



Title	An update on the aetiology of orofacial clefts
Author(s)	Wong, FK; Hägg, U
Citation	Hong Kong Medical Journal, 2004, v. 10 n. 5, p. 331-336
Issued Date	2004
URL	http://hdl.handle.net/10722/44554
Rights	Creative Commons: Attribution 3.0 Hong Kong License

FK Wong 王奉基
U Hägg 賀爾彬

An update on the aetiology of orofacial clefts

與口面裂病原有關的新近發現

Objective. To review recent data on the aetiology of cleft lip and palate.

Data sources. MEDLINE literature search (1986-2003).

Study selection. Literature and data on aetiology of cleft lip and palate using the following key words: 'cleft lip', 'cleft palate', 'aetiology', and 'genetics'.

Data extraction. Relevant information and data were reviewed by the authors.

Data synthesis. Cleft lip and palate is one of the most common types of congenital malformation. The aetiology seems complex, but genetics plays a major role. Recently several genes causing syndromic cleft lip and palate have been discovered. Three of them—namely T-box transcription factor-22 (*TBX22*), poliovirus receptor like-1 (*PVRL1*), and interferon regulatory factor-6 (*IRF6*)—are responsible for causing X-linked cleft palate, cleft lip/palate-ectodermal dysplasia syndrome, and Van der Woude's and popliteal pterygium syndromes, respectively; they are also implied in non-syndromic cleft lip and palate. The nature and function of these genes vary widely, illustrating high vulnerability within the craniofacial developmental pathways. The aetiological complexity of non-syndromic cleft lip and palate is also exemplified by the large number of candidate genes and loci.

Conclusions. The aetiology of non-syndromic cleft lip and palate is still largely unknown, but mutations in candidate genes have already been identified in a small proportion of cases of non-syndromic cleft lip and palate. Determining the relative risk of cleft lip and palate, on the basis of genetic background and environmental influence, including smoking, alcohol use, and dietary factors, will aid in genetic counselling and the development of future preventive measures.

目的：總覽新近取得與唇顎裂病原有關的資料。

資料來源：MEDLINE 文獻檢索（1986 年至 2003 年）。

研究範圍：以 'cleft lip'（唇裂）、'cleft palate'（顎裂）、'aetiology'（病原）和 'genetics'（遺傳）為關鍵詞，檢索與唇顎裂有關的文獻和數據。

資料選用：由本文作者回顧相關資料和數據。

資料綜合：唇顎裂是其中一種最常見的先天性畸形，病原相當複雜，而遺傳也是一個重要元素。最近醫學界發現了幾個造成綜合徵狀唇顎裂的遺傳基因，其中 T-box 基因家族轉錄調控因子 22 (*TBX22*)、骨髓灰質炎受體樣 1 (*PVRL1*)、干擾素調節因子 6 (*IRF6*)，按序分別是造成 X 連鎖顎裂、唇顎裂外胚層發育不良綜合症、Van der Woude 及腮翼狀肉綜合症的原因。此外，這三個基因亦可見於非綜合徵狀唇顎裂的病原。這些基因的性質和功能相差很大，反映出顱顏發展過程是非常脆弱的，而大量候選基因和基因座的存在又證明了非綜合徵狀唇顎裂病原的複雜性。

結論：造成非綜合徵狀唇顎裂的病原大部分至今未明，不過在少數非綜合徵狀唇顎裂病例中已確認了候選基因曾發生突變。從遺傳背景，以及吸煙、飲用酒精類飲品、飲食習慣等環境因素影響來判斷出現唇顎裂的機會率，有助於遺傳諮詢和未來預防措施的發展。

Key words:

Cleft lip;
Cleft palate;
Genetics;
Mutation;
Syndrome

關鍵詞：

唇裂；
顎裂；
遺傳學；
突變；
綜合徵狀

Hong Kong Med J 2004;10:331-6

Faculty of Dentistry, The University of
Hong Kong, Pokfulam Road, Hong Kong
FK Wong, Spec Orthod, PhD
U Hägg, OdontDr, FHKAM (Dental Surgery)

Correspondence to: Dr FK Wong
(e-mail: funwon@netvigator.com)

Introduction

Cleft lip and palate (CLP) comprises a group of the most common congenital malformations. Immediately after birth, individuals with CLP have facial deformation, feeding problems, and frequent middle ear infection, the treatments of which require interventions from multiple disciplines. At the age of speech

acquisition, speech therapy is often needed to correct problems resulting from muscular defects of the cleft. As the individual continues to grow, defects in tooth development and malocclusion require dental and sometimes surgical treatment. The lengthy series of treatment from birth to adulthood is a heavy burden for the patient, family, and society. Various efforts have been made to understand the aetiology of CLP so as to predict its occurrence and to prevent it from occurring in the future. In recent years, advances in genetics and molecular biology have begun to reveal the basis of craniofacial development, and a number of genes associated with CLP have been identified. Studies of the combined genetic and environmental cause of CLP are also growing. This article reviews the recently discovered genes involved in CLP, and provides an update on the aetiological factors underlying this common malformation.

Embryology

The lip and palatal regions are developed from the embryonic primary and secondary palates. The primary palate contributes to the lip, anterior tooth-bearing alveolus, and the anterior palate up to the incisal foramen. The secondary palate contributes to the remaining hard and soft palates. During the fourth week of gestation, neural crest cells from the anterior neural tube migrate to form the facial primordia, from which the nasal and the lateral maxillary processes fuse to form the primary palate. The secondary palate begins to form in the sixth week of gestation. Palatal shelves initially appear as two downward extensions from the inner side of the left and right maxilla along the lateral surface of the tongue. In the ninth week, the two palatal shelves undergo a rapid horizontal transformation, by moving over the tongue and fusing with each other and with the nasal septum. Failure in the fusion of primary or secondary palates leads to clefts of the respective areas. Thus, CLP appears commonly as cleft lip with or without cleft palate or isolated cleft palate.

Clinically, when CLP appears with other (usually two or more) malformations in recognisable patterns, it is classified as syndromic CLP (SCLP). If it appears as an isolated defect or if syndromes cannot be identified, the term non-syndromic CLP (NSCLP) is used. The number of CLP syndromes is large and still growing. A search of the *Online Mendelian Inheritance in Man* database using the term 'cleft lip' yielded more than 200 entries and a search using 'cleft palate' yielded close to 400.¹ The distinction between NSCLP and SCLP, however, is sometimes not clear-cut. In families with SCLP, some affected members may present with only CLP, because of variable expression of the syndrome. On the other hand, more than 20% of patients with NSCLP were found to have associated congenital malformations in one study.² Thus, some cases of SCLP and NSCLP might share a common aetiology.

Recently discovered genes causing orofacial cleft syndromes

T-box transcription factor-22

X-linked cleft palate (CPX) is characterised by isolated cleft palate and ankyloglossia (tongue-tie); clinical expression of CPX is highly variable. High-arched palate, bifid uvula, or ankyloglossia could be the only presenting sign in affected males. Female carriers could be asymptomatic or they could express full features of CPX. The syndrome has been found in a number of large families who show inheritance in a Mendelian X-linked semi-dominant pattern. By using genetic linkage analysis, Stanier et al³ located the disease gene locus to chromosome Xq21. Braybrook et al⁴ performed extensive mutation analysis of candidate genes in the region and found mutations of the T-box transcription factor-22 gene (*TBX22*) in a large Icelandic family with CPX and in several smaller families from other countries. Animal experiments showed that expression of *TBX22* was highly restricted to the palatal shelves just before their elevation to adopt a horizontal position, and at the base of the tongue corresponding to the frenulum, both of these expression patterns closely matched the clinical presentation of CPX. Involvement of *TBX22* in NSCLP has recently been indicated from a genome-wide sibling-pair analyses in which the chromosome Xcen-q region, where *TBX22* is located, showed promising multipoint logarithm of odds (LOD) scores.⁵ Mutation analysis of *TBX22* in these patients could reveal whether the gene is involved in NSCLP as well.

Poliovirus receptor like-1

Cleft lip/palate ectodermal dysplasia syndrome (CLPED) is characterised by cleft lip with or without cleft palate, hidrotic ectodermal dysplasia, syndactyly, and occasionally mental retardation. Two other syndromes—the Zlotogora-Ogur syndrome and Margarita Island ectodermal dysplasia—are also classified as CLPED. The inheritance of CLPED appears to be autosomal recessive. Using positional cloning, Suzuki et al⁶ identified mutations of the poliovirus receptor like-1 gene (*PVRL1*) in CLPED families from Margarita Island, Israel, and Brazil.

The protein product of *PVRL1* was initially identified as poliovirus receptor-related protein (PRR). Takahashi et al⁷ confirmed the function of PRR as a cell adhesion molecule, and they renamed it nectin-1. All three *PVRL1* mutations found in families with CLPED resulted in truncations in nectin-1, thereby destroying the nectin-afadin-ponsin (NAP)-dependent cell-adhesion system. In animal experiments, *PVRL1* was expressed at the medial edge epithelium of the palatal shelves and the skin surface epithelium—locations that corresponded to the clinical phenotypes of CLPED.⁶ Interestingly, heterozygous mutation of *PVRL1* (W185X) was associated with NSCLP in northern Venezuela.⁸ Thus, a certain proportion of NSCLP cases can be explained by *PVRL1* mutations.

Interferon regulatory factor-6

Van der Woude's syndrome (VDWS) is the most common form of NSCLP and accounts for 2% of all CLP cases.⁹ This syndrome is characterised by cleft lip with or without cleft palate, isolated cleft palate, pits or mucous cysts on the lower lip, and hypodontia. Popliteal pterygium syndrome (PPS) includes all the features of VDWS plus popliteal pterygium, syngnathia, distinct toe/nail abnormality, syndactyly, and genito-urinary malformations. Owing to their clinical similarities, VDWS and PPS were thought to be allelic—that is, caused by different mutations of the same gene.¹⁰ Clinical expressions of VDWS and PPS are also highly variable—for example, some family members with VDWS present only with hypodontia, and in PPS, popliteal pterygium is not always present.

The genetic locus for VDWS was localised to chromosome 1 back in 1990.¹¹ Through linkage and chromosomal analysis, the critical area for VDWS was gradually narrowed to 1q32-q41. In 1999, PPS was also linked to the same region.¹² In 2002, Kondo et al¹³ described a pair of monozygotic twins discordant for VDWS whose parents did not have the disorder. The VDWS in the affected twin was thought to arise from somatic mutation. Sequence analysis revealed a point mutation in the interferon regulatory factor-6 gene (*IRF6*), which is located within the VDWS critical region. Additional mutations of *IRF6* were found in 45 unrelated families with VDWS, as well as in 13 families with PPS, thereby confirming a common genetic aetiology in both syndromes.

In animal experiments, *IRF6* was expressed in tissues affected by both VDWS and PPS.¹³ The phenotypic heterogeneity of VDWS and PPS was shown to be due to different types of *IRF6* mutation. In most cases of VDWS, *IRF6* mutations produced a non-functional protein and haplo-insufficiency. The *IRF6* mutations, however, were missense mutations that affected the DNA binding domain and caused a dominant-negative effect, which resulted in severe phenotypes. A partial or modifying role of *IRF6* in NSCLP has been demonstrated in a study applying the transmission disequilibrium test, in which specific parental alleles at the VDWS locus were preferentially transmitted to the individuals with NSCLP.¹⁴

Candidate genes or loci for non-syndromic cleft lip and palate

Transforming growth factor-alpha

In 1989, Ardinger et al¹⁵ showed in a case-control study that transforming growth factor-alpha (TGFA) was associated with NSCLP. A number of follow-up studies in different populations provided mixed results. Machida et al¹⁶ sequenced the *TGFA* gene in a group of NSCLP patients and found five mutations that could be aetiological to orofacial clefts. The combined effect of *TGFA* mutation and environmental influence in NSCLP has been analysed by several groups of researchers. The rare *TGFA* variant

(*TaqI* C2 allele) and maternal smoking could increase the risk of cleft palate by 6 to 8 times¹⁷ and of cleft lip with or without cleft palate by 2 times.¹⁸ If multivitamins were not consumed during the first trimester of pregnancy and the baby is carrying the *TGFA TaqI* C2 allele, the relative risk for cleft lip with or without cleft palate increased by 3 to 8 times.¹⁹

Drosophila msh homeo box homolog-1

Mice lacking functional *Drosophila* msh homeo box homolog-1 (*MSX1*) develop a cleft of the secondary palate and tooth agenesis.²⁰ In humans, *MSX1* mutation was first shown to cause an autosomal dominant form of tooth agenesis.²¹ Subsequently, van den Boogaard et al²² described a family with a common pattern of tooth agenesis plus a mixture of cleft lip with or without cleft palate and cleft palate. Direct sequencing of *MSX1* revealed a disease-causing mutation. Recently, a large-scale sequence analysis of *MSX1* performed on 917 CLP patients identified mutations in 16 patients with cleft lip with or without cleft palate, or cleft palate alone, providing evidence that this gene could be involved in both forms of cleft.²³ The authors estimated that *MSX1* mutations contributed to 2% of all NSCLP cases. A recent study showed that the combined genetic background of rare variants of *TGFA* and *MSX1* could increase the risk of cleft palate by up to 9.7 times, demonstrating the significance of gene-gene interaction in the aetiology of NSCLP.²⁴

5,10-Methylenetetrahydrofolate reductase

The association between folic acid deficiency and neural tube defects has been well established. 5,10-Methylenetetrahydrofolate reductase (MTHFR) is the enzyme responsible for catalysing the conversion of 5,10-methylenetetrahydrofolate into 5-methyl-tetrahydrofolate in the folate metabolism pathway. The *MTHFR* C677T single-nucleotide polymorphism (SNP) is thermally labile and considered a risk factor of neural tube defects.²⁵ In NSCLP, the *MTHFR* C677T genotype in the mother conferred a risk of CLP in offspring that was increased by 4.6 times.²⁶ In peri-conceptual folic acid deficiency, the *MTHFR* thermally labile variant could lead to a risk of CLP that was increased by 10 times.²⁷

Transforming growth factor beta-3

Mice lacking functional gene encoding transforming growth factor beta-3 (*TGFB3*) displayed cleft palate because of defective adhesion of opposing palatal shelves.²⁸ In humans, *TGFB3* was associated with NSCLP in different populations.^{24,29} A newly discovered SNP of *TGFB3* (IVS5+104 A>G) increased the risk of CLP by up to 16 times in a Korean population.³⁰

Other genes and loci

The special AT-rich sequence-binding protein-2 gene (*SATB2*), located at chromosome 2q32-33, was disturbed in two unrelated patients with cleft palate.³¹ Involvement of

Table. Candidate genes or loci involved in the aetiology of non-syndromic cleft lip and palate, and available evidence

Chromosome	Gene*	Evidence			
		Mutation identified	Linkage	Linkage disequilibrium	Association
1p36	<i>MTHFR</i>			✓	✓
1q32	<i>IRF6</i>	✓			
2p13	<i>TGFA</i>			✓	✓
2q32	<i>SATB2</i>	✓			
4p26	<i>MSX1</i>	✓		✓	✓
4q21	<i>ACOD4</i>	✓			
6p23	-	✓	✓	✓	
11q23	<i>PVRL1</i>	✓			✓
14q24	<i>TGFB3</i>			✓	✓
19q13	<i>CLPTM1</i>	✓	✓	✓	
Xq12	<i>TBX22</i>	✓		✓	

* *MTHFR*: 5,10-methylenetetrahydrofolate reductase; *IRF6*: interferon regulatory factor-6; *TGFA*: transforming growth factor-alpha; *SATB2*: special AT-rich sequence-binding protein-2; *MSX1*: *Drosophila* msh homeo box homolog-1; *ACOD4*: acyl-coenzyme A desaturase-4; *PVRL1*: poliovirus receptor like-1; *TGFB3*: transforming growth factor beta-3; *CLPTM1*: cleft lip and palate-associated transmembrane protein-1; *TBX22*: T-box transcription factor-22

this gene in palatogenesis was confirmed by expression analysis. Although an initial search for *SATB2* mutations in 70 patients with cleft palate was negative, the gene could still be responsible for other CLP cases.

Markers from chromosome 4q21 have been linked to familial NSCLP.³² The acyl-coenzyme A desaturase-4 gene (*ACOD4*) from chromosome 4q21 was recently found disrupted in a family with cleft lip.³³ Mutation analysis of *ACOD4* in families with 4q-linked CLP or other patients with CLP might reveal more mutations.

Yoshiura et al³⁴ reported on a family with CLP in three generations; all affected members had a balanced translocation at chromosome 19q13. Breakpoint cloning revealed a novel gene termed 'cleft lip and palate-associated transmembrane protein-1' (*CLPTM1*). Eight rare variants of *CLPTM1* were found in 74 patients with NSCLP, but none was significantly associated with cleft lip or palate. The authors concluded that *CLPTM1* was not a major contributor to CLP. On the other hand, the same region of chromosome 19q13 has been implied in NSCLP through linkage³⁵ and transmission disequilibrium studies.³⁶ Thus, *CLPTM1* or other genes in this locus could still be associated with NSCLP.

Chromosome 6p23 has been indicated in NSCLP by linkage studies.^{5,37} Chromosomal aberrations involving 6p23 in patients with CLP have also been reported.³⁸ It is therefore likely that a yet unidentified CLP gene exists at chromosome 6p23. A summary of the above genes and loci implied in NSCLP, with chromosomal locations and available evidence, is shown in the Table.

Environmental factors

Smoking

The relationship between maternal smoking and CLP is not strong, but it is significant. Several studies have consistently yielded a relative risk of about 1.3 to 1.5.³⁹

When maternal smoking was considered together with certain genetic background, the combined effect was more significant. Furthermore, van Rooij et al⁴⁰ found that maternal glutathione s-transferase θ -1 (*GSTT1*) genotype, combined with smoking, could significantly increase the risk of CLP (odds ratio=4.9). And Beaty et al⁴¹ reported that maternal smoking and infant *MSX1* genotypes contributed to an elevated risk for CLP by 7.16 times.

Alcohol use

Heavy maternal drinking, apart from causing foetal alcohol syndrome, increases the risk of CLP. Munger et al⁴² showed that maternal drinking posted an increased risk from 1.5 to 4.7 times for CLP in a dose-dependent manner. The results were supported by Shaw and Lammer⁴³ that mothers who consumed more than five drinks per occasion had a 3.4 times the risk of CLP developing in the offspring. Low-level alcohol consumption, however, did not seem to increase the risk of orofacial clefts.⁴⁴ The link between alcohol consumption and genotypes on the risk of CLP has yet to be shown.

Use of folic acid and multivitamins

Shaw et al⁴⁵ reported that if vitamin supplements were not taken during early pregnancy, the risk for CLP could be tripled. Folic acid deficiency with the background of the *TGFA TaqI C2* genotype was also found to increase the risk of CLP.⁴⁶ In addition, defective maternal vitamin-dependent homocysteine metabolism is a risk factor for CLP in offspring: in a case-control study, mothers of patients with CLP had a significantly higher homocysteine level, lower level of whole-blood vitamin B6, and higher rate of hyperhomocysteinemia.⁴⁷ The role of folic acid supplementation in the prevention of CLP has been investigated in several studies. It seems that low-dose folic acid supplementation by fortifying cereal grain products could not protect against CLP.⁴⁸ Only a very high dose of supplementary folic acid (10 mg/d) could reduce the risk of CLP significantly (65%).⁴⁹

Discussion

The complexity and heterogeneity of CLP, as shown by its extensive involvement in craniofacial syndromes and the number of anticipated candidate genes, could be anticipated from the long developmental duration of the primary and secondary palates. Disruption of the coordinated migration and fusion of various facial processes by genetic, environmental, or combined factors at any timepoint could lead to CLP. Genes, such as those encoding transcription factors (eg *TBX22*, *MSX1*), growth factors (eg *TGFA*, *TGFB3*), and adhesion molecules (eg *PVRL1*), have all been implicated in the aetiology of CLP. In the search of genes causing syndromic CLP, current methods of positional cloning or positional candidate approaches could be applied to families or patients. With the completion of the human genome sequence, gene discovery is accelerated by the availability of target sequences. Close collaboration between clinicians and scientists is still essential, as illustrated by the example of VDWS.

The future search for genes in NSCLP will not be straightforward. Mutations in SCLP genes or other candidate genes so far could only be found in a handful of NSCLP cases or families. Promising NSCLP gene candidates, such as *MSX1*, was expected to cause up to 2% of NSCLP cases.²³ The proportion was remarkably similar to that of VDWS in all CLP cases. Therefore, *MSX1* mutations may represent a new syndrome, which has variable penetrance for the CLP and tooth agenesis phenotypes.

Instead of directly searching for disease-causing mutations in NSCLP, studies exploring the relative risk imparted by candidate genes and gene-environmental interactions are becoming popular approaches, for several reasons. Firstly, the amount of sequence data, such as SNPs, on candidate genes is constantly increasing. These sequence data not only allow more CLP-associated mutations to be identified, but functional correlation may also be attributed to some of these subtle variations. Secondly, high-throughput genotyping such as DNA microarray analyses is now more readily available, so that a large number of candidate genes and SNPs could be tested simultaneously. In the near future, the relative contribution from these candidate CLP genes could be integrated into a genetic test for the weighed risk of CLP. Such data could provide additional information on prospective parents in genetic counselling. Preventive measures, including dietary supplementation or lifestyle modification, could then be prescribed accordingly.

References

1. Online Mendelian Inheritance in Man. National Library of Medicine website: <http://www.ncbi.nlm.nih.gov/omim/>. Accessed 2 Feb 2004.
2. Stoll C, Alembik Y, Dott B, Roth MP. Associated malformations in cases with oral clefts. *Cleft Palate Craniofac J* 2000;37:41-7.
3. Stanier P, Forbes SA, Arnason A, et al. The localization of a gene causing X-linked cleft palate and ankyloglossia (CPX) in an Icelandic kindred is between DXS326 and DXYS1X. *Genomics* 1993;17:549-55.
4. Braybrook C, Doudney K, Marcano AC, et al. The T-box transcription factor gene *TBX22* is mutated in X-linked cleft palate and ankyloglossia. *Nat Genet* 2001;29:179-83.
5. Prescott NJ, Lees MM, Winter RM, Malcolm S. Identification of susceptibility loci for nonsyndromic cleft lip with or without cleft palate in a two stage genome scan of affected sib-pairs. *Hum Genet* 2000;106:345-50.
6. Suzuki K, Hu D, Bustos T, et al. Mutations of *PVRL1*, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. *Nat Genet* 2000;25:427-30.
7. Takahashi K, Nakanishi H, Miyahara M, et al. Nectin/PRR: an immunoglobulin-like cell adhesion molecule recruited to cadherin-based adherens junctions through interaction with Afadin, a PDZ domain-containing protein. *J Cell Biol* 1999;145:539-49.
8. Sozen MA, Suzuki K, Tolarova MM, Bustos T, Fernandez Iglesias JE, Spritz RA. Mutation of *PVRL1* is associated with sporadic, non-syndromic cleft lip/palate in northern Venezuela. *Nat Genet* 2001;29:141-2.
9. Burdick AB. Genetic epidemiology and control of genetic expression in van der Woude syndrome. *J Craniofac Genet Dev Biol Suppl* 1986;2:99-105.
10. Bixler D, Poland C, Nance WE. Phenotypic variation in the popliteal pterygium syndrome. *Clin Genet* 1973;4:220-8.
11. Murray JC, Nishimura DY, Buetow KH, et al. Linkage of an autosomal dominant clefting syndrome (Van der Woude) to loci on chromosome 1q. *Am J Hum Genet* 1990;46:486-91.
12. Lees MM, Winter RM, Malcolm S, Saal HM, Chitty L. Popliteal pterygium syndrome: a clinical study of three families and report of linkage to the Van der Woude syndrome locus on 1q32. *J Med Genet* 1999;36:888-92.
13. Kondo S, Schutte BC, Richardson RJ, et al. Mutations in *IRF6* cause Van der Woude and popliteal pterygium syndromes. *Nat Genet* 2002;32:285-9.
14. Houdayer C, Bonaiti-Pellie C, Erguy C, et al. Possible relationship between the van der Woude syndrome (vWS) locus and nonsyndromic cleft lip with or without cleft palate (NSCL/P). *Am J Med Genet* 2001;104:86-92.
15. Ardinger HH, Buetow KH, Bell GI, Bardach J, VanDemark DR, Murray JC. Association of genetic variation of the transforming growth factor-alpha gene with cleft lip and palate. *Am J Hum Genet* 1989;45:348-53.
16. Machida J, Yoshiura K, Funkhauser CD, Natsume N, Kawai T, Murray JC. Transforming growth factor-alpha (TGFA): genomic structure, boundary sequences, and mutation analysis in nonsyndromic cleft lip/palate and cleft palate only. *Genomics* 1999;61:237-42.
17. Hwang SJ, Beaty TH, Panny SR, et al. Association study of transforming growth factor alpha (TGF alpha) TaqI polymorphism and oral clefts: indication of gene-environment interaction in a population-based sample of infants with birth defects. *Am J Epidemiol* 1995;141:629-36.
18. Shaw GM, Wasserman CR, Lammer EJ, et al. Orofacial clefts, parental cigarette smoking, and transforming growth factor-alpha gene variants. *Am J Hum Genet* 1996;58:551-61.
19. Shaw GM, Wasserman CR, Murray JC, Lammer EJ. Infant TGF-alpha genotype, orofacial clefts, and maternal periconceptional multivitamin use. *Cleft Palate Craniofac J* 1998;35:366-70.
20. Satokata I, Maas R. *Msx1* deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* 1994;6:348-56.
21. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human *MSX1* homeodomain missense mutation causes selective tooth agenesis. *Nat Genet* 1996;13:417-21.
22. van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK. *MSX1* mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat Genet* 2000;24:342-3.
23. Jezewski PA, Vieira AR, Nishimura C, et al. Complete sequencing

- shows a role for MSX1 in non-syndromic cleft lip and palate. *J Med Genet* 2003;40:399-407.
24. Jugessur A, Lie RT, Wilcox AJ, et al. Variants of developmental genes (TGFA, TGFB3, and MSX1) and their associations with orofacial clefts: a case-parent triad analysis. *Genet Epidemiol* 2003;24:230-9.
 25. van der Put NM, Steegers-Theunissen RP, Frosst P, et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet* 1995;346:1070-1.
 26. Prescott NJ, Winter RM, Malcolm S. Maternal MTHFR genotype contributes to the risk of non-syndromic cleft lip and palate. *J Med Genet* 2002;39:368-9.
 27. van Rooij IA, Vermeij-Keers C, Kluijtmans LA, et al. Does the interaction between maternal folate intake and the methylenetetrahydrofolate reductase polymorphisms affect the risk of cleft lip with or without cleft palate? *Am J Epidemiol* 2003;157:583-91.
 28. Proetzl G, Pawlowski SA, Wiles MV, et al. Transforming growth factor-beta 3 is required for secondary palate fusion. *Nat Genet* 1995;11:409-14.
 29. Vieira AR, Orioli IM, Castilla EE, Cooper ME, Marazita ML, Murray JC. MSX1 and TGFB3 contribute to clefting in South America. *J Dent Res* 2003;82:289-92.
 30. Kim MH, Kim HJ, Choi JY, Nahm DS. Transforming growth factor-beta3 gene SfaNI polymorphism in Korean nonsyndromic cleft lip and palate patients. *J Biochem Mol Biol* 2003;36:533-7.
 31. FitzPatrick DR, Carr IM, McLaren L, et al. Identification of SATB2 as the cleft palate gene on 2q32-q33. *Hum Mol Genet* 2003;12:2491-501.
 32. Beiraghi S, Foroud T, Diouhy S, et al. Possible localization of a major gene for cleft lip and palate to 4q. *Clin Genet* 1994;46:255-6.
 33. Beiraghi S, Zhou M, Talmadge CB, et al. Identification and characterization of a novel gene disrupted by a pericentric inversion inv(4)(p13.1q21.1) in a family with cleft lip. *Gene* 2003;309:11-21.
 34. 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;54:231-40.
 35. Stein J, Mulliken JB, Stal S, et al. Nonsyndromic cleft lip with or without cleft palate: evidence of linkage to BCL3 in 17 multigenerational families. *Am J Hum Genet* 1995;57:257-72.
 36. Wyszynski DF, Maestri N, McIntosh I, et al. Evidence for an association between markers on chromosome 19q and non-syndromic cleft lip with or without cleft palate in two groups of multiplex families. *Hum Genet* 1997;99:22-6.
 37. Eiberg H, Bixler D, Nielsen LS, Conneally PM, Mohr J. Suggestion of linkage of a major locus for nonsyndromic orofacial cleft with F13A and tentative assignment to chromosome 6. *Clin Genet* 1987;32:129-32.
 38. Davies AF, Stephens RJ, Olavesen MG, et al. Evidence of a locus for orofacial clefting on human chromosome 6p24 and STS content map of the region. *Hum Mol Genet* 1995;4:121-8.
 39. Wyszynski DF, Wu T. Use of US birth certificate data to estimate the risk of maternal cigarette smoking for oral clefting. *Cleft Palate Craniofac J* 2002;39:188-92.
 40. van Rooij IA, Wegerif MJ, Roelofs HM, et al. Smoking, genetic polymorphisms in biotransformation enzymes, and nonsyndromic oral clefting: a gene-environment interaction. *Epidemiology* 2001;12:502-7.
 41. Beaty TH, Hetmanski JB, Zeiger JS, et al. Testing candidate genes for non-syndromic oral clefts using a case-parent trio design. *Genet Epidemiol* 2002;22:1-11.
 42. Munger RG, Romitti PA, Daack-Hirsch S, Burns TL, Murray JC, Hanson J. Maternal alcohol use and risk of orofacial cleft birth defects. *Teratology* 1996;54:27-33.
 43. Shaw GM, Lammer EJ. Maternal periconceptional alcohol consumption and risk for orofacial clefts. *J Pediatr* 1999;134:298-303.
 44. Natsume N, Kawai T, Ogi N, Yoshida W. Maternal risk factors in cleft lip and palate: case control study. *Br J Oral Maxillofac Surg* 2000;38:23-5.
 45. Shaw GM, Nelson V, Carmichael SL, Lammer EJ, Finnell RH, Rosenquist TH. Maternal periconceptional vitamins: interactions with selected factors and congenital anomalies? *Epidemiology* 2002;13:625-30.
 46. Jugessur A, Lie RT, Wilcox AJ, et al. Cleft palate, transforming growth factor alpha gene variants, and maternal exposures: assessing gene-environment interactions in case-parent triads. *Genet Epidemiol* 2003;25:367-74.
 47. Wong WY, Eskes TK, Kuijpers-Jagtman AM, et al. Nonsyndromic orofacial clefts: association with maternal hyperhomocysteinemia. *Teratology* 1999;60:253-7.
 48. Ray JG, Meier C, Vermeulen MJ, Wyatt PR, Cole DE. Association between folic acid food fortification and congenital orofacial clefts. *J Pediatr* 2003;143:805-7.
 49. Tolarova M, Harris J. Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology* 1995;51:71-8.