ORAL DISEASES

Oral Diseases (2011) 17, 304–308 doi:10.1111/j.1601-0825.2010.01741.x
© 2010 John Wiley & Sons A/S
All rights reserved
www.wiley.com

ORIGINAL ARTICLE

Replication of two novel susceptibility loci for non-syndromic orofacial clefts in a Chinese population

Y Pan[1], J Ma[1], W Zhang[1], Y Wang[1], Y Wang[2], H Zhang[2], M Wang[3], Z Zhang[3], L Wang[1]

[1]*Institute of Stomatology, Nanjing Medical University, Nanjing;* [2]*Xuzhou First People's Hospital, Xuzhou;* [3]*Department of Epidemiology, Nanjing Medical University, Nanjing, China*

OBJECTIVES: Non-syndromic orofacial clefts (NSOC) are the most common developmental disorders in human beings. Recently, two genome-wide association studies in European Caucasians identified three novel NSOC susceptibility loci: rs987525 on 8q24, rs7078160 on 10q25.3, and rs223371 on 17q22. The aim of this study was to determine the association of these polymorphisms with NSOC susceptibility and its subgroups in a Chinese Han population.

MATERIAL AND METHODS: In this study, 199 NSOC patients and 210 healthy individuals were recruited. SNP rs987525 was not genotyped because of its low frequency in the study subjects. The other two polymorphisms (rs7078160 and rs223371) were respectively genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and Taqman-MGB assay.

RESULTS:Overall genotype distributions of rs7078160 and rs223371 polymorphisms were consistent with Hardy–Weinberg equilibrium test. The allele and genotype frequencies of the two polymorphisms were not significantly different between cases and controls. Further analysis indicated that none of the genotypes was associated with increased risk of NSOC. Similar results were also found when all cleft cases were stratified by cleft types.

CONCLUSION: Our findings are consistent with a lack of involvement of the rs7078160 and rs223371 polymorphisms in the development of NSOC in the Chinese Han population.

Oral Diseases (2011) **17**, 304–308

Keywords: genome-wide association studies; non-syndromic orofacial clefts; polymorphism; molecular epidemiology

Correspondence: Lin Wang, Stomatological Hospital of JiangSu Province, 136 Hanzhong Rd, Nanjing 210029, China. Tel: +86 25 850 31847, Fax: +86 25 865 16414, E-mail: lw603@njmu.edu.cn
Received 26 March 2010; revised 30 June 2010; accepted 7 July 2010

Introduction

Non-syndromic orofacial clefts (NSOC) are the most common developmental disorders in human beings, which may lead to not only masticatory dysfunction, speech alteration, esthetic problems, malocclusion and potential psychiatric disorders to the patients, but also long-term burdens to their families and society (Stanier and Moore, 2004; Vieira, 2008). They may occur as sporadic forms or present with familial aggregation, inherited in a dominant or recessive pattern (Mossey *et al*, 2009). In most circumstances, these defects can be anatomically divided into three subgroups: the lip only (CLO), both the lip and palate (CLP), and the palate only (CPO) (Letra *et al*, 2007a,b). CLO and CLP are traditionally collapsed to a single group of cleft lip with or without cleft palate (CL/P). However, recent studies indicate that these two categories may have distinct genetic origins and should, when feasible, be analyzed separately (Harville *et al*, 2005; Rahimov *et al*, 2008).

The incidence of NSOC occurs in a wide geographic distribution from 1/500 to 1/2000 (Harville *et al*, 2007), with Asian and Native American populations having the highest rates and African populations the lowest (Huang *et al*, 2009). The Chinese people are susceptible to these defects with a high incidence of 1.82/1000 livebirths (Dai *et al*, 2009). The incidence variation of NSOC among ethnic groups also indicates that different susceptibility loci might be involved.

Both environmental and genetic risk factors are responsible for NSOC (Carinci *et al*, 2000). Genetic linkage and association analysis as well as genome-wide family-based scans have identified several potential causative genes (e.g. *MSX1, BMP4, TBX22, MTHFR* and *TGFA*) and chromosomal regions (e.g. 1q, 2p, 4q, 6p, 14q, 17q and 19q) for these defects (Grant *et al*, 2009). Syndromic forms of clefts have also provided much insight into the genetic architecture of non-syndromic forms (Vieira *et al*, 2008). For example, the *IRF6* gene, responsible for van der Woude's syndrome and popliteal pterygium syndrome (PPS), is also strongly associated with the isolated form of clefts (Marazita *et al*, 2004; Zucchero *et al*, 2004). Further-

more, animal models such as mouse (Liu *et al*, 2007) and zebrafish (Eberhart *et al*, 2008) have also been described where the causative genes have been identified.

During the past few years, genome-wide association studies (GWAS), by tagging polymorphisms across the whole genome and usually accompanied by large sample size and multiple-stage replications, are emerging as a powerful technique in complex diseases. This approach relies on the foundation of the international human HapMap Project and the fact that genetic variation at one locus can predict with high probability genetic variation at an adjacent locus. This haplotypic structure of the human genome indicates that it is possible to survey the genome for common variability associated with disease susceptibility simply by genotyping approximately 500 000 judiciously chosen markers in the genome of several thousand cases and controls (Hardy and Singleton, 2009).

To date, there have been four GWAS concerning NSOC (Birnbaum *et al*, 2009; Jugessur *et al*, 2009; Mangold *et al*, 2009; Marazita *et al*, 2009). Among them, two large-scale GWAS had been recently reported. Birnbaum *et al* conducted the first GWAS of NSOC in European Caucasians and found a major susceptibility locus (SNP rs987525) for NSOC on 8q24.21 (Birnbaum *et al*, 2009). Recently, another GWAS, also in European Caucasians, successfully identified two novel NSOC susceptibility loci on 17q22 (SNP rs223371) and 10q25.3 (SNP rs7078160), both of which are located in close vicinity to the promising candidate genes for these defects (Mangold *et al*, 2009).

The encouraging results in the GWAS thus impel us to investigate the association between the SNPs reported in Caucasians and NSOC susceptibility in the Chinese populations. Therefore, in this study, we recruit three risk loci (i.e. rs987525, rs7078160 and rs223371) mentioned in the above GWAS and replicate their associations with susceptibility of NSOC in a hospital-based case–control study in a Chinese Han population.

Materials and methods

Study subjects

All the participants were genetically unrelated Han Chinese in Nanjing and its surrounding regions. In brief, 199 NSOC cases without the presence of associated congenital anomalies or syndromes and 210 healthy controls, frequency-matched to the cases on gender, age, and residential area, were consecutively recruited from the Stomatological Hospital of JiangSu Province and Nanjing Children's Hospital, from August 2008 to January 2010. All the participants were carefully examined by two experienced doctors based on detailed diagnostic information from medical records. In addition, general characteristics, including age, gender, ethnicity, birth defects were documented. After written informed consent was obtained from each participant or their guardians, about 3–5 ml of venous blood sample was collected for genetic analysis. This hospital-based case–control study was approved by the institutional review board of Nanjing Medical University.

Single nucleotide polymorphisms re-selection

By examining the international HapMap Project (http://hapmap.ncbi.nlm.nih.gov/), however, we found that rs987525 identified in the first GWAS was extremely rare (minor allele frequency: 0.044) in CHB (Han Chinese in Beijing), suggesting that the role this SNP played in the etiology of NSOC in Chinese Han populations, if any, may not be as important as they did in Caucasians. Therefore, in the following study, we recruited the other two SNPs in the second GWAS (i.e. rs7078160 and rs223371) and replicated their associations with risk of NSOC.

DNA extraction and genotyping analysis

As previously described (Pan *et al*, 2008), genomic DNA was extracted from a leukocyte pellet by proteinase K digestion and followed by phenol–chloroform extraction and ethanol precipitation. The DNA pellet was resuspended in TE (Tris-EDTA) buffer and the purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm.

The PCR-restriction fragment length polymorphism (RFLP) assay was designed to detect SNP rs7078160 (G > A) with the following primers: 5′-AGAGCCA TAGGAAGTTG AA-3′ (forward) and 5′-TTCCCATT-TCTCGTCTGC-3′ (reverse). The140 bp PCR products were generated and then digested overnight with the restriction enzyme Hpy188 I (New England BioLabs, Beverly, MA, USA) and separated on a 3% agarose gel. The G allele of rs7078160, creating a restriction endonuclease site, resulted in two fragments of 97 and 43 bp, whereas the A allele did not produce such a site and appeared as a single 140 bp fragment (Figure 1).

The SNP rs223371 (C > A) polymorphism was analyzed by the conventional TaqMan-MGB method. PCR was performed using the following primers: 5′-AATCTAGATAACCTTGAGTATGGTGATGAC-3′ (forward) and 5′-CAGTTTGTGACTTGTCTTCT-CTTTCTTTAG-3′ (reverse) and probes for detection of the polymorphism were FAM-ATACCACAAT-AAAAGC-MGB and VIC-TACCACACTAAAAGC-MGB.

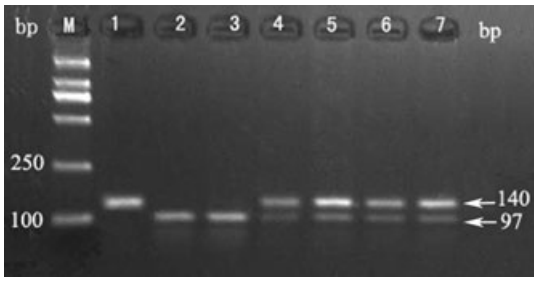

Figure 1 PCR-RFLP genotyping of rs7078160. Lane M: DL2000 DNA marker; lane 1: AA genotype; lane 2, 3: GG genotype; lane 4, 5, 6, 7: AG genotype

Genotyping was performed without knowing the subject's case or control status. To validate genotype identification, 10% of the samples were randomly repeated and the results were totally concordant.

Statistical analysis

Differences in demographic characteristics and frequencies of the genotypes and alleles of the two SNPs between cases and controls were evaluated by using chi-squared test (for categorical variables) or Student's *t*-test (for continuous variables). Hardy–Weinberg equilibrium of the genotype distribution of controls was tested by a goodness-of-fit χ^2 test. The association between the SNPs and NSOC risk was estimated by computing the odds ratios (ORs) and 95% confidence interval (CI) from logistic regression analyses. All tests were two-sided by using the SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA). A *P*-value of <0.05 (two-sided) was used as the criterion of statistical significance.

Results

The demographic characteristics of the study subjects are shown in Table 1. As expected, there were no significant differences on age and gender distributions between cases and controls ($P = 0.631$ and 0.776, respectively), suggesting that our frequency matching of the demographic characteristics was satisfactory. Among the total 199 NSOC cases, 90 were cleft lip only (CLO), 98 were cleft lip with cleft palate (CLP), and 11 were cleft palate only (CPO).

The genotype and allelic distributions of the two SNPs in cases and controls are shown in Tables 2 and 3. The observed genotype frequencies in controls were all in Hardy–Weinberg equilibrium ($P = 0.61$ for rs7078160 and $P = 0.77$ for rs223371, respectively), suggesting good homogeneity within the study subjects.

For rs7078160, 13 (6.1%) controls for rs7078160 failed to genotype because of poor DNA quantity and/or quality. As shown in Table 2, the frequencies of the rs7078160 genotypes, GG, AG, and AA, in the controls were 25.3, 51.7, and 23.0%, respectively. The genotype frequencies in all cleft cases were similar to those in the controls: GG, AG, and AA genotypes were 27.2, 44.5, and 28.3%, respectively ($P = 0.23$). Similarly, for rs223371, the frequencies of the AA, AC, and CC genotypes were 42.3, 44.7, and 13.0%, respectively, among the controls and 46.7, 37.2, and 16.1%, respectively, among the cases. These differences were not statistically significant ($P = 0.27$) (Table 3).

The potential risk allele frequency of rs7078160 and rs223371 (i.e. rs7078160 A allele and rs223371 C allele) was 0.49 and 0.35, respectively, among the controls and 0.48 and 0.34, respectively, among the cases, but the differences were not statistically significant ($P = 0.48$ for rs7078160 and $P = 0.87$ for rs223371).

Further logistic regression analysis revealed that neither the heterozygote (i.e. rs7078160 AG genotype and rs223371 AC genotype) nor the mutated homozygote (i.e. rs7078160 AA and rs223371 CC) of these two SNPs was associated with increased risk of NSOC, compared with their corresponding wild homozygote (i.e. rs7078160 GG and rs223371 AA). When we combined the heterozygote and mutated homozygote assuming a dominant model (i.e. rs7078160 AG/AA and rs223371 AC/CC), non-significant associations were observed between the variant genotypes and NSOC risk (Tables 2 and 3).

In the stratified analysis, four specific subgroups such as CL/P (cleft lip with or without cleft palate), CLO (cleft lip only), CLP (cleft lip with cleft palate), and CPO (cleft palate only) were analyzed. Again, we found no significant differences in genotype and allele distributions between subgroup cases and controls. Furthermore, none of the genotypes was associated with increased risk of any specific subgroup (Tables 2 and 3).

Table 1 Characteristic information of non-syndromic orofacial clefts cases and controls

Variables	*All cleft cases (n = 199)*	*Controls (n = 210)*	P
Age, mean (s.d.)	9.58 ± 0.72	7.36 ± 0.56	0.631[a]
Gender			
Male	92	88	0.776[b]
Female	107	122	
Subgroups			
CLO	90		
CLP	98		
CPO	11		

CLO, cleft lip only; CLP, cleft lip with cleft palate; CPO, cleft palate only;
[a]Independent-samples *T*-test;
[b]Two-sided chi-squared test.

Discussion

The NSOC are complex and heterogeneous diseases. The past few years have witnessed great advances in gene-identification for these complex birth defects, providing an unprecedented opportunity to identify genetically susceptible individuals (Jugessur *et al*, 2008).

There have been four GWAS concerning NSOC, and two recent GWAS in European Caucasians revealed three most promising risk variants for NSOC susceptibility. Thus, replication studies are necessary and exigent to elucidate the significance of these SNPs in NSOC risk in a Chinese Han population.

Given the rarity of rs987525 on 8q24 in the subjects, however, this variant was not recruited in the subsequent study. We successfully genotyped the other two variants (rs7078160 and rs223371), unfortunately, on the basis of 199 NSOC cases and 210 healthy controls derived from Southern Chinese Han populations, similar frequencies of the alleles and genotypes were found and none of the genotypes was associated with increased risk of NSOC, suggesting neither of them might contribute to the development of NSOC in the Chinese Han population.

We subsequently stratified all cleft cases into four subgroups (i.e. CL/P, CLO, CLP, and CPO), with the purpose of improving the power of genetic analyses and

Table 2 Genotype and allelic distributions of rs7078160 in non-syndromic orofacial clefts cases and controls

rs7078160 (G > A)	*Controls (n = 197, %)*	*All cleft cases (n = 199, %)*	*CL/P (n = 188, %)*	*CLP (n = 98, %)*	*CLO (n = 90, %)*	*CPO (n = 11, %)*
Genotypes						
GG	50 (25.3)	53 (27.2)	52 (27.7)	33 (33.7)	19 (21.1)	1 (9.1)
GA	102 (51.7)	88 (44.5)	81 (43.1)	41 (41.8)	40 (44.4)	7 (63.6)
AA	45 (23.0)	58 (28.3)	55 (29.2)	24 (24.5)	31 (34.5)	3 (27.3)
P^a	–	0.23	0.20	0.22	0.11	0.47
OR (95% CI)						
AG *vs* GG	–	0.81 [0.50, 1.32]	0.76 [0.47, 1.24]	0.61 [0.34, 1.08]	1.03 [0.54, 1.96]	3.43 [0.41, 28.66]
AA *vs* GG	–	1.22 [0.70, 2.10]	1.18 [0.68, 2.04]	0.81 [0.42, 1.57]	1.81 [0.90, 3.65]	3.33 [0.33, 33.21]
GA/AA *vs* GG	–	0.94 [0.60, 1.47]	0.89 [0.57, 1.40]	0.67 [0.40, 1.14]	1.27 [0.70, 2.31]	3.40 [0.42, 27.24]
Alleles						
G	0.51	0.52	0.48	0.55	0.43	0.41
A	0.49	0.48	0.52	0.45	0.57	0.59
P^b	–	0.48	0.57	0.45	0.08	0.34

CL/P, cleft lip with or without cleft palate; CLP, cleft lip with cleft palate; CLO, cleft lip only; CPO, cleft palate only; OR, odds ratio; CI, confidence interval.
[a,b]Two-sided chi-squared test for the genotype and allele distributions between cases and controls.

Table 3 Genotype and allelic distributions of rs223371 in non-syndromic orofacial clefts cases and controls

rs223371 (A > C)	*Controls (n = 210, %)*	*All cleft cases (n = 199, %)*	*CL/P (n = 188, %)*	*CLP (n = 98, %)*	*CLO (n = 90, %)*	*CPO (n = 11, %)*
Genotypes						
AA	89 (42.3)	93 (46.7)	89 (47.3)	44 (45.4)	45 (49.5)	4 (36.4)
AC	94 (44.7)	74 (37.2)	69 (36.7)	41 (41.2)	28 (31.9)	5 (45.5)
CC	27 (13.0)	32 (16.1)	30 (16.0)	13 (13.4)	17 (18.6)	2 (18.1)
P^a	–	0.27	0.25	0.84	0.09	0.85
OR (95% CI)						
AC *vs* AA	–	0.75 [0.49, 1.15]	0.73 [0.48, 1.13]	0.86 [0.51, 1.44]	0.61 [0.35, 1.06]	1.18 [0.31, 4.55]
CC *vs* AA	–	1.13 [0.63, 2.04]	1.11 [0.61, 2.02]	0.97 [0.46, 2.07]	1.25 [0.62, 2.52]	1.65 [0.29, 9.50]
AC/CC *vs* AA	–	0.84 [0.57, 1.24]	0.82 [0.55, 1.22]	0.89 [0.55, 1.44]	0.75 [0.46, 1.23]	1.29 [0.37, 4.53]
Alleles						
A	0.65	0.66	0.66	0.66	0.65	0.59
C	0.35	0.34	0.34	0.34	0.35	0.41
P^b	–	0.87	0.78	0.77	0.88	0.59

CL/P, cleft lip with or without cleft palate; CLP, cleft lip with cleft palate; CLO, cleft lip only; CPO, cleft palate only; OR, odds ratio; CI, confidence interval.
[a,b]Two-sided chi-squared test for the genotype and allele distributions between cases and controls.

the accuracy of risk estimates for genetic counseling (Letra *et al*, 2007a,b). However, the genotype and allele frequencies are similarly distributed between each of the subgroups and the controls. In addition, none of the risk genotypes was found in association with any subgroup of NSOC.

These results seem to be a little frustrating; however, given the complicated heterogeneous nature of NSOC and the number of confounding factors (Carinci *et al*, 2003), this is not unexpected. In fact, accumulating inconsistent results have been obtained from genetic studies of complex traits, without exception of NSOC (Scapoli *et al*, 2005). For example, Rahimov *et al* recently reported an impressive association between a common SNP rs642961 (G > A, that disrupts the binding site of the transcription factor AP-2a) in the *IRF6* enhancer and NSOC (Rahimov *et al*, 2008). However, in a replication study in a Brazilian population, its association was not confirmed. It was presumed that the complex ethnic admixture of the Brazilian population may contribute to such result (Paranaiba *et al*, 2009).

Several limitations in our study should be addressed. First of all, the study was a hospital-based case–control study, the selection bias might not be avoidable and the subjects may not be representative of the general population. Secondly, we only recruited 199 cases and 210 controls in the study therefore the sample size might not be large enough to detect small effects from low penetrance genes. We only had 60% power at a 0.05 level or a smaller with level to detect an OR of 1.58 or greater, and 0.60 or smaller with an exposure frequency of 30%, given our study sample size.

Taken together, we fail to confirm the association between the two SNPs rs7078160 and rs223371 and NSOC risk, even its subgroups, in a Chinese Han population. The substantially different genetic backgrounds and environmental exposures between the

populations may make the results different. In the future, other well-designed studies with different ethnic populations are warranted to verify these findings.

Acknowledgements

We gratefully appreciated the support of the subjects who participated in this study. This study was supported in part by the National Natural Science Foundation of China (no: 30973361), China Postdoctoral Science Foundation (20090451235) and Postdoctoral Science Foundation of Jiangsu Province (0802001B).

References

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

Carinci F, Pezzetti F, Scapoli L *et al* (2000). Genetics of nonsyndromic cleft lip and palate: a review of international studies and data regarding the Italian population. *Cleft Palate Craniofac J* **37:** 33–40.

Carinci F, Pezzetti F, Scapoli L *et al* (2003). Recent developments in orofacial cleft genetics. *J Craniofac Surg* **14:** 130–143.

Dai L, Zhu J, Mao M *et al* (2009). Time trends in oral clefts in Chinese newborns: data from the Chinese national birth defects monitoring network. *Birth Defects Res A Clin Mol Teratol* **88:** 41–47.

Eberhart JK, He X, Swartz ME *et al* (2008). MicroRNA mirn140 modulates Pdgf signaling during palatogenesis. *Nat Genet* **40:** 290–298.

Grant SF, Wang K, Zhang H *et al* (2009). A genome-wide association study identifies a locus for nonsyndromic cleft lip with or without cleft palate on 8q24. *J Pediatr* **155:** 909–913.

Hardy J, Singleton A (2009). Genomewide association studies and human disease. *N Engl J Med* **360:** 1759–1768.

Harville EW, Wilcox AJ, Lie RT *et al* (2005). Cleft lip and palate versus cleft lip only: are they distinct defects? *Am J Epidemiol* **162:** 448–453.

Harville EW, Wilcox AJ, Lie RT *et al* (2007). Epidemiology of cleft palate alone and cleft palate with accompanying defects. *Eur J Epidemiol* **22:** 389–395.

Huang Y, Wu J, Ma J *et al* (2009). Association between IRF6 snps and oral clefts in West China. *J Dent Res* **88:** 715–718.

Jugessur A, Rahimov F, Lie RT *et al* (2008). Genetic variants in IRF6 and the risk of facial clefts: single-marker and haplotype-based analyses in a population-based case-control study of facial clefts in Norway. *Genet Epidemiol* **32:** 413–424.

Jugessur A, Shi M, Gjessing HK *et al* (2009). Genetic determinants of facial clefting: analysis of 357 candidate genes using two national cleft studies from scandinavia. *PLoS One* **4:** e5385.

Letra A, Menezes R, Granjeiro JM *et al* (2007a). Defining subphenotypes for oral clefts based on dental development. *J Dent Res* **86:** 986–991.

Letra A, da Silva RA, Menezes R *et al* (2007b). Studies with MMP9 gene promoter polymorphism and nonsyndromic cleft lip and palate. *Am J Med Genet A* **143:** 89–91.

Liu KJ, Arron JR, Stankunas K *et al* (2007). Chemical rescue of cleft palate and midline defects in conditional gsk-3beta mice. *Nature* **446:** 79–82.

Mangold E, Ludwig KU, Birnbaum S *et al* (2009). Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. *Nat Genet* **42:** 24–26.

Marazita ML, Murray JC, Lidral AC *et al* (2004). Meta-analysis of 13 genome scans reveals multiple cleft lip/palate genes with novel loci on 9q21 and 2q32-35. *Am J Hum Genet* **75:** 161–173.

Marazita ML, Lidral AC, Murray JC *et al* (2009). 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* **68:** 151–170.

Mossey PA, Little J, Munger RG *et al* (2009). Cleft lip and palate. *Lancet* **374:** 1773–1785.

Pan Y, Wang L, Ma J *et al* (2008). PAX9 polymorphisms and susceptibility to sporadic tooth agenesis: a case-control study in southeast china. *Eur J Oral Sci* **116:** 98–103.

Paranaiba LM, Bufalino A, Martelli-Junior H *et al* (2009). Lack of association between IRF6 polymorphisms (rs2235371 and rs642961) and non-syndromic cleft lip and/or palate in a Brazilian population. *Oral Dis* **16:** 193–197.

Rahimov F, Marazita ML, Visel A *et al* (2008). Disruption of an ap-2alpha binding site in an IRF6 enhancer is associated with cleft lip. *Nat Genet* **40:** 1341–1347.

Scapoli L, Palmieri A, Martinelli M *et al* (2005). 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* **76:** 180–183.

Stanier P, Moore GE (2004). Genetics of cleft lip and palate: syndromic genes contribute to the incidence of non-syndromic clefts. *Hum Mol Genet* **13 Spec No 1:** R73–R81.

Vieira AR (2008). Unraveling human cleft lip and palate research. *J Dent Res* **87:** 119–125.

Vieira AR, McHenry TG, Daack-Hirsch S *et al* (2008). Candidate gene/loci studies in cleft lip/palate and dental anomalies finds novel susceptibility genes for clefts. *Genet Med* **10:** 668–674.

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

Copyright of Oral Diseases is the property of Wiley-Blackwell and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.