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Evidence of Gene-Environment Interaction for the *RUNX2* Gene and Environmental Tobacco Smoke in Controlling the Risk of Cleft Lip with/without Cleft Palate

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Abstract

This study examined the association between 49 markers in the Runt-related transcription factor 2 (*RUNX2*) gene and nonsyndromic cleft lip with/without cleft palate (CL/P) among 326 Chinese case-parent trios, while considering gene-environment (GxE) interaction and parent-of-origin effects. Five single-nucleotide polymorphisms (SNPs) showed significant evidence of linkage and association with CL/P and these results were replicated in an independent European sample of 825 case-parent trios. We also report compelling evidence for interaction between markers in *RUNX2* and environmental tobacco smoke (ETS). Although most marginal SNP effects (i.e., ignoring maternal exposures) were not statistically significant, eight SNPs were significant when considering possible interaction with ETS when testing for gene (G) and GxE interaction simultaneously or when considering GxE alone. Independent samples from European populations showed consistent evidence of significant GxETS interaction at two SNPs (*rs6904353* and *rs7748231*). Our results suggest genetic variation in *RUNX2* may influence susceptibility to CL/P through interacting with ETS.

Keywords

RUNX2; oral clefts; gene-environment interaction; parent-of-origin effects; imprinting

INTRODUCTION

Nonsyndromic cleft lip with or without cleft palate (CL/P), which includes cases with cleft lip alone plus cases with cleft lip and cleft palate is one of the most common human birth defects (Mossey, 2007). Chinese people have higher prevalence rates of CL/P among liveborn infants compared to other racial groups (Cooper et al., 2006). CL/P is considered to be a complex disease because both genetic and environmental risk factors contribute to its etiology (Mossey et al., 2009). The Runt-related transcription factor 2 (*RUNX2*) gene is a member of the RUNX family of transcription factors, and its gene product is involved in osteoblast differentiation and bone morphogenesis (Komori et al., 1997). Mutations in *RUNX2* cause cleidocranial dysplasia, a rare autosomal dominant inherited skeleton condition where clefts of the palate or submucous palate can be present (Lee et al., 1997; Mundlos et al., 1997; Otto et al., 1997; Cooper et al., 2001). Previous studies of CL/P multiplex families yielded some evidence of linkage on chromosome 6p, where this gene is located (Eiberg et al., 1987). An association analyses of case-parent trios from four populations also suggested *RUNX2* may influence risk of CL/P through imprinting effects (Sull et al., 2008). In this study, case-parent trios from Maryland, Taiwan, Singapore, and Korea were genotyped for the *RUNX2* gene and three single nucleotide polymorphisms (SNPs) showed significant excess maternal transmission. Gene-environment (GxE) interaction has been suggested for several genes associated with CL/P (Beaty et al., 2002; Mossey, 2007; Shi et al., 2008; Sull et al., 2009; Zhu et al., 2009), although to our knowledge no studies have investigated this question for *RUNX2*.

In this study, we tested for association between SNPs in *RUNX2* and CL/P using 326 Chinese case-parent trios, which are independent of the previous report by Sull et al. (2008), which considered maternal genotypic effects. Here we explicitly test for imprinting effects and GxE interaction with common maternal exposures, including environmental tobacco smoke (ETS) and multivitamin supplementation.

SUBJECTS AND METHODS

Sample description

The “International Genetic Epidemiology of Oral Clefts” study is a multicenter, international family-based study initiated in 2003 to investigate the genetic etiology of oral clefts. As part of this study, case-parent trios were recruited from three sites in mainland China (Weifang, Shandong Province; Wuhan, Hubei Province; Chengdu, Sichuan Province) and Taiwan. Research protocols were reviewed and approved by institutional review boards at each institution. Informed consent was obtained from parents. The majority of cases were infants seen during a surgical or postsurgical visit. All probands were examined for other congenital anomalies or major developmental delays, and were classified as having an isolated nonsyndromic CL/P. Ethnicity and other demographic data were obtained through structured interviews. Maternal exposure information, including cigarette smoking, ETS, multivitamin supplementation, and alcohol consumption was collected through direct interview of the mothers. Environmental exposures were defined as being exposed from 3 months before pregnancy through the first trimester, except for ETS whereas exposure was defined as being exposed at any time from 3 months before pregnancy through the entire pregnancy. The proportion of infants exposed to maternal cigarette smoking and alcohol consumption was very low (around 1%), so only maternal ETS and multivitamin supplementation were analyzed. Table 1 presents information on gender, family history, and maternal exposures among CL/P probands. None of the parents of these CL/P cases were themselves affected. The proportion of exposure to ETS and multivitamin supplementation were 40.5 and 8.6%, respectively.

Single-Nucleotide Polymorphism Selection and Genotyping

SNPs in and around *RUNX2* on chromosome 6p21 were identified from the literature, dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), and based on results of our previous study (Sull et al., 2008). A set of 69 SNPs was chosen based on the criteria of high “design scores” as provided by Illumina (San Diego, CA), high heterozygosity, and HapMap validation (<http://www.hapmap.org/index.html.en>). Genomic DNA samples were prepared from peripheral blood lymphocytes by the protein precipitation method (Bellus et al., 1995). Primers for each SNP were synthesized using the Oligator technology by Illumina as part of an oligo pool for the BeadLab 1000 system (San Diego, California). A 4 µg aliquot of each genomic DNA sample was dispensed into a barcoded 96-well microtiter plate at a concentration of 100 ng/µl and genotyped using the Illumina Golden-Gate chemistry at the SNP Center of the Genetic Resources Core Facility, a part of the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine. The average distance between neighboring markers was 4.814 kb (based on Build 36.1 of dbSNP). From these 69 selected SNPs, 20 SNPs were monomorphic in this sample and were dropped. No Mendelian inconsistencies were found for the remaining 49 SNPs. Two duplicates and four Centre d’Etude du Polymorphisme Humain control DNA samples were included to evaluate genotyping consistency within and between plates and to insure correct plate orientation. The minor allele frequencies of the 49 SNPs ranged from 5.0% to 47.6% (Supplementary Table S1).

Statistical analysis

Minor allele frequencies were computed using the parents. Pairwise linkage disequilibrium (LD) was measured as r^2 for all SNPs using the Haploview program, and was used to identify LD blocks (Barrett et al., 2005). Nine independent SNPs and eight blocks of LD were identified (Fig. 1). Clayton’s extension of the Transmission Disequilibrium Test (TDT) incorporated into STATA 10 was used on individual SNPs to test for evidence of linkage or LD (Spielman et al., 1993; Cordell et al., 2004). To assess the significance of TDT findings while considering multiple tests, the permutation test in PLINK was used to get corrected p values (PLINK, <http://pngu.mgh.harvard.edu/purcell/plink/>; Purcell et al., 2007). A Bonferroni test would be too conservative because there is strong LD among these markers in *RUNX2*. In the permutation test, the LD structure was preserved and the permuted p values represent the chance of getting a test statistic as large as, or larger than the observed one over 10,000 tests. The family-based association test program (FBAT, <http://www.biostat.harvard.edu/fbat/default.html>) was used for haplotype analysis (Rabinowitz and Laird, 2000). FBAT uses a score test to compare the observed-to-expected genotypes among offspring under the null hypothesis of no association between observed genotype counts and the phenotype. This approach has been extended to consider haplotypes of several SNPs (Laird et al., 2000; Rabinowitz and Laird, 2000; Horvath et al., 2004). We used a sliding window approach for haplotypes to systematically analyze all adjacent 2-SNP and 3-SNP combinations. Plots of $-\log_{10}(p \text{ value})$ for individual SNPs and haplotypes were generated using the R package *snp.plotter* (Luna and Nicodemus, 2007).

We screened for possible GxE interaction between all SNPs in *RUNX2* and two common maternal exposures: ETS and multivitamin supplementation. We used conditional logistic regression under additive models to estimate the relative risk (RR) for being a case with or without environmental exposures for each SNP (Vansteelandt et al., 2008). Estimates of RR (CL/P|G without E) and RR (CL/P|G with E) provide an estimate of the allelic impact of an SNP on the risk of having CL/P. To ensure validly testing for GxE interaction, we also used conditional logistic regression to test for a log-additive GxE interaction while saturating the genetic main effects (by allowing two RR parameters in the model, one for heterozygotes and one for homozygotes). We also used the general strategy proposed by Vansteelandt et

al. (2008) to estimate the haplotype-environment interaction, where family-based association tests are evaluated while allowing for a potential GxE interaction in a 2 degree of freedom (df) test, followed by a separate 1 df test for GxE interaction alone (Schaid, 1996; Kraft et al., 2007). This approach is implemented in the PBAT package (v 3.6; <http://www.biostat.harvard.edu/~clange/default.htm>). The 2 df test examines the genetic effect of a haplotype while taking into account possible effects of GxE interaction, while the 1 df test investigates the effect of GxE interaction alone.

Parent-of-origin effects were also tested using several methods. First, the transmission asymmetry test (TAT) was used to check for potential parent-of-origin effects (Weinberg et al., 1998). A significant TAT could reflect an imprinting effect or a maternal genotype effect. Therefore, we used the max_Z² test implemented in the TRIMM package to test for maternal genotypic effects separately from effects of the child's genotype (Shi et al., 2007). This approach maintains LD patterns among SNPs, and generates empiric *p* values through permutation. Finally, we used the parent-of-origin likelihood ratio test (PO-LRT) developed by Weinberg (1999) to test for imprinting effects as implemented in the program LEM (van Den Oord and Vermunt, 2000).

Replication Study

We carried out an in silico replication study using samples of European and Asian ancestry drawn from a genome-wide association study (GWAS) of nonsyndromic CL/P using the case-parent design (Beaty et al., 2010). The Chinese samples presented here were part of this International Cleft Consortium GWAS, but were dropped for this replication study to insure an independent sample.

Seventy-eight SNPs in *RUNX2* were genotyped in the independent replication samples from the International Cleft Consortium with 2491 individuals from 825 European CL/P families and 2151 individuals from 717 Asian CL/P trios (Supplementary Table S2, online only). Samples were genotyped using the Illumina Human610-Quad v.1_B BeadChip (Illumina). Supplementary Table S3, online only, presents detailed information on these 78 SNPs. TDT analysis in PLINK, tests of GxE interaction using conditional logistic regression models, and PBAT were performed to confirm the findings seen among these Chinese trios.

RESULTS

No SNPs showed evidence of deviating from Hardy-Weinberg equilibrium (all were *p* > 0.10). Figure 1 shows results of the FBAT analyses on individual SNPs and haplotypes. Among 49 SNPs in *RUNX2*, five SNPs showed a statistically significant association with CL/P at a nominal value of *p* < 0.05 (Table 2). SNP *rs545289* remained significant after permutation test with permuted *p* value = 0.035. Sliding windows of haplotypes consisting of two, three, four, and five SNPs were also tested. All haplotypes, including *rs545289*, also showed nominally significant *p* values (0.035 < *p* < 0.05 in Fig. 1), possibly reflecting effects of this single SNP. In the replication sample, five SNPs (*rs2153277*, *rs1200426*, *rs1200425*, *rs9395112*, and *rs2782660*) yielded significant evidence of association in the combined European samples (Supplementary Table S4, online only). SNP *rs1200425* was identified in both original Chinese samples and independent European replication samples, and it was in high LD with *rs545289*, the most significant SNP in the Chinese data, based on LD information of Hap-Map CEU samples (*D'* = 1, *r*² = 0.924). However, none of these SNPs showed significant association in TDT among the remaining Asian samples in the International Cleft Consortium.

Conditional logistic regression under an additive model was used to test for possible GxE interaction and estimate exposure-specific RRs. Twelve SNPs yielded nominal significance

in the likelihood ratio test (LRT) examining G effects and GxETS interaction with 2 df, although eight of these did not show significant SNP effects in the TDT alone. Figure 2A shows estimated RRs and their 95% confidence intervals (CIs) in exposed and unexposed groups for these SNPs. Among these 12 SNPs, 8 SNPs (rs910586, rs765724, rs2790103, rs9472494, rs2396442, rs6904353, rs7748231, and rs10948237) also showed significant p values in the 1 df test for GxETS alone. For SNP rs765724, being a heterozygous child of an exposed mother was associated with a slightly increased risk (RR = 5.00; 95% CI = 1.45-17.27), but not among children of unexposed mothers (RR = 0.57; 95% CI = 0.31-1.06). The LRT for GxETS interaction in this conditional logistic regression model was also statistically significant ($p = 0.00052$). We also used a saturated conditional logistic regression model with two genotypic RR parameters in the model to validate these results, but again saw no substantial difference (data not shown).

Figure 2B shows results of screening for GxETS interaction between SNPs in *RUNX2* using PBAT. Three 2-SNP haplotypes and six 3-SNP haplotypes yielded $p < 0.01$ in the 2 df test for G and GxETS interaction, although these haplotypes did not include any SNP showing significant individual effects.

SNPs rs6930053, rs2772395, and rs485817 showed nominally significant p values for Gx(vitamin) interaction using the LRT ($0.027 < p < 0.05$). Haplotypes involving these three SNPs also yielded significant evidence of interaction with exposure. Like ETS, SNPs with no significant marginal effects also gave intriguing evidence of GxE interaction. For example, two 2-SNP haplotypes and three 3-SNP haplotypes (none of which contained SNPs which were individually significant) gave nominally significant p values in both the 2 df and 1 df tests for Gx(vitamin) interaction ($0.003 < p < 0.05$).

We replicated the intriguing results of GxE interaction among the International Cleft Consortium samples. Five SNPs in *RUNX2* yielded significant p values in the LRT with 2 df for effects of G and GxETS jointly among European trios, although none of them showed significant gene effect ignoring exposures (Supplementary Table S5, online only). Among these markers, four SNPs also showed significant p values in the 1 df test for GxE effect alone. In the test of Gx(vitamin), two SNPs in the European trios and one SNP in Asian trios were statistically significant in the 2 df LRT. PBAT was used to test for haplotype-environment interactions. In the test of Gx(ETS) interaction, two 3-SNP haplotypes among the European samples, and two 2-SNP haplotypes and one 3-SNP haplotype among the Asian samples yielded $p < 0.01$ in the 2 df test for G and GxE interaction, although these haplotypes did not include any SNPs showing significant individual effects. The results of tests for haplotype-vitamin interaction also confirmed these findings from the Chinese samples. Three 2-SNP and two 3-SNP haplotypes without SNPs showing any individual gene effects gave significant p values < 0.01 in the 1 df test for GxE interaction alone among European trios.

Parent-of-origin effects were first investigated by stratifying informative transmissions (T) and non-transmissions (NT) by parental source in the TAT. Among SNPs not in perfect LD (i.e., those with $r^2 < 0.8$), three SNPs showed excess maternal transmission at $p < 0.05$ level (Table 3). TRIMM was used to test for maternal genotype effects (Shi et al., 2007). However, none showed any evidence of maternal genotype effect. The PO-LRT was used to test for imprinting effects controlling for maternal genotype effect. SNP rs675613 yielded a marginally significant p value (RR = 2.2; $p = 0.046$), suggesting the maternally derived copy of the minor allele at rs675613 was associated with a two-fold increase in risk compared to receiving a paternally derived copy of this allele (Table 3).

DISCUSSION

Our results showed consistent evidence of linkage in the presence of association (LD) for five SNPs in the *RUNX2* gene (nominal $p < 0.05$) and one SNP *rs545289* remained significant in permutation tests (empiric $p = 0.035$). Single SNP analysis using conditional logistic regression models and haplotype analysis using PBAT suggested the *RUNX2* gene may also influence the risk of CL/P through interaction with ETS and multivitamin supplementation. We also found one SNP (*rs675613*) showed marginally significant maternal over-transmission after considering maternal genotypic effects (nominal $p = 0.046$).

The *RUNX2* gene is located on chromosome 6p21, and encodes a transcription factor critical for osteoblastic differentiation (Zelzer and Olsen, 2003; Schroeder et al., 2005; Komori, 2010). It has been suggested that *RUNX2* may be under the regulation of estrogen in both osteoblasts and osteoclastogenesis (Mundlos et al., 1997; McCarthy et al., 2003). Mutations in *RUNX2* lead to cleidocranial dysplasia, a heritable congenital skeletal disorder characterized by partly or completely missing clavicles, dental anomalies, and craniofacial abnormalities including submucous CL/P and cleft lip (Lee et al., 1997; Otto et al., 1997; Cooper et al., 2001; Yamachika et al., 2001; Aberg et al., 2004). Animal models have suggested mutations or expression levels of *RUNX2* may be involved in the development of several craniofacial defects (Aberg et al., 2004; Eswarakumar et al., 2004).

Our study found five SNPs in the *RUNX2* gene that showed significant evidence of linkage and association with nonsyndromic CL/P among Chinese case-parent trios. In our study of 326 Chinese CL/P case-parent trios, *rs545289* yielded marginal association possibly due to LD with untyped variant influencing risk of nonsyndromic CL/P (empiric $p = 0.035$), and this finding was confirmed among independent Europeans trios. Five SNPs in *RUNX2* yielded statistical significance in TDT analysis among these trios of European ancestry. SNP *rs1200425* was statistically significant in both the Chinese samples and the independent replication samples, and was in high LD with the most significant SNP (*rs545289*) among Chinese trios. Under-transmission of the minor allele (T allele) at *rs1200425* was associated with decreased risk of CL/P among the Chinese and the replication European trios. However, the association was not replicated in the Asian subjects. The combined Asian replication samples were from different regions (Singapore, Korea, and Philippines). Principal component analysis showed genetic distances between Singapore and Philippines were relatively large compared to the distances among other East Asian samples, which might explain why none of the SNPs yielded significant evidence of association among Asians drawn from the International Cleft Consortium. Most Chinese trios of current study were also genotyped for 78 SNPs in *RUNX2* in the GWAS. TDT analysis (Supplementary Table S6, online only) showed *rs16873396* yielded significant association with CL/P (nominal $p = 0.008$) in the GWAS and this further confirmed the finding of the original analysis (nominal $p = 0.012$; Table 2).

The interaction between susceptibility genes and common environment factors can also influence the etiology of nonsyndromic oral clefts (Graham and Shaw, 2005; Young et al., 2007). Considering GxE interaction will identify more risk loci, lead to better understanding of underlying biologic mechanisms, and provide information for designing effective preventive strategies. However, there is little consistent evidence of interactions between genes and environment risk factors of nonsyndromic CL/P (Mossey et al., 2009; Shi et al., 2008). Moreover, to our knowledge, no study to date has focused on whether GxE interaction influences the association between *RUNX2* and the risk of isolated nonsyndromic CL/P. Our study found intriguing evidence of GxETS and Gx(vitamin) interaction among both the Chinese trios and independent replication samples.

Several studies have shown ETS is not only an independent risk factor of CL/P, but may interact with genetic variants (*MSX1*, *CYP1A1*, *BMP4T538C*, and *MYH9*) to increase risk (Chevrier et al., 2008; Jia et al., 2010; Jianyan et al., 2010). Our study suggests an interaction between ETS and markers in *RUNX2*. Both single marker and haplotype analysis yielded consistent results of Gx(ETS) interaction among Chinese trios and replication trios. The 2 df test identified SNPs influencing risk of disease when considering maternal exposure. Eight SNPs in *RUNX2* showing no significant individual effects yielded nominal significance in both the 2 df and 1 df tests when exposure to ETS was included in the model. This result illustrates the importance of considering possible GxE interaction in the etiology of CL/P.

Maternal vitamin use has also been reported to be inversely associated with the risk of CL/P, and potential interaction between candidate genes and vitamin supplementation has been suggested (Shaw et al., 1998; Johnson and Little, 2008). We also found evidence of GxE interaction for maternal multivitamin supplementation at three SNPs in *RUNX2* and these findings were replicated in single SNP and haplotype analysis among the replication samples. These results suggest being exposed to maternal multivitamin supplementation and carrying certain genotypes may lower the risk of the baby developing CL/P.

Maternal genotypic effects for nonsyndromic CL/P have also been reported for several candidate genes (*RUNX2*, *TGFA*, and *CBS*; Rubini et al., 2005; Sull et al., 2008; Sull et al., 2009). Several studies using cellular models or animal models suggested *RUNX2* may function through an epigenetic mechanism or by regulating expression of a maternally imprinted gene (Prescott et al., 2001; Flores et al., 2004; Weinstein et al., 2004; Bertaux et al., 2006). Sull et al. (2008) found several SNPs in *RUNX2* suggesting possible imprinting effects in a 46.7 kb region (from *rs910586* to *rs2396442*). In our study, we found suggestive evidence of excess maternal transmission for three SNPs (*rs16873396*, *rs16873401*, and *rs675613*) using the TAT, but only *rs675613* showed significant imprinting effects (with an estimated RR = 2.2 by the PO-LRT). This SNP is located about 67 kb downstream of the region previously reported by Sull et al. (2008). We did not find evidence for imprinting using the most significant SNPs in Sull's study and the effect size for *rs675613* was smaller in these data. Subjects in Sull's study were drawn from four populations including Maryland, Singapore, Taiwan, and Korea, which might account for this difference. The PO-LRT estimates an imprinting effect while controlling for possible maternal genotypic effects. By conditioning on the child's genotype, it also removes dependence on genotype effects of the cases, and remains valid when applied to markers in LD with an unobserved causal variant (Shi et al., 2007). These results suggest that *RUNX2* may influence the risk of CL/P through possible maternal-specific effects, although further studies are clearly needed to confirm this finding.

The case-parent trio design is robust to population stratification, and provides a unique opportunity to investigate parent-of-origin effects (Cordell et al., 2004; Starr et al., 2005). The present study found some evidence of potential maternal over-transmission for markers in *RUNX2* and risk of nonsyndromic CL/P. Moreover, these results showed evidence for association in the context of GxE interaction between markers in *RUNX2* and both ETS and maternal multivitamin supplementation. ETS, in particular, seems to increase the risk of nonsyndromic CL/P in cases carrying certain genotypes. The exposure rate of ETS in this sample was high (40.5%), reflecting the high prevalence of smoking among Chinese men (about 60%; Gu et al., 2004). In addition, mothers in mainland China had much lower use of multivitamin supplementation compared to those in Taiwan (6.8 vs. 14.1%). If this observation is confirmed, such a GxE interaction will create opportunities for effective intervention to reduce the risk of nonsyndromic CL/P.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Significance of individual single-nucleotide polymorphisms (SNPs) and sliding window haplotypes for the *RUNX2* gene in 326 nonsyndromic cleft lip with or without cleft palate trios. The $-\log_{10}(p\text{ value})$ for the overall test for an individual SNP (dots) and for sliding windows of haplotypes of two to five SNPs (dots connected by lines) is presented. Only haplotypes with $p\text{ values} < 0.1$ are shown here. The plot was produced using R package *snp.plotter* (Luna and Nicodemus, 2007).

Figure 2.

Testing for gene-environment (GxE) interaction for common maternal exposures in 326 cleft palate (CL/P) case-parent trios from Chinese populations. **(A)** Single-nucleotide polymorphisms (SNPs) with nominal significant p values in the likelihood ratio test (LRT) with 2 df testing gene (G) and G-environmental tobacco smoke (GxETS) were plotted here. Odds ratios and confidence intervals (CIs) are drawn on a logarithmic scale. The relative risks (RRs) and 95% CIs were obtained from conditional logistic regression using additive model. Solid circles represent estimated RRs for a heterozygous genotype among those cases whose mothers were not exposed to ETS (for *rs16873396*, *rs16873401*, *rs545289*, and *rs1200425*, the reference allele was the minor allele; for the other eight SNPs, the reference allele was the major allele). Open circles represent RRs among those cases with a mother who was exposed to ETS. The p values from LRT with 2 df or 1 df are listed for each SNP. **(B)** Triangles represent the GxE interaction 2 df test of G and GxE interaction, squares represent the 1 df test of GxE only. Haplotypes of 2-loci and 3-loci are connected by dashed lines (only nominally significant single SNPs [2 df $p < 0.05$ or 1 df $p < 0.05$] and haplotypes [$p < 0.01$] were plotted). SNPs showing evidence of linkage in the presence of linkage disequilibrium (LD) without considering GxE are noted in bold.

Table 1

Characteristics of 326 Chinese Nonsyndromic Cleft Lip with or without Cleft Palate (CL/P) Cases

Number of probands	Cleft lip only	Cleft lip with cleft palate	Total
Gender			
Male	60	165	225
Female	36	65	101
Exposure			
Multivitamin supplementation	9	19	28
Environmental tobacco smoke	45	87	132

Table 2

TDT analysis of 5 significant SNPs in *RUNX2* gene among 326 Chinese non-syndromic CL/P case parent trios

No.	SNP name	Position ^a	MAF			OR ^b	Chi-square	<i>p</i> value	TDT
				T	NT				Permuted <i>p</i> value ^c
1	<i>rs16873396</i>	45481992	0.103	67	41	1.634	6.259	0.012	0.257
2	<i>rs10948234</i>	45482586	0.182	104	75	1.387	4.698	0.030	0.476
3	<i>rs16873401</i>	45487619	0.102	67	41	1.634	6.259	0.012	0.257
4	<i>rs545289</i>	45632924	0.325	120	175	0.686	10.254	0.001	0.035
5	<i>rs1200425</i>	45635110	0.304	121	158	0.766	4.907	0.027	0.461

^aBased on National Center for Biotechnology Information Human Genome build 36.1.

^bOdds ratio of transmission of minor allele.

^cPermuted *p* value using PLINK (v 1.06) based on 10,000 times permutation.

TDT, transmission disequilibrium test; SNPs, single nucleotide polymorphisms; CL/P, cleft lip with or without cleft palate; MAF, minor allele frequency; T, transmitted; NT, not transmitted; OR, odds ratio.

Table 3

Parental transmission and maternal genotypic effect of three SNPs in RUNX2 in 326 CL/P trios^a

SNP name	Paternal				Maternal				PO-LRT	
	TAT				TAT				OR (imprinting)	
	T	NT	OR ^a	p value	T	NT	OR ^a	p value	OR (imprinting)	p value
rs16873401	27	16	1.688	0.093	26	13	2.000	0.037	1.20	0.699
rs675613	29	34	0.853	0.529	30	16	1.875	0.039	2.20	0.046
rs545289	35	55	0.636	0.035	26	55	0.473	0.001	1.46	0.345

^aOR(transmission): odds ratio of transmission of minor allele.

SNPs, single nucleotide polymorphisms; CL/P, cleft lip with or without cleft palate; TAT, transmission asymmetry test; PO-LRT, parent-of-origin likelihood ratio test; T, transmitted; NT, not transmitted; OR, odds ratio.