called successfully >95% of the time, 14 were called successfully 93–95% of the time, and the remaining two SNPs, *CRISPLD2* S105G (rs12051468) and *SATB2* Intron 9 T>G (rs4675475), were called successfully 91% and 90% of the time, respectively. Tests of Hardy-Weinberg Equilibrium (HWE) were carried out for all SNPs (independently for cases, case-mothers, case-fathers, and controls). The level of agreement for repeat genotyping was >96% for all SNPs.

## Exclusions

Of the 1,088 cases recruited, 196 were excluded from analyses for the following reasons (number of cases in parenthesis): maternal diabetes (5), maternal epilepsy (12), maternal teratogenic drug exposure (27), chromosomal abnormalities (9), known syndromes (49), type of cleft could not be classified (3), possible non-Mendelian inheritance (8), having two or more SNPs with discordant results upon repeat genotyping (1), and samples could not be genotyped (82). Among the 926 controls, two that had two or more SNPs with discordant

results upon repeat genotyping and 22 that had samples that could not be genotyped were excluded from the analysis.

### Statistical analysis

Patients with isolated oral clefts (absence of other non-cleft defects) were used for the main analysis and were categorized into isolated CLP and isolated CP case groups. We also divided the CLP case group into cleft lip only and cleft lip with cleft palate, and performed separate analyses for these subgroups, because epidemiological and molecular data indicate that these could be distinct conditions (Harville et al., 2005; Rahimov et al., 2008; Genisca et al., 2009). Analyses were repeated adding cases with multiple defects (presence of other non-cleft defects) to determine if they influenced the results. Tests of HWE were performed using a chi-squared test for genotype proportions. Allele frequencies were compared between cases and controls using a chi-squared test. Case-control comparisons of genotype frequency were performed using logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for each SNP. Effect estimates were reported separately for being heterozygous and homozygous for the minor allele, with homozygosity for the major allele as the reference category. We also used the multiplicative disease model in logistic regression analyses to generate *P*-values for the overall effect of minor allele copy number on the association. The transmission disequilibrium test (TDT) was used to assess allele transmission distortion among case-parent trios. Adjustment for multiple comparisons was done using permutation (N=99,999), separately by gene, because the associations between oral clefts and each gene were considered to be distinct *a priori* hypotheses derived from the literature. Multivariate permuting of the trios for the TDT was performed by treating the test as a one-sample test, and permuting the allele of interest. Permutations of cases and controls were independent of permutations of trios, and the results combined by Bonferroni adjustment so that the resulting adjusted *P*-values accounted for all comparisons on that gene while controlling the probability of any false-positives (family-wise error rate). Gene-gene interactions were examined between *IRF6* SNPs and those in genes associated with oral clefts in individual analyses. A disease inheritance model for each SNP was estimated from the OR and 95% CI for heterozygotes and homozygotes in individual analyses and was then used in logistic regression analysis to test gene-gene interaction.

### Linkage disequilibrium

Measures of linkage disequilibrium (LD) were estimated using Haploview (http://www.broad.mit.edu/mpg/haploview/) (Barrett et al., 2005). LD measures were based on genotypes of control samples (N=902) from this study or publicly available data (www.hapmap.org) as indicated in Results and discussion.

## RESULTS

There were 892 cases (765 of them isolated), 838 case-mothers, 719 case-fathers, and 902 controls included in the analysis (Table 1). The birth year of the cases ranged from 1931–2004. The proportion of case-mothers who reported smoking and alcohol use during the affected pregnancy was 38% and 55%, respectively. There was a positive family history of oral clefts among first-degree relatives for 7.5% (67/892) of cases.

The selected SNPs and their minor allele frequencies among controls and patients with isolated CLP or CP are presented in Table 2. Genotype distributions among study groups were in HWE with one exception: *PTCH1* N555N (rs1805155) among controls ($P=9\times10^{-14}$). This could have been due to genotyping error; therefore, we did not report any results for this SNP. Quality control results indicated that genotyping was performed satisfactorily on the other two *PTCH1* SNPs.

Case-control comparisons of genotype frequency indicated statistically significant associations between *PTCH1* SNPs and CLP (Table 3). The *PTCH1* A562A (rs2066836) minor allele was associated with reduced odds of CLP among homozygotes (OR: 0.29, 95% CI: 0.13–0.64; corrected *P*=0.038) which is equivalent to the major allele increasing the odds of CLP (OR: 3.51, 95% CI: 1.56–7.87). The *PTCH1* P1315L (rs357564) minor allele was associated with increased odds of CLP (OR: 1.36, 95% CI: 1.07–1.74 for heterozygotes and OR: 1.56, 95% CI: 1.09–2.24 for homozygotes; corrected *P*=0.023). There was little linkage disequilibrium between these two *PTCH1* SNPs ($r^2$ = 0.10). The minor allele of the *SUMO1* intron 2 G>A SNP (rs3769817) was associated with increased odds of CP among heterozygotes (OR: 1.45, 95% CI: 1.06–1.99; corrected *P*=0.038) but among homozygotes the confidence interval included the null (OR: 1.62, 95% CI: 0.65–4.03) (Table 4). The minor allele of the other *SUMO1* SNP tested (intron 1 G>A, rs12470401) had a similar OR estimate for CP that was of borderline significance (OR = 1.44, 95% CI = 1.05–1.97 for heterozygotes; corrected *P*=0.056); it was in strong LD ($r^2$= 0.98) with rs3769817. Based on the TDT, there was no transmission distortion of *PTCH1* SNPs to CLP cases or of *SUMO1* SNPs to CP cases (Table 4). TDT results indicated that the *TGFA* V159V (rs2166975) minor allele was over-transmitted to CP cases (corrected *P*=0.041) (Table 4). SNPs in the other nine genes were not associated with CLP or CP. When the CLP case group was divided into isolated cleft lip only and isolated cleft lip with cleft palate, the only statistically significant finding was for the association of the *PTCH1* P1315L (rs357564) minor allele with cleft lip and palate in case-control comparisons (OR = 1.67, 95% CI = 1.13–2.49 for homozygotes; corrected *P*=0.040) (Table 5). Repeating the analyses after adding patients with multiple birth defects to the case groups produced no important changes in the results. The numbers of cases and controls in Table 1 are different to those in Table 3 due to the numbers of subjects with genotype data available. *IRF6* S153S (rs2013162) was used in tests of gene-gene interaction because it was the only *IRF6* SNP to show an association with CLP, based on its uncorrected *P*-value (*P*=0.046). There was no evidence of gene-gene interaction between this SNP and those in *PTCH1*, *SUMO1*, and *TGFA*.

## DISCUSSION

In a large, ethnically homogenous study population, we tested 12 genes that previously were suggested to be risk factors for oral clefts and found that SNPs in three genes had statistically significant associations with oral clefts, after adjusting for multiple comparisons. *PTCH1* A562A (rs2066836) and P1315L (rs357564) were associated with CLP while a *SUMO1* non-coding SNP (rs3769817) and *TGFA* V159V (rs2166975) were associated with CP. When the cleft lip only and cleft lip with cleft palate subgroups were examined separately, it was found that *PTCH1* P1315L was associated with cleft lip with cleft palate.

*PTCH1* is a receptor for sonic hedgehog (*SHH*), an important regulator of craniofacial morphogenesis and an etiologic factor in holoprosencephaly, a phenotype that often includes oral clefts (Cohen Jr., 2006). *PTCH1* mutations have been identified in patients with holoprosencephaly, some with oral clefts (Ming et al., 2002). *PTCH1* mutations also cause nevoid basal cell carcinoma syndrome, a condition in which approximately 3–5% of affected patients have oral clefts (Kimonis et al., 1997), a prevalence that is 30–50 times greater than in the general population. A study of CLP and CP cases combined observed that *PTCH1* P1315L (rs357564) was associated with nonsyndromic oral clefts in Caucasians (71 cases, 66 controls) but not in Filipinos (84 cases, 414 controls) (Mansilla et al., 2006). *PTCH1* A562A (rs2066836) was not tested in that study but based on analyses in 220 Filipino families it also found an association between oral clefts and a haplotype composed of two *PTCH1* SNPs, C89T (rs2297088) and T86C (rs2236407), that was of borderline significance. The larger sample size in the current study permitted separate analyses to be

conducted for CLP and CP and the results confirmed the association of CLP with *PTCH1* P1315L observed previously among Caucasians.

Reports of *SUMO1* haploinsufficiency (Alkuraya et al., 2006) and deletion (Shi et al., 2009) in patients with oral clefts implicate this gene in nonsyndromic oral clefts. *SUMO1* participates in the post-translational modification of many target proteins including the products of genes with evidence of a role in oral clefts in humans: msh homeobox1 (*MSX1*) (Lee et al., 2006); *SATB2* (Dobreva et al., 2003); T-box 22 (*TBX22*) (Andreou et al., 2007); and tumor protein 63 (*TP63*) (Ghioni et al., 2005). A study of 181 cases and 162 controls from China that examined four *SUMO1* SNPs found an association with CLP for one haplotype but not for any individual SNPs (Song et al., 2008). HapMap data from a Caucasian population shows that one of the haplotype SNPs from that study (rs6761131) is in perfect LD ($D' = r^2 = 1$) with the two *SUMO1* SNPs that we tested.

A number of studies have examined whether *TGFA* polymorphisms are related to oral clefts in humans but have produced conflicting results. However, these studies varied according to the type of study population, study design, sample size, and *TGFA* variants used (Vieira, 2006). The authors of a meta-analysis stated that the evidence concerning *TGFA* polymorphisms and oral clefts was inconclusive, and suggested that the different study outcomes could be due to variations in the clinical characteristics of cases and in the proportion of cases with a positive family history (Mitchell, 1997). *TGFA* V159V (rs2166975) was associated with CP in a study of 94 CLP trios and 18 CP trios from Lithuania (Morkuniene et al., 2007). The association with CP was confirmed by the current study in which the analysis was based on a larger sample size from another European population: 133 informative CP trios. Based on HapMap data for Caucasians, *TGFA* V159V was not in strong LD with two *TGFA* variants that have previously been examined for an association with oral clefts: the BamHI polymorphism (rs11466297; $r^2 = 0.253$) and the T3851C polymorphism of Marker H2A (rs11466285; $r^2 = 0.021$). Other *TGFA* variants that have been examined in studies of oral clefts, including the TaqI polymorphism, were not found in the HapMap database.

The V274I (rs2235371) variant and other SNPs in *IRF6* have been shown to be associated with nonsyndromic CLP, and although the European populations studied were rarely polymorphic for V274I, there was a suggestive positive association between the V allele and CLP in these populations (Zucchero et al., 2004). Less than 3% of our study population had the I allele of the V274I variant, resulting in too few informative subjects. *IRF6* S153S (rs2013162) has also been associated with nonsyndromic CLP in previous reports (Scapoli et al., 2005). In our study, case-control and TDT association tests for *IRF6* S153S produced uncorrected *P*-values <0.05 for isolated CLP; however, the results were not statistically significant after adjustment for multiple comparisons. HapMap data for Caucasians indicated that the four *IRF6* SNPs we tested were not in strong LD with *IRF6* rs642961 (rs2235371, $r^2 = 0.014$; rs2013162, $r^2 = 0.203$; rs7552506, $r^2 = 0.171$; rs2235377, $r^2 = 0.010$), a common polymorphism (minor allele frequency in Europeans = 0.25) which is associated with cleft lip only and cleft lip with cleft palate in Europeans (Rahimov et al., 2008).

Previous reports have implicated a role in oral clefts for the other eight genes that we tested: *CLPTM1* (Yoshiura et al., 1998); *CRISPLD2* (Chiquet et al., 2007); *FGFR2* (Riley et al., 2007); *GABRB3* (Scapoli et al., 2002; Inoue et al., 2008; Vieira et al., 2008); *GLI2* (Roessler et al., 2003); *RARA* (Chenevix-Trench et al., 1992; Maestri et al., 1997); *RYK* (Watanabe et al., 2006); and *SATB2* (Fitzpatrick et al., 2003; Beaty et al., 2006). In contrast, several studies did not find strong evidence of an association between oral clefts and variants in some of these genes. A study of Japanese patients found no association between

*CLPTM1* and oral clefts (Ichikawa et al., 2006). Two *FGFR2* SNPs showed no association with nonsyndromic CLP in 294 Filipino families (Riley et al., 2007). There was only a suggestive association between *GABRB3* SNPs and CP for patients from Iowa (Vieira et al., 2008) and between *SATB2* variants and oral clefts based on another Iowa study population (Vieira et al., 2005). Some studies observed no association between *RARA* polymorphisms and oral clefts (Kanno et al., 2002; Mitchell et al., 2003). Most of these findings were reported after adjustment for multiple comparisons and it is possible that some true positive associations became statistically non-significant in an attempt to reduce false-positive reports. We found no evidence of an association with oral clefts among the SNPs that we selected for testing in these eight genes however other variants in these genes should be examined for a possible role in oral clefts.

With the exception of *CRISPLD2*, the genes we selected were also included in a recent report that examined 357 candidate genes in two European study populations (Jugessur et al., 2009). There were only two SNPs in common between the two studies: *IRF6* rs2013162 and *RYK* rs1131262. Their study found that *IRF6* was one of seven genes associated with CLP. The other six genes found to be associated with CLP were not among the genes that we selected. Another recent study that examined SNPs in or near a number of candidate genes found that *IRF6* rs2013162 was associated with cleft lip only and that forkhead box E1 (*FOXE1*) was associated with cleft lip with cleft palate (Marazita et al., 2009). These reports provide further evidence that *IRF6* is involved in nonsyndromic oral clefts but, along with the results of our study, also implicate a role for other genes. Other recent reports include the first genome-wide association studies of oral clefts. Two such studies in people with European ancestry found that a SNP in the chromosome 8q24 region (rs987525) is associated with CLP, although the SNP is not located near any known genes (Birnbaum et al., 2009; Grant et al., 2009).

An advantage of our study was the large number of CLP and CP cases and parents available from an ethnically homogenous study population. A limitation of our study was that only one or a few SNPs were tested in each gene. Many haplotype blocks were not examined in the genes we selected because we did not use haplotype-tagging SNPs. Therefore, failure to find an association for SNPs in some of the genes studied does not provide conclusive evidence about whether the genes play a role in oral clefts. Within each gene, we considered whether SNPs in different LD blocks could represent separate *a priori* hypotheses regarding an association between possible functional effects of the SNP on the gene and oral clefts. Had we adjusted for multiple comparisons separately for SNPs in different LD blocks, without accounting for the other SNPs examined in the same gene, then the only altered finding would be that *CRISPLD2* rs4783099 would be associated with cleft lip with cleft palate. We chose to be more conservative when adjusting for multiple comparisons and therefore, within each gene, adjusted for all comparisons performed on the SNPs selected for the gene. This was done to limit the possibility of a false-positive finding in our study. We used a permutation procedure to control the probability of a false-positive association at 5%, separately for each gene. Therefore it was expected that the chance of a Type I error would be small. It is difficult to determine whether our adjustment was over-conservative. Another shortcoming was that most of the SNPs (in *PTCH1*, *SUMO1*, *TGFA*) associated with clefts in this report alter codons but do not result in an amino acid change. However, synonymous changes can have a direct impact on gene function (that is, via splicing, altering translation rates). It is also possible that other variants in these genes are associated with oral clefts and were missed by our study.

Our control group was comprised of women only whereas the case group included both males and females. If the allele frequencies in the control group were not representative of those in the source population that gave rise to the cases then this would produce bias in the

results. However, we had no reason to expect that genotype frequencies would differ by sex because the genes we selected were located on autosomes and not sex chromosomes. We compared the case-control and TDT results for SNPs in *PTCH1*, *SUMO1*, and *TGFA* to determine whether the findings based on use of our control group were in agreement with another test of association. The effect estimates for the association between the *TGFA* SNP (rs2166975) and CP were similar using the case-control and TDT methods, although the result from the case-control method was not statistically significant after adjustment for multiple comparisons. This similarity suggests that the use of our control group did not result in biased estimates of effect.

For 10 of the 12 candidate genes, ours was the largest study to have examined associations between SNPs and nonsyndromic oral clefts. We observed associations with SNPs in *PTCH1, SUMO1*, and *TGFA. PTCH1* A562A (rs2066836) is located in the protein's sterol-sensing domain and P1315L (rs357564) is in the C-terminal domain; both domains are required for transduction of the *SHH* signal (Johnson et al., 2000; Martin et al., 2001). P1315L alters the third non-conserved amino acid in a motif, PPxY, that is a potential target for Nedd4 ubiquitin ligases, proteins that regulate the trafficking of intracellular cargo (Ingham et al., 2004). Mutation studies of the PPxY motif suggest that it is important for the regulation of *PTCH1* turnover (Lu et al., 2006). *TGFA* V159V (rs2166975) encodes a terminal valine in the *TGFA* precursor protein and this residue is required for glycosylation leading to mature protein and for *TGFA* localization to the cell surface (Briley et al., 1997). The work reported here suggests that most of the 31 SNPs we examined do not play a role in oral clefts but that three genes, *PTCH1*, *SUMO1*, and *TGFA*, warrant further investigation as risk factors for oral clefts. We recommend that future research investigates whether the SNPs that showed positive associations with oral clefts in this study actually alter gene function.

## Acknowledgments

This work was supported by the Intramural Research Programs of the National Institutes of Health, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and the National Human Genome Research Institute.

The authors gratefully acknowledge the patients and families who participated in the study, the Cleft Lip and Palate Association of Ireland, and the Dublin Cleft Center team.

## References

Alkuraya FS, Saadi I, Lund JJ, et al. SUMO1 haploinsufficiency leads to cleft lip and palate. Science. 2006; 313:1751. [PubMed: 16990542]

Andreou AM, Pauws E, Jones MC, et al. TBX22 missense mutations found in patients with X-linked cleft palate affect DNA binding, sumoylation, and transcriptional repression. Am J Hum Genet. 2007; 81:700–712. [PubMed: 17846996]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

Beaty TH, Hetmanski JB, Fallin MD, et al. Analysis of candidate genes on chromosome 2 in oral cleft case-parent trios from three populations. Hum Genet. 2006; 120:501–518. [PubMed: 16953426]

Birnbaum S, Ludwig KU, Reutter H, et al. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. Nat Genet. 2009; 41:473–477. [PubMed: 19270707]

Briley GP, Hissong MA, Chiu ML, et al. The carboxyl-terminal valine residues of proTGF alpha are required for its efficient maturation and intracellular routing. Mol Biol Cell. 1997; 8:1619–1631. [PubMed: 9285829]

Chenevix-Trench G, Jones K, Green AC, et al. 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]

Chiquet BT, Lidral AC, Stal S, et al. CRISPLD2: a novel NSCLP candidate gene. Hum Mol Genet. 2007; 16:2241–2248. [PubMed: 17616516]

Cohen MM Jr. Holoprosencephaly: clinical, anatomic, and molecular dimensions. Birth Defects Res A Clin Mol Teratol. 2006; 76:658–673. [PubMed: 17001700]

Dobreva G, Dambacher J, Grosschedl R. SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin mu gene expression. Genes Dev. 2003; 17:3048–3061. [PubMed: 14701874]

Fitzpatrick DR, Carr IM, McLaren L, et al. Identification of SATB2 as the cleft palate gene on 2q32-q33. Hum Mol Genet. 2003; 12:2491–2501. [PubMed: 12915443]

Genisca AE, Frias JL, Broussard CS, et al. Orofacial clefts in the National Birth Defects Prevention Study, 1997–2004. Am J Med Genet A. 2009; 149A:1149–1158. [PubMed: 19441124]

Ghioni P, D'Alessandra Y, Mansueto G, et al. The protein stability and transcriptional activity of p63alpha are regulated by SUMO-1 conjugation. Cell Cycle. 2005; 4:183–190. [PubMed: 15611636]

Grant SF, Wang K, Zhang H, et al. A genome-wide association study identifies a locus for nonsyndromic cleft lip with or without cleft palate on 8q24. J Pediatr. 2009 (In Press).

Harville EW, Wilcox AJ, Lie RT, et al. Cleft lip and palate versus cleft lip only: are they distinct defets? Am J Epidemiol. 2005; 162:448–453. [PubMed: 16076837]

Hashmi SS, Waller DK, Langlois P, et al. Prevalence of nonsyndromic oral clefts in Texas: 1995–1999. Am J Med Genet A. 2005; 134A:368–372. [PubMed: 15779018]

Ichikawa E, Watanabe A, Nakano Y, et al. PAX9 and TGFB3 are linked to susceptibility to nonsyndromic cleft lip with or without cleft palate in the Japanese: population-based and family-based candidate gene analyses. J Hum Genet. 2006; 51:38–46. [PubMed: 16247549]

Ingham RJ, Gish G, Pawson T. The Nedd4 family of E3 ubiquitin ligases: functional diversity within a common modular architecture. Oncogene. 2004; 23:1972–1984. [PubMed: 15021885]

Inoue H, Kayano S, Aoki Y, et al. Association of the GABRB3 gene with nonsyndromic oral clefts. Cleft Palate Craniofac J. 2008; 45:261–266. [PubMed: 18452349]

Johnson RL, Milenkovic L, Scott MP. In vivo functions of the patched protein: requirement of the C terminus for target gene inactivation but not Hedgehog sequestration. Mol Cell. 2000; 6:467–478. [PubMed: 10983992]

Jugessur A, Shi M, Gjessing HK, et al. Genetic determinants of facial clefting: analysis of 357 candidate genes using two national cleft studies from Scandinavia [electronic article]. PLoS One. 2009; 4:e5385. [PubMed: 19401770]

Kanno K, Suzuki Y, Yang X, et al. Lack of evidence for a significant association between nonsyndromic cleft lip with or without cleft palate and the retinoic acid receptor alpha gene in the Japanese population. J Hum Genet. 2002; 47:269–274. [PubMed: 12111375]

Kimonis VE, Goldstein AM, Pastakia B, et al. Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. Am J Med Genet. 1997; 69:299–308. [PubMed: 9096761]

Kirke PN, Mills JL, Molloy AM, et al. Impact of the MTHFR C677T polymorphism on risk of neural tube defects: a case-control study. BMJ. 2004; 328:1535–1536. [PubMed: 15155469]

Lee H, Quinn JC, Prasanth KV, et al. PIAS1 confers DNA-binding specificity on the Msx1 homeoprotein. Genes Dev. 2006; 20:784–794. [PubMed: 16600910]

Lu X, Liu S, Kornberg TB. The C-terminal tail of the Hedgehog receptor Patched regulates both localization and turnover. Genes Dev. 2006; 20:2539–2551. [PubMed: 16980583]

Maestri NE, Beaty TH, Hetmanski J, et al. Application of transmission disequilibrium tests to nonsyndromic oral clefts: including candidate genes and environmental exposures in the models. Am J Med Genet. 1997; 73:337–344. [PubMed: 9415696]

Mansilla MA, Cooper ME, Goldstein T, et al. Contributions of PTCH gene variants to isolated cleft lip and palate. Cleft Palate Craniofac J. 2006; 43:21–29. [PubMed: 16405370]

Marazita ML, Mooney MP. Current concepts in the embryology and genetics of cleft lip and cleft palate. Clin Plast Surg. 2004; 31:125–140. [PubMed: 15145658]

Marazita ML, Lidral AC, Murray JC, et al. Genome scan, fine-mapping, and candidate gene analysis of non-syndromic cleft lip with or without cleft palate reveals phenotype-specific differences in linkage and association results. Hum Hered. 2009; 68:151–170. [PubMed: 19521098]

Martin V, Carrillo G, Torroja C, et al. The sterol-sensing domain of Patched protein seems to control Smoothened activity through Patched vesicular trafficking. Curr Biol. 2001; 11:601–607. [PubMed: 11369205]

Mills JL, Molloy AM, Parle-McDermott A, et al. Folate-related gene polymorphisms as risk factors for cleft lip and cleft palate. Birth Defects Res A Clin Mol Teratol. 2008; 82:636–643. [PubMed: 18661527]

Ming JE, Kaupas ME, Roessler E, et al. Mutations in PATCHED-1, the receptor for SONIC HEDGEHOG, are associated with holoprosencephaly. Hum Genet. 2002; 110:297–301. [PubMed: 11941477]

Mitchell LE. Transforming growth factor alpha locus and nonsyndromic cleft lip with or without cleft palate: a reappraisal. Genet Epidemiol. 1997; 14:231–240. [PubMed: 9181353]

Mitchell LE, Murray JC, O'Brien S, et al. Retinoic acid receptor alpha gene variants, multivitamin use, and liver intake as risk factors for oral clefts: a population-based case-control study in Denmark, 1991–1994. Am J Epidemiol. 2003; 158:69–76. [PubMed: 12835288]

Morkuniene A, Steponaviciut D, Utkus A, et al. Few associations of candidate genes with nonsyndromic orofacial clefts in the population of Lithuania. J Appl Genet. 2007; 48:89–91. [PubMed: 17272867]

Parle-McDermott A, Mills JL, Kirke PN, et al. Analysis of the MTHFR 1298A-->C and 677C-->T polymorphisms as risk factors for neural tube defects. J Hum Genet. 2003; 48:190–193. [PubMed: 12730722]

Rahimov R, Marazita ML, Visel A, et al. Disruption of an AP-2α binding site in an IRF6 enhancer is associated with cleft lip. Nat Genet. 2008; 40:1341–1347. [PubMed: 18836445]

Riley BM, Mansilla MA, Ma J, et al. Impaired FGF signaling contributes to cleft lip and palate. Proc Natl Acad Sci USA. 2007; 104:4512–4517. [PubMed: 17360555]

Roessler E, Du YZ, Mullor JL, et al. Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. Proc Natl Acad Sci USA. 2003; 100:13424–13429. [PubMed: 14581620]

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

Scapoli L, Palmieri A, Martinelli M, et al. 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]

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]

Shi M, Mostowska A, Jugessur A, et al. Identification of microdeletions in candidate genes for cleft lip and/or palate. Birth Defects Res A Clin Mol Teratol. 2009; 85:42–51. [PubMed: 19137569]

Song T, Li G, Jing G, et al. SUMO1 polymorphisms are associated with non-syndromic cleft lip with or without cleft palate. Biochem Biophys Res Commun. 2008; 377:1265–1268. [PubMed: 18983974]

Vieira AR, Avila JR, Daack-Hirsch S, et al. Medical sequencing of candidate genes for nonsyndromic cleft lip and palate [electronic article]. PLoS Genet. 2005; 1:e64. [PubMed: 16327884]

Vieira AR. Association between the transforming growth factor alpha gene and nonsyndromic oral clefts: a HuGE review. Am J Epidemiol. 2006; 163:790–810. [PubMed: 16495466]

Vieira AR, Howe A, Murray JC. Studies of gamma-aminobutyric acid type A receptor beta3 (GABRB3) and glutamic acid decarboxylase 67 (GAD67) with oral clefts. Am J Med Genet A. 2008; 146A:2828–2830. [PubMed: 18837046]

Watanabe A, Akita S, Tin NT, et al. A mutation in RYK is a genetic factor for nonsyndromic cleft lip and palate. Cleft Palate Craniofac J. 2006; 43:310–316. [PubMed: 16681403]

Yoshiura K, Machida J, Daack-Hirsch S, et al. Characterization of a novel gene disrupted by a balanced chromosomal translocation t(2;19)(q11.2;q13.3) in a family with cleft lip and palate. Genomics. 1998; 54:231–240. [PubMed: 9828125]

Zucchero TM, Cooper ME, Maher BS, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. N Engl J Med. 2004; 351:769–780. [PubMed: 15317890]

## Table 1

Irish case families.

| Oral cleft case group | Family member | Isolated defect | Multiple defects | Total |
|---|---|---|---|---|
| Cleft lip with or without cleft palate | Case | 472 | 37 | 509 |
| | Mother | 442 | 32 | 474 |
| | Father | 370 | 28 | 398 |
| Cleft lip only | Case | 147 | 5 | 152 |
| | Mother | 140 | 5 | 145 |
| | Father | 123 | 3 | 126 |
| Cleft lip with cleft palate | Case | 325 | 32 | 357 |
| | Mother | 302 | 27 | 329 |
| | Father | 247 | 25 | 272 |
| Cleft palate only | Case | 293 | 90 | 383 |
| | Mother | 275 | 89 | 364 |
| | Father | 243 | 78 | 321 |

## Table 2

Allele frequencies of single nucleotide polymorphisms (SNPs) in controls and patients with isolated cleft lip with or with cleft palate (CLP) or isolated cleft palate only (CP).

| Gene | SNP | Description | Alleles[a] | Minor allele frequency | | |
|---|---|---|---|---|---|---|
| | | | | Controls (N=902) | CLP (N=472) | CP (N=293) |
| ***CLPTM1*** | rs3786505 | P309P | A/G | 0.316 | 0.288 | 0.293 |
| | rs204468 | G331G | C/T | 0.366 | 0.345 | 0.350 |
| | rs7257610 | Intron 7, splice site acceptor | G/A | 0.036 | 0.029 | 0.042 |
| ***CRISPLD2*** | rs12051468 | S105G | A/G | 0.460 | 0.463 | 0.423 |
| | rs8061351 | P157P | T/C | 0.239 | 0.233 | 0.285[b] |
| | rs721005 | T322S | C/G | 0.393 | 0.402 | 0.382 |
| | rs1546124 | 5′ untranslated region | C/G | 0.329 | 0.334 | 0.358 |
| | rs16974880 | 3′ untranslated region | T/G | 0.338 | 0.357 | 0.314 |
| | rs4783099 | 3′ untranslated region | C/T | 0.394 | 0.428 | 0.377 |
| ***FGFR2*** | rs1047100 | V232V | G/A | 0.197 | 0.211 | 0.185 |
| ***GABRB3*** | rs2017247 | 3′ untranslated region | G/A | 0.256 | 0.271 | 0.257 |
| | rs11637141 | 3′ untranslated region | C/T | 0.229 | 0.236 | 0.233 |
| ***GLI2*** | rs12711538 | D1306N | A/G | 0.280 | 0.287 | 0.319 |
| ***IRF6*** | rs2235371 | V274I | C/T | 0.014 | 0.004[b] | 0.009 |
| | rs2013162 | S153S | C/A | 0.356 | 0.318 | 0.376 |
| | rs7552506 | Intron 3, splice site acceptor | G/C | 0.339 | 0.323 | 0.377 |
| | rs2235377 | Intron 1, splice site acceptor | T/C | 0.013 | 0.006 | 0.007 |
| ***PTCH1*** | rs2066836 | A562A | C/T | 0.211 | 0.165[c] | 0.219 |
| | rs357564 | P1315L | G/A | 0.323 | 0.379[c] | 0.350 |
| ***RARA*** | rs2229773 | 3′ untranslated region | C/T | 0.031 | 0.032 | 0.040 |
| ***RYK*** | rs1131262 | Stop *98* | G/A | 0.115 | 0.111 | 0.117 |
| | rs4470517 | Intron 13 | T/C | 0.356 | 0.383 | 0.349 |
| ***SATB2*** | rs4673313 | Intron 8 | T/C | 0.103 | 0.121 | 0.115 |
| | rs17199393 | Intron 3 | G/A | 0.038 | 0.052 | 0.047 |

| Gene | SNP | Description | Alleles[a] | Minor allele frequency | | |
|---|---|---|---|---|---|---|
| | | | | Controls (N=902) | CLP (N=472) | CP (N=293) |
| | rs16831366 | Intron 6 | G/T | 0.061 | 0.084[b] | 0.086[b] |
| | rs1446636 | Intron 10 | G/T | 0.120 | 0.147 | 0.133 |
| | rs4675475 | Intron 9 | T/G | 0.224 | 0.254 | 0.242 |
| ***SUMO1*** | rs3769817 | Intron 2 | G/A | 0.118 | 0.143 | 0.158[b] |
| | rs12470401 | Intron 1 | G/A | 0.116 | 0.138 | 0.153[b] |
| ***TGFA*** | rs2166975 | V159V | G/A | 0.254 | 0.245 | 0.291 |

[a] Major allele is listed first

[b] Significant difference in minor allele frequency compared with controls; $P<0.05$, uncorrected

[c] Significant difference in minor allele frequency compared with controls; $P<0.01$, uncorrected

## Table 3

Genotype distributions for single nucleotide polymorphisms (SNPs) in 12 candidate genes and tests of association in controls and patients with isolated cleft lip with or with cleft palate (CLP) or isolated cleft palate only (CP).

| SNPs | Control Genotypes[a] | CLP Genotypes[a] | CLP OR (95% CI)[b] | | Corrected P-Value[d] | CP Genotypes[a] | CP OR (95% CI)[b] | | Corrected P-value[d] |
|---|---|---|---|---|---|---|---|---|---|
| | | | Heterozygotes | Homozygotes[c] | | | Heterozygotes | Homozygotes[c] | |
| ***CLPTM1*** | | | | | | | | | |
| rs3786505 | 414/375/91 | 235/172/44 | 0.81 (0.64, 1.03) | 0.85 (0.57, 1.26) | 0.523 | 146/108/29 | 0.82 (0.61, 1.09) | 0.90 (0.57, 1.43) | 1.000 |
| rs204468 | 360/401/123 | 196/203/55 | 0.93 (0.73, 1.19) | 0.82 (0.57, 1.18) | 0.981 | 118/128/34 | 0.97 (0.73, 1.30) | 0.84 (0.55, 1.30) | 1.000 |
| rs7257610 | 819/64/0 | 423/26/0 | 0.79 (0.49, 1.26) | - | 1.000 | 261/22/1 | 1.08 (0.65, 1.79) | - | 1.000 |
| ***CRISPLD2*** | | | | | | | | | |
| rs12051468 | 234/406/170 | 125/209/93 | 0.96 (0.73, 1.27) | 1.02 (0.73, 1.43) | 1.000 | 87/142/45 | 0.94 (0.69, 1.29) | 0.71 (0.47, 1.07) | 0.850 |
| rs8061351 | 492/328/42 | 270/156/28 | 0.87 (0.68, 1.10) | 1.22 (0.74, 2.00) | 1.000 | 139/125/18 | 1.35 (1.02, 1.78) | 1.52 (0.85, 2.72) | 0.217 |
| rs721005 | 324/393/140 | 161/215/73 | 1.10 (0.86, 1.42) | 1.05 (0.75, 1.48) | 1.000 | 108/134/41 | 1.02 (0.76, 1.37) | 0.88 (0.58, 1.33) | 1.000 |
| rs1546124 | 385/369/95 | 193/201/47 | 1.09 (0.85, 1.39) | 0.99 (0.67, 1.46) | 1.000 | 110/137/31 | 1.30 (0.97, 1.73) | 1.14 (0.72, 1.81) | 1.000 |
| rs16974880 | 382/398/97 | 180/215/52 | 1.15 (0.90, 1.46) | 1.14 (0.78, 1.67) | 1.000 | 133/121/28 | 0.87 (0.66, 1.16) | 0.83 (0.52, 1.32) | 1.000 |
| rs4783099 | 321/425/134 | 143/224/79 | 1.18 (0.92, 1.53) | 1.32 (0.94, 1.86) | 0.840 | 110/134/40 | 0.92 (0.69, 1.23) | 0.87 (0.58, 1.32) | 1.000 |
| ***FGFR2*** | | | | | | | | | |
| rs1047100 | 575/298/29 | 287/150/22 | 1.01 (0.79, 1.29) | 1.52 (0.86, 2.69) | 0.745 | 193/85/11 | 0.85 (0.64, 1.14) | 1.13 (0.55, 2.31) | 1.000 |
| ***GABRB3*** | | | | | | | | | |
| rs2017247 | 496/329/63 | 244/168/38 | 1.04 (0.82, 1.32) | 1.23 (0.80, 1.89) | 1.000 | 156/110/18 | 1.06 (0.80, 1.41) | 0.91 (0.52, 1.58) | 1.000 |
| rs11637141 | 527/323/43 | 269/163/27 | 0.99 (0.78, 1.26) | 1.23 (0.74, 2.04) | 1.000 | 174/94/20 | 0.88 (0.66, 1.17) | 1.41 (0.81, 2.46) | 1.000 |
| ***GLI2*** | | | | | | | | | |
| rs12711538 | 448/336/71 | 219/177/35 | 1.08 (0.85, 1.38) | 1.01 (0.65, 1.56) | 1.000 | 131/122/29 | 1.24 (0.93, 1.65) | 1.40 (0.87, 2.24) | 0.176 |
| ***IRF6*** | | | | | | | | | |
| rs2235371 | 869/25/0 | 456/4/0 | 0.31 (0.11, 0.88) | - | 0.103 | 281/3/1 | 0.37 (0.11, 1.24) | - | 1.000 |
| rs2013162 | 350/409/102 | 204/202/41 | 0.85 (0.67, 1.08) | 0.69 (0.46, 1.03) | 0.275 | 109/129/40 | 1.01 (0.76, 1.36) | 1.26 (0.82, 1.93) | 1.000 |
| rs7552506 | 379/406/95 | 201/202/43 | 0.94 (0.74, 1.19) | 0.85 (0.57, 1.27) | 1.000 | 110/129/41 | 1.10 (0.82, 1.46) | 1.49 (0.97, 2.27) | 0.480 |
| rs2235377 | 847/23/0 | 440/5/0 | 0.42 (0.16, 1.11) | - | 0.338 | 271/4/0 | 0.54 (0.19, 1.59) | - | 1.000 |
| ***PTCH1*** | | | | | | | | | |
| rs2066836 | 559/284/45 | 305/133/7 | 0.86 (0.67, 1.10) | 0.29 (0.13, 0.64) | 0.038 | 172/101/12 | 1.16 (0.87, 1.54) | 0.87 (0.45, 1.68) | 1.000 |

| SNPs | Control Genotypes[a] | CLP Genotypes[a] | CLP OR (95% CI)[b] | | Corrected P-Value[d] | CP Genotypes[a] | CP OR (95% CI)[b] | | Corrected P-value[d] |
|---|---|---|---|---|---|---|---|---|---|
| | | | Heterozygotes | Homozygotes[c] | | | Heterozygotes | Homozygotes[c] | |
| rs357564 | 415/382/98 | 174/218/64 | 1.36 (1.07, 1.74) | 1.56 (1.09, 2.24) | 0.023 | 116/137/31 | 1.28 (0.97, 1.70) | 1.13 (0.72, 1.78) | 0.947 |
| ***RARA*** | | | | | | | | | |
| rs2229773 | 825/53/1 | 429/29/0 | 1.05 (0.66, 1.68) | - | 1.000 | 262/23/0 | 1.37 (0.82, 2.27) | - | 0.637 |
| ***RYK*** | | | | | | | | | |
| rs1131262 | 699/179/13 | 354/99/1 | 1.09 (0.83, 1.44) | 0.15 (0.02, 1.17) | 1.000 | 228/51/8 | 0.87 (0.62, 1.23) | 1.89 (0.77, 4.61) | 1.000 |
| rs4470517 | 354/376/111 | 178/198/73 | 1.05 (0.82, 1.34) | 1.31 (0.93, 1.85) | 0.597 | 119/120/36 | 0.95 (0.71, 1.27) | 0.97 (0.63, 1.48) | 1.000 |
| ***SATB2*** | | | | | | | | | |
| rs4673313 | 694/147/15 | 338/94/6 | 1.31 (0.98, 1.75) | 0.82 (0.32, 2.14) | 1.000 | 216/60/2 | 1.31 (0.94, 1.84) | 0.43 (0.10, 1.89) | 1.000 |
| rs17199393 | 797/65/0 | 409/39/4 | 1.17 (0.77, 1.77) | - | 0.733 | 252/26/0 | 1.27 (0.79, 2.04) | - | 1.000 |
| rs16831366 | 762/96/5 | 367/68/3 | 1.47 (1.05, 2.06) | 1.25 (0.30, 5.24) | 0.309 | 232/46/1 | 1.57 (1.08, 2.30) | 0.66 (0.08, 5.65) | 0.408 |
| rs1446636 | 673/174/17 | 327/110/11 | 1.30 (0.99, 1.71) | 1.33 (0.62, 2.88) | 0.536 | 215/64/6 | 1.15 (0.83, 1.59) | 1.11 (0.43, 2.84) | 1.000 |
| rs4675475 | 530/291/50 | 223/158/24 | 1.29 (1.01, 1.66) | 1.14 (0.68, 1.90) | 0.877 | 152/87/19 | 1.04 (0.77, 1.41) | 1.33 (0.76, 2.32) | 1.000 |
| ***SUMO1*** | | | | | | | | | |
| rs3769817 | 684/177/15 | 332/118/6 | 1.37 (1.05, 1.79) | 0.82 (0.32, 2.14) | 0.159 | 197/74/7 | 1.45 (1.06, 1.99) | 1.62 (0.65, 4.03) | 0.038 |
| rs12470401 | 694/117/14 | 337/114/6 | 1.33 (1.01, 1.74) | 0.88 (0.34, 2.32) | 0.227 | 199/73/6 | 1.44 (1.05, 1.97) | 1.50 (0.57, 3.94) | 0.056 |
| ***TGFA*** | | | | | | | | | |
| rs2166975 | 472/338/49 | 253/161/28 | 0.89 (0.70, 1.13) | 1.07 (0.65, 1.74) | 1.000 | 138/107/25 | 1.08 (0.81, 1.45) | 1.75 (1.04, 2.93) | 0.165 |

[a] Genotype values are numbers of individuals with homozygous major allele/heterozygous/homozygous minor allele

[b] OR – odds ratio; CI – confidence interval; homozygous major allele was reference category

[c] Homozygous for minor allele; homozygote odds ratios were not obtained when there were no cases and/or controls that were homozygous for the minor allele

[d] Corrected P-value after adjustment for multiple comparisons; testing for the overall effect of minor allele copy number on the outcome based on the multiplicative disease model.

**Table 4**

Test for distortion of allele transmission to patients that have isolated cleft lip with or with cleft palate or isolated cleft palate only.

| SNPs[a] | Cleft lip with or without cleft palate | | | Cleft palate only | | |
|---|---|---|---|---|---|---|
| | N[b] | T/NT[c] | RR (95% CI)[d] | N[b] | T/NT[c] | RR (95% CI)[d] |
| ***CLPTM1*** | | | | | | |
| rs3786505 | 216 | 128/148 | 0.87 (0.68, 1.10) | 149 | 93/97 | 0.96 (0.72, 1.27) |
| rs204468 | 229 | 137/160 | 0.86 (0.68, 1.08) | 156 | 106/100 | 1.06 (0.81, 1.40) |
| rs7257610 | 35 | 14/21 | 0.67 (0.34, 1.31) | 30 | 17/15 | 1.13 (0.57, 2.27) |
| ***CRISPLD2*** | | | | | | |
| rs12051468 | 224 | 158/133 | 1.19 (0.94, 1.50) | 157 | 106/93 | 1.14 (0.86, 1.51) |
| rs8061351 | 212 | 118/137 | 0.86 (0.67, 1.10) | 130 | 86/72 | 1.19 (0.87, 1.63) |
| rs721005 | 241 | 167/156 | 1.07 (0.86, 1.33) | 154 | 103/96 | 1.07 (0.81, 1.42) |
| rs1546124 | 244 | 149/169 | 0.88 (0.71, 1.10) | 131 | 88/81 | 1.09 (0.80, 1.47) |
| rs16974880 | 212 | 153/118 | 1.30 (1.02, 1.65) | 136 | 94/90 | 1.04 (0.78, 1.39) |
| rs4783099 | 221 | 159/131 | 1.21 (0.96, 1.53) | 150 | 108/91 | 1.19 (0.90, 1.57) |
| ***FGFR2*** | | | | | | |
| rs1047100 | 203 | 124/125 | 0.99 (0.77, 1.27) | 121 | 66/74 | 0.89 (0.64, 1.24) |
| ***GABRB3*** | | | | | | |
| rs2017247 | 193 | 124/123 | 1.01 (0.79, 1.29) | 148 | 87/96 | 0.91 (0.68, 1.21) |
| rs11637141 | 198 | 117/128 | 0.91 (0.71, 1.17) | 144 | 88/83 | 1.06 (0.79, 1.43) |
| ***GLI2*** | | | | | | |
| rs12711538 | 213 | 132/125 | 1.06 (0.83, 1.35) | 142 | 93/90 | 1.03 (0.77, 1.38) |
| ***IRF6*** | | | | | | |
| rs2235371 | 8 | 3/5 | 0.60 (0.14, 2.51) | 3 | 1/2 | 0.50 (0.05, 5.51) |
| rs2013162 | 236 | 128/171 | 0.75 (0.60, 0.94) | 130 | 100/75 | 1.33 (0.99, 1.80) |
| rs7552506 | 238 | 136/168 | 0.81 (0.65, 1.02) | 135 | 104/76 | 1.37 (1.02, 1.84) |
| rs2235377 | 8 | 2/6 | 0.33 (0.07, 1.65) | 3 | 2/1 | 2.00 (0.18, 22.06) |
| ***PTCH1*** | | | | | | |
| rs2066836 | 161 | 89/100 | 0.89 (0.67, 1.18) | 120 | 78/66 | 1.18 (0.85, 1.64) |
| rs357564 | 258 | 167/167 | 1.00 (0.81, 1.24) | 165 | 111/106 | 1.05 (0.80, 1.37) |

| SNPs[a] | Cleft lip with or without cleft palate | | | Cleft palate only | | |
|---|---|---|---|---|---|---|
| | N[b] | T/NT[c] | RR (95% CI)[d] | N[b] | T/NT[c] | RR (95% CI)[d] |
| ***RARA*** | | | | | | |
| rs2229773 | 45 | 26/20 | 1.30 (0.73, 2.33) | 33 | 21/13 | 1.62 (0.81, 3.23) |
| ***RYK*** | | | | | | |
| rs1131262 | 135 | 69/77 | 0.90 (0.65, 1.24) | 78 | 45/47 | 0.96 (0.64, 1.44) |
| rs4470517 | 231 | 157/156 | 1.01 (0.81, 1.26) | 154 | 93/110 | 0.85 (0.64, 1.11) |
| ***SATB2*** | | | | | | |
| rs4673313 | 119 | 68/63 | 1.08 (0.77, 1.52) | 76 | 41/43 | 0.95 (0.62, 1.46) |
| rs17199393 | 51 | 24/29 | 0.83 (0.48, 1.42) | 38 | 19/21 | 0.91 (0.49, 1.68) |
| rs16831366 | 81 | 42/43 | 0.98 (0.64, 1.49) | 60 | 28/34 | 0.82 (0.50, 1.36) |
| rs1446636 | 144 | 86/73 | 1.18 (0.86, 1.61) | 98 | 57/58 | 0.98 (0.68, 1.42) |
| rs4675475 | 171 | 105/99 | 1.06 (0.81, 1.40) | 112 | 69/71 | 0.97 (0.70, 1.35) |
| ***SUMO1*** | | | | | | |
| rs3769817 | 133 | 82/77 | 1.07 (0.78, 1.45) | 102 | 65/53 | 1.23 (0.85, 1.76) |
| rs12470401 | 141 | 84/80 | 1.05 (0.77, 1.43) | 98 | 67/46 | 1.46 (1.00, 2.12) |
| ***TGFA*** | | | | | | |
| rs2166975 | 177 | 111/109 | 1.01 (0.78, 1.33) | 133 | 102/72 | 1.42 (1.05, 1.92)[e] |

[a] SNPs – single nucleotide polymorphisms

[b] N – number of informative trios

[c] T/NT – transmission/non-transmission of minor allele

[d] RR – risk ratio; CI – confidence interval

[e] Significant difference in allele transmission, corrected $P<0.05$, after adjustment for multiple comparisons



*Birth Defects Res A Clin Mol Teratol*. Author manuscript; available in PMC 2012 November 21.

$watermark-text

$watermark-text

## Table 5

Tests of association between SNPs in 12 candidate genes and subgroups of isolated cleft lip with or without cleft palate: cleft lip only and cleft lip with cleft palate.

| | Cleft Lip Only | | | Cleft Lip with Cleft Palate | | |
|---|---|---|---|---|---|---|
| | Case-Control OR (95% CI)[b] | | | Case-Control OR (95% CI)[b] | | |
| SNPs[a] | Heterozygotes | Homozygotes[c] | TDT RR (95% CI)[d] | Heterozygotes | Homozygotes[c] | TDT RR (95% CI)[d] |
| ***CLPTM1*** | | | | | | |
| rs3786505 | 0.81 (0.56, 1.19) | 0.95 (0.52, 1.73) | 0.86 (0.58, 1.29) | 0.81 (0.61, 1.06) | 0.81 (0.51, 1.28) | 0.87 (0.65, 1.16) |
| rs204468 | 0.83 (0.57, 1.21) | 0.75 (0.43, 1.33) | 0.70 (0.47, 1.04) | 0.98 (0.74, 1.29) | 0.86 (0.57, 1.30) | 0.95 (0.72, 1.26) |
| rs7257610 | 0.68 (0.30, 1.51) | - | 0.57 (0.17, 1.95) | 0.84 (0.49, 1.42) | - | 0.71 (0.32, 1.61) |
| ***CRISPLD2*** | | | | | | |
| rs12051468 | 0.94 (0.62, 1.43) | 0.94 (0.56, 1.58) | 0.94 (0.63, 1.40) | 0.97 (0.71, 1.33) | 1.07 (0.73, 1.56) | 1.33 (1.00, 1.77) |
| rs8061351 | 0.88 (0.60, 1.28) | 1.53 (0.76, 3.10) | 0.80 (0.53, 1.21) | 0.86 (0.66, 1.14) | 1.07 (0.60, 1.93) | 0.90 (0.66, 1.22) |
| rs721005 | 1.09 (0.74, 1.61) | 0.98 (0.57, 1.67) | 1.06 (0.73, 1.53) | 1.10 (0.83, 1.47) | 1.08(0.74, 1.60) | 1.08 (0.82, 1.41) |
| rs1546124 | 1.09 (0.75, 1.59) | 0.98 (0.53, 1.80) | 0.86 (0.60, 1.23) | 1.08 (0.82, 1.43) | 0.99 (0.63, 1.55) | 0.89 (0.68, 1.18) |
| rs16974880 | 0.87 (0.60, 1.27) | 0.82(0.44, 1.53) | 1.14 (0.76, 1.72) | 1.31 (0. 99, 1.73) | 1.32 (0.86, 2.04) | 1.39 (1.03, 1.87) |
| rs4783099 | 0.82 (0.56, 1.20) | 0.80 (0.46, 1.38) | 0.98 (0.66, 1.45) | 1.45 (1.07, 1.96) | 1.70 (1.15, 2.52) | 1.36 (1.02, 1.81) |
| ***FGFR2*** | | | | | | |
| rs1047100 | 0.99 (0.67, 1.45) | 1.98 (0.91, 4.33) | 1.05 (0.69, 1.59) | 1.02 (0.77, 1.34) | 1.31 (0.67, 2.57) | 0.96 (0.71, 1.31) |
| ***GABRB3*** | | | | | | |
| rs2017247 | 0.88 (0.60, 1.29) | 1.26 (0.67, 2.40) | 0.92 (0.59, 1.45) | 1.12 (0.85, 1.47) | 1.21 (0.74, 1.98) | 1.05 (0.78, 1.41) |
| rs11637141 | 0.73 (0.49, 1.09) | 2.34 (1.28, 4.29) | 0.98 (0.65, 1.47) | 1.12 (0.85, 1.46) | 0.68 (0.34, 1.38) | 0.88 (0.64, 1.21) |
| ***GLI2*** | | | | | | |
| rs12711538 | 1.04 (0.72, 1.51) | 0.95 (0.48, 1.88) | 1.22 (0.79, 1.90) | 1.10 (0.83, 1.45) | 1.04 (0.63, 1.71) | 0.99 (0.74, 1.33) |
| ***IRF6*** | | | | | | |
| rs2235371 | 0.48 (0.11, 2.06) | - | 0.33 (0.03, 3.20) | 0.22 (0.05, 0.95) | - | 1.00 (0.14, 7.10) |
| rs2013162 | 0.95 (0.66, 1.38) | 0.78 (0.42, 1.44) | 0.75 (0.50, 1.10) | 0.80 (0.61, 1.06) | 0.65 (0.41, 1.04) | 0.75 (0.57, 1.00) |
| rs7552506 | 0.98 (0.67, 1.42) | 0.89 (0.48, 1.65) | 0.79 (0.54, 1.15) | 0.92 (0.70, 1.21) | 0.84 (0.53, 1.33) | 0.82 (0.62, 1.09) |
| rs2235377 | 0.53 (0.12, 2.27) | - | 0.50 (0.05, 5.51) | 0.37 (0.11, 1.23) | - | 0.25 (0.03, 2.24) |
| ***PTCH1*** | | | | | | |

| SNPs[a] | Cleft Lip Only | | | Cleft Lip with Cleft Palate | | |
|---|---|---|---|---|---|---|
| | Case-Control OR (95% CI)[b] | | | Case-Control OR (95% CI)[b] | | |
| | Heterozygotes | Homozygotes[c] | TDT RR (95% CI)[d] | Heterozygotes | Homozygotes[c] | TDT RR (95% CI)[d] |
| rs2066836 | 0.83 (0.56, 1.23) | 0.39 (0.12, 1.29) | 0.97 (0.59, 1.60) | 0.87 (0.66, 1.16) | 0.24 (0.08, 0.67) | 0.86 (0.60, 1.21) |
| rs357564 | 1.67 (1.14, 2.45) | 1.27 (0.69, 2.36) | 0.88 (0.60, 1.28) | 1.24 (0.94, 1.63) | 1.67 (1.13, 2.49)[e] | 1.06 (0.82, 1.38) |
| ***RARA*** | | | | | | |
| rs2229773 | 1.68 (0.90, 3.11) | - | 1.86 (0.74, 4.65) | 0.78 (0.43, 1.41) | - | 1.00 (0.46, 2.16) |
| ***RYK*** | | | | | | |
| rs1131262 | 0.96 (0.62, 1.50) | - | 0.64 (0.36, 1.16) | 1.16 (0.85, 1.58) | 0.22 (0.03, 1.72) | 1.04 (0.70, 1.54) |
| rs4470517 | 1.10 (0.74, 1.62) | 1.33 (0.78, 2.27) | 1.11 (0.74, 1.66) | 1.03 (0.77, 1.36) | 1.30 (0.88, 1.92) | 0.96 (0.74, 1.26) |
| ***SATB2*** | | | | | | |
| rs4673313 | 1.31 (0.84, 2.04) | 0.43(0.06, 3.28) | 0.89 (0.51, 1.54) | 1.31 (0.95, 1.83) | 1.01 (0.36, 2.80) | 1.22 (0.79, 1.90) |
| rs17199393 | 1.25 (0.67, 2.32) | - | 0.64 (0.25, 1.64) | 1.14 (0.71, 1.82) | - | 0.94 (0.49, 1.83) |
| rs16831366 | 1.72 (1. 05, 2.80) | 1.37 (0.16, 11.86) | 1.00 (0.49, 2.05) | 1.36 (0.93, 2.00) | 1.19 (0.23, 6.17) | 0.96 (0.57, 1.64) |
| rs1446636 | 1.33 (0.88, 2.01) | 0.76 (0.17, 3.31) | 1.13 (0.65, 1.95) | 1.29 (0.94, 1.76) | 1.61 (0.71, 3.65) | 1.20 (0.82, 1.76) |
| rs4675475 | 1.41 (0.96, 2.06) | 0.91 (0.38, 2.20) | 0.97 (0.61, 1.56) | 1.24 (0.93, 1.65) | 1.25 (0.71, 2.20) | 1.11 (0.79, 1.56) |
| ***SUMO1*** | | | | | | |
| rs3769817 | 1.20 (0.79, 1.84) | 1.29 (0.37, 4.53) | 1.00 (0.57, 1.74) | 1.45 (1.08, 1.96) | 0.61 (0.17, 2.11) | 1.10 (0.75, 1.60) |
| rs12470401 | 1.21 (0.79, 1.85) | 1.39 (0.39, 4.92) | 1.04 (0.61, 1.78) | 1.38 (1.02, 1.87) | 0.65 (0.18, 2.27) | 1.06 (0.73, 1.53) |
| ***TGFA*** | | | | | | |
| rs2166975 | 0.68 (0.46, 0.99) | 0.85 (0.39, 1.85) | 0.88 (0.56, 1.38) | 1.01 (0.77, 1.33) | 1.19 (0.69, 2.06) | 1.10 (0.80, 1.53) |

[a] SNPs – single nucleotide polymorphisms

[b] OR – odds ratio; CI – confidence interval; homozygous major allele was reference category

[c] Homozygous for minor allele; homozygote odds ratios were not obtained when there were no cases and/or controls that were homozygous for the minor allele

[d] TDT – transmission disequilibrium test; RR – risk ratio; CI – confidence interval

[e] Significant difference for test of association, corrected $P<0.05$, after adjustment for multiple comparisons

*Birth Defects Res A Clin Mol Teratol*. Author manuscript; available in PMC 2012 November 21.