

# Genetics of cleft lip and palate: syndromic genes contribute to the incidence of non-syndromic clefts

Philip Stanier\* and Gudrun E. Moore

Institute of Reproductive and Developmental Biology, Imperial College London, London W12 0NN, UK

Received December 1, 2003; Revised and Accepted December 12, 2003

---

Clefts of the lip and/or palate (CL/P) are among the most common birth defects worldwide. The majority are non-syndromic where CL/P occurs in isolation of other phenotypes. Where one or more additional features are involved, clefts are referred to as syndromic. Collectively CL/P has a major clinical impact requiring surgical, dental, orthodontic, speech, hearing and psychological treatments or therapies throughout childhood. The etiology of CL/P is complex and thought to involve both major and minor genetic influences with variable interactions from environmental factors. Using a combination of gene targeting technology and traditional developmental techniques in both mouse and chick, significant progress has been made in the identification of numerous genes and gene pathways critical for craniofacial development. Despite this, it has been a particular source of frustration that mutation screening of specific candidates, association studies and even genome-wide scans have largely failed to reveal the molecular basis of human clefting. Nevertheless, some important findings have recently come from studies involving syndromic forms of the disorder. These include several genes which have now been shown to contribute a major effect on the etiology of CL/P. Furthermore, these genes can also be used to demonstrate a significant overlap between syndromic and non-syndromic CL/P. The study of these syndromic genes and their molecular pathways will provide a useful and informative route with which to gain a better understanding of human craniofacial pathology.

---

## INTRODUCTION

Development of the head and face comprises one of the most complex events during embryonic development, coordinated by a network of transcription factors and signalling molecules together with proteins conferring cell polarity and cell–cell interactions. Disturbance of this tightly controlled cascade can result in a facial cleft where the facial primordia ultimately fail to meet and fuse or form the appropriate structures. Collectively, craniofacial abnormalities are among the most common features of all birth defects. The most frequent of these are the orofacial clefts, cleft lip and/or cleft palate (CL/P). CL/P results in complications affecting feeding, speech, hearing and psychological development. Patients will undergo multiple rounds of surgical repair starting in the first year of life and may continue until 18 or 20 years old. Frequently, extensive dental and orthodontic treatment, speech and hearing therapy may be required as well as referral for psychotherapy and genetic counselling. Occurrence estimates range between 1/300 and 1/2500 births for cleft lip with or without cleft palate

(CLP) and around 1/1500 births for cleft palate alone (CP) (1). It has been reported that CLP occurs more frequently in males, while the sex bias is reversed for CP, which is more common in females (2). Approximately 50% of CL patients also have CP, which is thought to be a secondary effect resulting from the defect in facial prominence fusion that precedes palate formation. CP occurring alone is therefore considered to be etiologically distinct from CLP. The majority of CL/P (~70%) are regarded as non-syndromic, where the clefts occur without other anomalies. The remaining syndromic cases have additional characteristic features that can be subdivided into categories of chromosomal abnormalities, recognizable Mendelian single gene syndromes, teratogenic effects and various unknown syndromes.

The high familial aggregation rates, recurrence risks and elevated concordance rates in monozygous versus dizygous twins provide evidence for a strong genetic component in CL/P (3). Despite this, familial inheritance is complex with simple Mendelian inheritance considered uncommon. As a general model, it is thought that both genes and environmental factors, acting either independently or in combination, are responsible

---

\*To whom correspondence should be addressed: Tel: +44 2075942124; Fax: +44 2075942129; Email: pstanier@imperial.ac.uk

for facial clefting. While numerous non-genetic risk factors have been identified such as use of anti-epileptic drugs, maternal alcohol or cigarette use (4), much effort has been concentrated on identifying the genetic contribution. This has taken the form of direct analysis of candidate genes, association studies with candidate genes or loci and genome-wide scans using large collections of CL/P families. Many mouse mutants with isolated clefts have been described where the specific gene is known (5), and these too contribute to the pool of candidate genes. We can therefore confidently predict that many individual genes, acting either alone or within gene networks, will be responsible for the heterogeneous causality observed in humans. It has been predicted that CLP would best fit an oligogenic model where one or a few major genes were influenced by a small number of modifiers (3,6–8). Nevertheless, the intense efforts of current screening programmes have not revealed major risk factors for human clefting, and the identification of causative mutations has remained elusive. This general failure probably reflects a more complicated and diverse etiology than these studies suggested. It is therefore encouraging that several important risk factors have recently been identified directly from human analyses. Interestingly, this has been achieved using syndromic CL/P patients, where the additional phenotypic features have allowed patients to be subdivided into more homogeneous and readily analysable groups.

## EMBRYONIC DEVELOPMENT

Development of the human face begins in the fourth week of gestation when migrating neural crest cells from the dorsal region of the anterior neural tube (cranial neural crest, CNC) combine with mesodermal cells to establish the facial primordia. The maxillary prominences enlarge and grow towards each other and the nasal prominences. During the sixth to seventh weeks, the nasal prominences merge to form the intermaxillary segment resulting in both the filtrum and primary palate. This region then fuses to the maxillary prominences, which form the lateral parts of the upper lip (9). The secondary palate is also CNC derived and forms the palatal shelves, which grow out from the maxillary prominences. Mouse mutants with disruption in genes such as *Gli2*, *Gli3*, *Tgfb2* and *Hoxa2* result in CL/P through disturbance to CNC migration and differentiation (10–12).

In the mouse the palatal shelves first appear at around E12.5 (approximately 6 weeks post conception in human) and rapidly grow in a vertical plane flanking the developing tongue (Fig. 1). A key stage in mouse palatogenesis occurs during E12.5–E13.5, when the shelves, consisting of rapidly proliferating mesenchymal cells, undergo a sudden elevation to bring them into horizontal apposition above the flattening tongue. Several genes have been implicated in palatal mesenchymal proliferation such as *Msx1* and *Lhx8*, where CP is seen in the respective null mice due to the palatal shelves failing to meet in the horizontal plane (13,14). As a general model, insufficient mesenchyme is believed to be the most common reason for CP in mice (5).

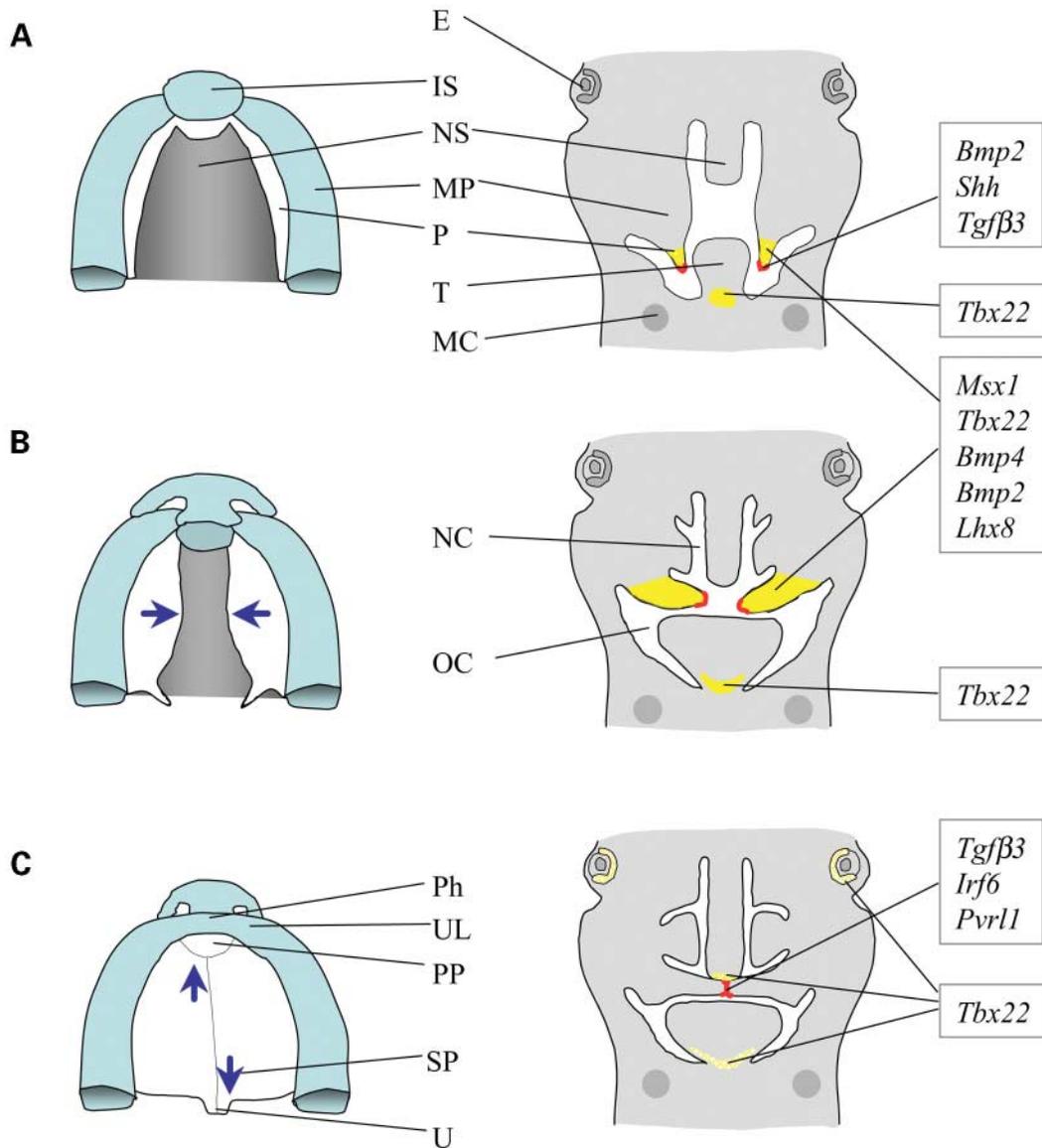
Although several theories exist for how the palatal shelves elevate, the actual mechanisms responsible are unclear.

Movement from the vertical to the horizontal is likely to be a consequence of an intrinsic force resulting from increased turgidity through recruitment of water in response to elevated levels of glycosaminoglycans such as hyaluronan (15). This occurs concomitantly with rapid remodelling of the extracellular matrix (ECM) (16). The shelves require the ability to hinge and maintenance of the appropriate structural shape is important during changes to the ECM and proliferating mesenchyme. *Pax9* mutant mice, for example, exhibit CP due to an abnormal morphology of the palatal shelves. The shelves are shorter and broader than the wild-type, which causes mechanical inhibition of shelf reorientation (17). Following elevation, the medial edge epithelia (MEE) of the opposing palatine shelves fuse in the midline through interactions of cell adhesion molecules and desmosomes. The palatal shelves initially contact in the mid portion and then zipper closed towards both the primary palate and the uvula. The resulting epithelial seam is rapidly removed through a combination of programmed cell death, epithelial cell migration and transdifferentiation (18,19). Palatogenesis is considered to be complete in mouse by around E15.5 or 12 weeks in the human.

## DEVELOPMENTAL GENE NETWORKS

A variety of molecules have been implicated in signalling facial primordia identity, epithelial differentiation and shelf remodelling (Table 1). These include ECM molecules and growth factors, which act as inductive signals such as sonic hedgehog (Shh), bone morphogenetic proteins (Bmp), fibroblast growth factors (Fgf) and members of the transforming growth factor  $\beta$  (Tgfb) superfamily. Shh plays an important role in the early induction of facial primordia in addition to expression in the palatal MEE (20). *Bmp2* and *Bmp4* on the other hand, are expressed more specifically within the epithelia and mesenchyme of the palatal shelves. The *Msx1* homeobox gene, which is also expressed in the facial primordia, is required for expression of *Bmp2* and *Bmp4* in the palatal mesenchyme and *Shh* in the MEE (21). Epidermal growth factor (Egf) stimulates glycosaminoglycan production within the palatal shelves while Tgfb, expressed throughout the palatal mesenchyme and epithelia, stimulates extracellular matrix biosynthesis (22). Fibronectin and collagen III act as modulating factors on hyaluronate expansion during shelf reorientation while collagen IX plays a critical role in signalling epithelial–mesenchymal interactions, appearing in the MEE cell surface just prior to shelf elevation (23). Transcription factors such as the distal-less (Dlx), Hox, Gli and T-box families also play key roles in maxillary and mandibular specification and are regulated by Shh, Bmps and Fgf signals (24). Clearly epithelial–mesenchymal interactions are crucial in craniofacial development and specific sites of expression such as the tooth buds may function as inductive signalling centres influencing palate morphogenesis.

The Tgfb family is particularly interesting in palate development and isoforms 1, 2 and 3 are all expressed during this process. Recent evidence suggests that their function in the embryonic palate is at least in part mediated through the Smad signalling system (25). *Tgfb3* is expressed earliest and is found in the epithelial component of the vertical shelves. It is also



**Figure 1.** Critical stages of lip and palate development. (A) Illustrations depicting paired horizontal and coronal sections of the head at mouse E12.5 (human week ~7). The medial nasal prominences merge to form the intermaxillary segment (IS) while lateral parts of the upper lip form from the maxillary prominences (MP). The palatal shelves (P) also bud from the maxillary prominences (MP) and grow vertical to the tongue (T). (B) At mouse E13.5 (human week ~8), the palatal shelves undergo epithelial remodelling and elevate to a horizontal position above the tongue. The blue arrows indicate the position of initial shelf contact and fusion. (C) By mouse E14.5 (human week ~9–10), the intermaxillary segment becomes the philtrum (Ph) of the upper lip (UL) and the primary palate (PP). The palatal shelves have fused in both anterior and posterior directions (blue arrows), together with the nasal septum. The epithelial seam disrupts and ossification begins in the anterior (hard) palate. Sites of expression for some of the key genes affecting palatogenesis are indicated in the coronal sections: yellow shading corresponds to *Tbx22* expression; red indicates the palatal medial edge epithelium characterized by *Tgfβ3* expression. Genes such as *Pvr11* and *p63* are expressed through the oral epithelia. E = eye; MC = Meckel's cartilage; NC = nasal cavity; OC = oral cavity; SP = soft palate; U = uvula.

expressed later in the horizontal shelves and MEE, but expression is undetected once the epithelial seam disrupts. *Tgfβ1* expression is limited to the horizontal shelves but like *Tgfβ3*, switches off soon after epithelial seam disruption. While *Tgfβ1* and 2 accelerate palatal shelf fusion (26), *Tgfβ3* may play a role in growth inhibition and is crucial for the first adhesive interaction. Indeed the *Tgfβ3* knock out mouse exhibits an isolated CP through failure of palatal shelf fusion (27,28). Although the palatal shelves otherwise develop normally, they show a marked reduction in the filopodia

present on the MEE surface (29,30) and show down-regulation of chondroitin sulphate proteoglycan on the apical surface of the MEE (31). Both of these are required for efficient MEE adhesion. In the mouse, antisense oligos, isoform-specific antibodies and gene knockouts show that palatal shelf fusion fails in the absence of *Tgfβ3* but not *Tgfβ1* or 2 (32). Tissue remodelling during palatal fusion involves a combination of basement membrane degradation and epithelio-mesenchymal transformation, both of which are under the control of specific matrix metalloproteinases (MMPs) and tissue inhibitors of

**Table 1.** Some of the key genes required for craniofacial morphogenesis

|                              |   |
|------------------------------|---|
| Polarizing signals           | <i>Shh, Bmp2, Bmp4 and Bmp7, Wnt5a, Smad2-4</i>   |
| Growth factors and receptors | <i>Egf, Egfr, Tgfa, Tgfb1-3, Fgf1, Fgf2, Fgf8, Fgfr1, Fgfr2</i>                             |
| Transcription factors        | <i>Ap2a, Dlx1-6, Gli2-3, Hoxa2, Irf6, Lhx8, Pax9, Pitx1, Pitx2, Prx1, Msx1, Tbx1, Tbx22</i> |
| Cell adhesion molecules      | <i>Pvrl1, Connexin43, E-cadherin</i>  |
| Extracellular matrix         | <i>Col2A1, Col11A1 and Col11A2, Mmp2, Mmp3, Mmp9, Mmp13, Timp1-3, Fibronectin</i>           |

metalloproteinases (TIMPs). In the *Tgfb3*<sup>-/-</sup> mouse, the palatal expression levels of *Timp-2* and *Mmp-13* are markedly reduced and their expression is dependent on *Tgfb3* (33). This model shows that proteolytic degradation of the ECM is essential for palatal fusion. Overall it is clear that tight control of a cascade of genes is required to complete normal palatogenesis.

### GENETIC ANALYSIS OF CL/P

Craniofacial development is highly complex with a large array of genes implicated. Combined with multigenic inheritance and the influence of non-genetic factors, identifying the key genes in human CL/P represents a major challenge. In addition to the direct analysis of functional candidates, much effort has gone into linkage and/or candidate gene directed association studies. A major drawback lies in the analysis of patients with heterogeneous etiology, since this dilutes the chances of finding positive gene-phenotype correlations. To date four genome-wide scans for CL/P have been published, one using sib pair analysis in an English population (34), one using multiplex families of Chinese origin (35), one using multiplex families from North and South America (36) and the other using two large Syrian families (37). The four studies do not generally concur on significant or highly suggestive regions, probably reflecting the diverse populations investigated. An exception to this is a region on 2q, which overlaps between the Chinese study and a subset of the American families. A number of new genome scans have been presented at the American Society of Human Genetics 2003 meeting, including a meta-analysis totalling 11 studies (38). Some regions consistent with previous linkage or candidate gene association studies (39) have been highlighted such as 2p13 (TGFA), 6p21.3-21.1, 17q12 (RARA), 1q22.3-41 (IRF6), and 2q35-36, as well as several new regions including, 7p13-15, 7q22-qter and 12q24-qter. It is hoped that this coordinated effort will provide the necessary power to fine map and identify the major genetic CL/P candidates.

### Syndromic models for non-syndromic CL/P

Recently, positional cloning has been successful for several forms of syndromic CL/P either where rare large Mendelian families were available or where specific associated features allowed refinement of the study population. This produces a higher chance of identifying single gene causation. While these subgroups are referred to as syndromic clefts, it is now becoming apparent that the same genes contribute to the population of non-syndromic clefts, perhaps through variable penetrance or the action of different modifiers.

### TBX22

We have been specifically interested in the X-linked Mendelian inherited form of CP (CPX), who exhibit a high degree of penetrance. This has been extensively studied as a rare but strongly genetic influence for nonsyndromic CP (40-49). In addition to CP or in some cases bifid or absent uvula, the majority of these patients also display ankyloglossia (tongue-tie). This minor feature is frequently missed or unreported; however, when noted in addition to X-linked inheritance, it is an important diagnostic marker for CPX. Positional cloning identified the CPX locus as the gene encoding T-BOX 22 (*TBX22*) (50). *TBX22* is a recently described member of the T-box containing transcription factor gene family that is conserved throughout metazoan evolution. These genes play essential roles in early development and in particular mesoderm specification. The first T-box gene, Brachyury or T, was originally identified in mice (51), where heterozygous animals have short tails and homozygotes lack a notochord and mesoderm posterior to somite 7 (52). Subsequently, a family of T-box proteins have been described including 18 in humans, all characterized by a similar DNA binding domain. In addition to *TBX22*, several other T-box genes have been implicated in human syndromes, emphasizing their importance in development. For example, haploinsufficiency of *TBX3* or *TBX5* causes ulnar-mammary and Holt-Oram syndromes respectively (53,54); *TBX1* is deleted in DiGeorge syndrome (55,56); and *TBX19* is mutated in isolated ACTH deficiency (57).

Although no gene deletions have yet been described for *TBX22*, a variety of point mutations have been identified. These include nonsense, splice site, frameshift and missense changes, with the latter affecting highly conserved residues within the T-box DNA-binding domain (58). It is interesting to note that the G118C missense mutation found in a Canadian CPX family (50,59) is at the equivalent position within the T-box domain to the G80R change seen in a Holt-Oram syndrome patient (60). This position is predicted to interact with the major groove of target DNA and has been demonstrated to result in loss of DNA binding (61,62). Taken together with the X-chromosomal localization, CP in males is likely to result from complete loss of *TBX22* function, while haploinsufficient females frequently exhibit a milder phenotype.

In addition to families with clear X-linked inheritance, mutations were also found in smaller families where ankyloglossia is a diagnostic feature (50,63). *TBX22* expression correlates precisely with the phenotype seen in CPX patients, both in the vertical palatal shelves and the base of the tongue corresponding to the frenulum (63). A similar expression pattern is seen in mouse (63,64) and in chick (65). The latter is interesting since birds have a constitutional cleft and one could speculate that *Tbx22* expression is important in palatal shelf outgrowth rather than fusion. Despite the frequent concordance of CP and ankyloglossia in CPX patients, the phenotype can vary even within single families (Fig. 2). Males usually have both CP and ankyloglossia (CPA) but occasionally CP (17%) or ankyloglossia (4%) alone. Female carriers may vary from fully affected to being phenotypically normal (CPA = 11%, CP = 6%, A = 43%, unaffected = 40%). A recent study of unselected Brazilian and North American CP patients, ascertained without bias for inheritance or ankyloglossia, showed that up to 4%



**Figure 2.** X-linked CP (CPX) phenotype is variable and overlaps with non-syndromic CP. Upper panel: left picture shows the isolated cleft soft palate in the mother carrying a single base deletion (664delC) in the coding region of *TBX22*. The son (centre) has a less severe partial cleft but also has ankyloglossia (not shown). The daughter (right) has a similar partial cleft to her brother but is shown in the process of repair. Like the mother, she has no ankyloglossia and is a phenocopy for non-syndromic CP. Lower panel: the classic male CPX phenotype is a complete cleft of the secondary palate (left picture) and ankyloglossia (middle picture). Right picture shows the ankyloglossia in a related male with the less severe bifid uvula (not shown). His brother also carries the same mutation (*TBX22* IVS6+1G>C) but has ankyloglossia only with no CP (not shown).

resulted from *TBX22* mutations (58). These include several sporadic patients with no ankyloglossia and a patient with isolated CP but with ankyloglossia only seen in the extended family. Furthermore, we have recently identified a DNA binding domain missense mutation in a five generation CP family with no evidence of ankyloglossia (unpublished data). This demonstrates that *TBX22* (and therefore CPX) plays a more important role in the combined incidence of non-syndromic CP than previously expected.

### ***PVRL1***

Autosomal recessive CLP with ectodermal dysplasia (CLPED1) is generally rare but occurs with a much higher frequency on Margarita Island (north of Venezuela). Positional cloning mapped the locus to 11q23 and mutations were identified in the cell adhesion molecule *PVRL1* (Nectin-1), which is expressed in the developing face and palate (66). On Margarita Island, CLPED1 is generally caused by homozygosity of the nonsense mutation W185X, while heterozygosity is high in the unaffected population. It has been speculated that, since Nectin-1 is the principle cell surface receptor for  $\alpha$ -herpes viruses, the high frequency of heterozygotes might have resulted from relative resistance to infection by viruses such as HSV1 and HSV2 (67,68). The same mutation is also present on the Venezuelan mainland, where heterozygosity was found to be a significant risk factor for non-syndromic CLP (69). It will be important to investigate whether *PVRL1* mutations contribute to non-syndromic clefts in other geographical locations.

### ***IRF6***

Van der Woude syndrome (VWS) provides one of the best models for non-syndromic CLP since most patients have only minor additional phenotypes of lip pits and occasional hypodontia, while 15% have isolated CL/P. Linkage analysis localized the gene to 1q32–41 and mutations were identified in the interferon regulatory factor 6 gene *IRF6* (70). In the mouse, *Irf6* expression is restricted to the palatal MEE immediately prior to and during fusion. This markedly overlaps with the site of *Tgfb3* expression and may suggest a potential interaction. A common *IRF6* variant (V274I) within the protein binding domain was identified in the VWS studies and has since been evaluated as a potential modifier in isolated CL/P. Transmission disequilibrium testing of >8000 individuals from 10 different populations for V274I and additional SNPs in the vicinity, shows strong evidence that *IRF6* is a modifier of CLP (71). Whilst sequence analysis in this cohort has not yet identified changes with clear causative function, a second reported study has identified an *IRF6* missense mutation in a two-generation apparently non-syndromic CLP family (72).

### ***P63***

EEC syndrome is an autosomal dominant disorder of ectrodactyly, ectodermal dysplasia and CL/P. EEC syndrome was mapped to 3q27 and heterozygous mutations were identified in the *p63* gene (73). One unusual phenomenon with *p63* is that mutation to different parts of the gene can influence the cleft

phenotype. Missense mutation of the conserved DNA binding domain region gives CLP while C-terminal mutations give CL or CP. Mutation at the N-terminal end outside of the conserved domains gives rise to CP or no clefting at all. Only a small number of non-syndromic CL/P patients have been screened for mutations to date and no mutations have been found (74). Not only is a larger study warranted but further investigation of downstream targets might be revealing. In particular *Jagged2*, a ligand in the notch signalling pathway, is known to act downstream of p63 and homozygous mouse knockouts of *Jagged2* exhibit CP (75).

### **MSX1**

*MSX1* first came to prominence as a candidate for CL/P following the generation of a gene knockout with cleft palate and oligodontia (13). A candidate gene-based association study reported significant linkage disequilibrium between both CLP and CP with polymorphisms in *MSX1* (76). In the same report, a cohort of non-syndromic CL/P patients were analysed for coding region mutations without success. An *MSX1* mutation was reported in a Dutch family with tooth agenesis and a mixture of CLP and CP, providing another rare example of where a single gene, and in this case single mutation, can give rise to a mixed clefting phenotype (77). Subsequently, conflicting reports have been published, some with evidence of linkage or association to either CLP or CP (78–81) and some with no association to either (82,83). Despite these conflicts, which may arise due to variations caused by ethnic population differences, the strongest evidence for a role of *MSX1* in CL/P has now been obtained by direct sequencing. Jezewski *et al.* (84) analysed a large cohort of CL/P patients from a variety of different ethnic origins and demonstrated that up to 2% of patients, predominantly with CLP, carried *MSX1* mutations.

### **Other genes . . .**

A variety of other genes causing syndromic CL/P are currently being analysed in extended cohorts as candidates for non-syndromic clefting. Examples of these include *FGFR1*, where mutations result in autosomal recessive Kallmann syndrome (85). As well as the characteristic hypogonadotropic hypogonadism and anosmia, five of the 13 patients with mutations had clefts of the lip or palate. Several forkhead genes represent good candidates, not only because of their craniofacial expression pattern but also because mutations give rise to clefts, e.g. *TTF-2* mutations cause thyroid abnormalities and CP (86), while *FOXC2* mutations lead to distichiasis, lymphoedema and cleft palate (87). In addition, two genes have recently been identified through chromosome rearrangements in cleft patients. These are the putative transcription factor *SATB2* on 2q32–q33, which is disrupted in two unrelated patients with non-syndromic CP (88) and a novel acyl-CoA desaturase *ACOD4* on 4q21, which is disrupted in a single two-generation family with CL (89).

### **FUTURE DIRECTIONS**

The completion of the genome sequence has contributed to recent successes in identifying novel CL/P genes. The direct

study of non-syndromic CL/P has previously been hampered due to the general lack of well-defined multiplex families with sufficient power to enable a genome-wide linkage study to provide a localisation. The use of model organisms and in particular the mouse, has for some time been a rich source of information for craniofacial development. Transgenic and knockout technology has often, and sometimes quite unexpectedly, provided a long list of genes that confer an CL/P phenotype. The number and diversity of targeted genes that result in a cleft probably reflects why CL/P is one of the most common features seen in human birth defects. The use of mutant inbred strains to tease out causative genes and provide models is an exceptionally powerful tool. Nevertheless, the problem is to directly relate them to the complex human situation where genetic heterogeneity and varying environmental/socio-economic status is found.

Our general failure to pinpoint the precise molecular events that lead to human CL/P most likely stems from our lack of knowledge about the gene networks and regulation of gene expression during palatal development. Clearly we are now in a far better position to address this. We now have several genes that play strong genetic roles in human craniofacial development and contribute to the incidence of non-syndromic CL/P. These genes will provide tools to study and elucidate the genetic pathways that they function in. This can be combined with a second powerful approach, using the latest generation of expression profiling techniques. In conjunction with the genome sequence and virtually a complete list of genes, detailed information about the genes that are expressed and those that are switched on or off at different stages of craniofacial development can be determined. Recent examples of this are the ongoing studies of COGENE (<http://hg.wustl.edu/COGENE/>), where expression profiling using both microarray and SAGE technologies are being used to generate profiles of a variety of human orofacial structures between 4 and 8.5 weeks of development. Similarly, a recent study from Brown *et al.* (90) used microarray analysis to look at expression profiles of mouse vertical, horizontal and fused (E13.5, 14.5 and 15.5) palatal shelves. An alternative approach has been reported by Fowles *et al.* (91) who performed a subtractive hybridization screen to enrich for E10.5 mouse embryonic branchial arches 1- and 2-specific genes. The validity of this approach was demonstrated by identifying a variety of genes with established roles in craniofacial development in a random sampling.

There is now every reason to be optimistic about our future understanding of human CL/P. Whilst syndromic gene mutations may contribute no more than 10% to non-syndromic CL/P, these findings will lead to a better understanding of the gene pathways, interactions and novel candidates. For many, the benefits of precise diagnosis, accurate risk assessment and genetic counselling can be achieved. When integrated with tissue specific expression profiling and targeted developmental studies, the potential for treatments and preventative therapies may also become a reality.

### **ACKNOWLEDGEMENTS**

The authors wish to thank Ana Marçano, Kit Doudney, Olafur Jensson, Alfred Arnason, Arnie Bjornsson and Jeff Murray for

use of the images, unpublished information and helpful comments on the manuscript. We are also grateful to all of the patients and families who have participated in these studies.

## REFERENCES

- Wyszynski, D.F., Beaty, T.H. and Maestri, N.E. (1996) Genetics of nonsyndromic oral clefts revisited. *Cleft Palate-Cranio. J.*, **33**, 406–417.
- Fraser, F.C. (1970) The genetics of cleft lip and cleft palate. *Am. J. Hum. Genet.*, **22**, 336–352.
- Mitchell, L.E. and Risch, N. (1992) Mode of inheritance of nonsyndromic cleft lip with or without cleft palate: a reanalysis. *Am. J. Hum. Genet.*, **51**, 323–332.
- Wyszynski, D.F. and Beaty, T.H. (1996) Review of the role of potential teratogens in the origin of human nonsyndromic oral clefts. *Teratology*, **53**, 309–317.
- Wilkie, A.O. and Morriss-Kay, G.M. (2001) Genetics of craniofacial development and malformation. *Nat. Rev. Genet.*, **2**, 458–468.
- Marazita, M.L., Goldstein, A.M., Smalley, S.L. and Spence, M.A. (1986) Cleft lip with or without cleft palate: reanalysis of a three-generation family study from England. *Genet. Epidemiol.*, **3**, 335–342.
- Farrall, M. and Holder, S. (1992) Familial recurrence-pattern analysis of cleft lip with or without cleft palate. *Am. J. Hum. Genet.*, **50**, 270–277.
- Schliekelman, P. and Slatkin, M. (2002) Multiplex relative risk and estimation of the number of loci underlying an inherited disease. *Am. J. Hum. Genet.*, **71**, 1369–1385.
- Moore, K.L. (1988) *The Developing Human*, 3rd edn. WB Saunders, Philadelphia, PA, pp. 197–213.
- Mo, R., Freer, A.M., Zinyk, D.L., Crackower, M.A., Michaud, J., Heng, H.H., Chik, K.W., Shi, X.M., Tsui, L.C., Cheng, S.H. *et al.* (1997) Specific and redundant functions of *Gli2* and *Gli3* zinc finger genes in skeletal patterning and development. *Development*, **124**, 113–123.
- Sanford, L.P., Ormsby, I., Gittenberger-de Groot, A.C., Sariola, H., Friedman, R., Boivin, G.P., Cardell, E.L. and Doetschman, T. (1997) *TGFβ2* knockout mice have multiple developmental defects are non-overlapping with other *TGFβ* knockout phenotypes. *Development*, **124**, 2659–2670.
- Rijli, F.M., Mark, M., Lakkaraju, S., Dierich, A., Dolle, P. and Chambon, P. (1993) A homeotic transformation is generated in the rostral branchial region of the head by disruption of *Hoxa-2*, which acts as a selector gene. *Cell*, **75**, 1333–1349.
- Satokata, I. and Maas, R. (1994) *Msx1* deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat. Genet.*, **6**, 348–355.
- Zhao, Y., Guo, Y.J., Tomac, A.C., Taylor, N.R., Grinberg, A., Lee, E.J., Huang, S. and Westphal, H. (1999) Isolated cleft palate in mice with a targeted mutation of the LIM homeobox gene *Lhx8*. *Proc. Natl Acad. Sci. USA*, **96**, 15002–15006.
- Ferguson, M.W. (1988) Palate development. *Development*, **103** (suppl.), 41–60.
- Morris-Wiman, J. and Brinkley, L. (1992) An extracellular matrix infrastructure provides support for murine secondary palatal shelf remodelling. *Anat. Rec.*, **234**, 575–586.
- Peters, H., Neubuser, A., Kratochwil, K. and Balling, R. (1998) *Pax9*-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Genes Dev.*, **12**, 2735–2747.
- Kaartinen, V., Cui, X., Heisterkamp, N., Groffen, J. and Shuler, C.F. (1997) Transforming growth factor- $\beta$ 3 regulates transdifferentiation of medial edge epithelium during palatal fusion and associated degradation of the basement membrane. *Dev. Dyn.*, **209**, 255–260.
- Martinez-Alvarez, C., Tudela, C., Perez-Miguelsanz, J., O'Kane, S., Puerta, J. and Ferguson, M.W. (2000) Medial edge epithelial cell fate during palatal fusion. *Dev. Biol.*, **220**, 343–357.
- Bitgood, M.J. and McMahon, A.P. (1995) *Hedgehog* and *Bmp* genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. *Dev. Biol.*, **172**, 126–138.
- Zhang, Z., Song, Y., Zhao, X., Zhang, X., Fermin, C. and Chen, Y. (2002) Rescue of cleft palate in *Msx1*-deficient mice by transgenic *Bmp4* reveals a network of BMP and Shh signaling in the regulation of mammalian palatogenesis. *Development*, **129**, 4135–4146.
- Dixon, M.J., Carette, M.J., Moser, B.B. and Ferguson, M.W. (1993) Differentiation of isolated murine embryonic palatal epithelium in culture: exogenous transforming growth factor alpha modulates matrix biosynthesis in defined experimental conditions. *In Vitro Cell Dev. Biol.*, **29A**, 51–61.
- Ferguson, M.W. and Honig, L.S. (1984) Epithelial-mesenchymal interactions during vertebrate palatogenesis. *Curr. Top. Dev. Biol.*, **19**, 137–164.
- Richman, J.M. and Lee, S.-H. (2003) About face: signals and genes controlling jaw patterning and identity in vertebrates. *BioEssays*, **25**, 554–568.
- Greene, R.M., Nugent, P., Mukhopadhyay, P., Warner, D.R. and Pisano, M.M. (2003) Intracellular dynamics of Smad-mediated TGF $\beta$  signalling. *J. Cell. Physiol.*, **197**, 261–271.
- Fitzpatrick, D.R., Denhez, F., Kondaiah, P. and Akhurst, R.J. (1990) Differential expression of TGF beta isoforms in murine palatogenesis. *Development*, **109**, 585–595.
- Proetzel, G., Pawlowski, S.A., Wiles, M.V., Yin, M., Boivin, G.P., Howles, P.N., Ding, J., Ferguson, M.W.J. and Doetschman, T. (1995) Transforming growth factor- $\beta$ 3 is required for secondary palate fusion. *Nat. Genet.*, **11**, 409–414.
- Kaartinen, V., Voncken, J.W., Shule, C., Warburton, D., Bu, D., Heisterkamp, N. and Groffen, J. (1995) Abnormal lung development and cleft palate in mice lacking *TGFβ3* indicates defects of epithelial-mesenchymal interaction. *Nat. Genet.*, **11**, 415–421.
- Taya, Y., O'Kane, S. and Ferguson, M.W. (1999) Pathogenesis of cleft palate in TGF- $\beta$ 3 knockout mice. *Development*, **126**, 3869–3879.
- Martinez-Alvarez, C., Bonelli, R., Tudela, C., Gato, A., Mena, J., O'Kane, S. and Ferguson, M.W. (2000) Bulging medial edge epithelial cells and palatal fusion. *Int. J. Dev. Biol.*, **44**, 331–335.
- Gato, A., Martinez, M.L., Tudela, C., Alonso, I., Moro, J.A., Formoso, M.A., Ferguson, M.W. and Martinez-Alvarez, C. (2002) TGF- $\beta$ (3)-induced chondroitin sulphate proteoglycan mediates palatal shelf adhesion. *Dev. Biol.*, **250**, 393–405.
- Brunet, C.L., Sharpe, P.M. and Ferguson, M.W. (1995) Inhibition of TGF- $\beta$ 3 (but not TGF- $\beta$ 1 or TGF- $\beta$ 2) activity prevents normal mouse embryonic palate fusion. *Int. J. Dev. Biol.*, **39**, 345–355.
- Blavier, L., Lazaryev, A., Groffen, J., Heisterkamp, N., DeClerck, Y.A. and Kaartinen, V. (2001) TGF- $\beta$ 3-induced palatogenesis requires matrix metalloproteinases. *Mol. Biol. Cell.*, **12**, 1457–1466.
- Prescott, N.J., Lees, M.M., Winter, R.M. and Malcolm, S. (2000) 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.*, **106**, 345–350.
- Marazita, M.L., Field, L.L., Cooper, M.E., Tobias, R., Maher, B.S., Peachtlerkajom, S. and Liu, Y.E. (2002) Genome scan for loci involved in cleft lip with or without cleft palate, in Chinese multiplex families. *Am. J. Hum. Genet.*, **71**, 349–364.
- Zeiger, J.S., Hetmanski, J.B., Beaty, T.H., VanderKolk, C.A., Wyszynski, D.F., Bailey-Wilson, J.E., De Luna, R.O., Perandones, C., Tolarova, M.M., Mosby, T. *et al.* (2003) Evidence for linkage of nonsyndromic cleft lip with or without cleft palate to a region on chromosome 2. *Eur. J. Hum. Genet.*, **11**, 835–839.
- Wyszynski, D.F., Albacha-Hejazi, H., Aldirani, M., Hammoud, M., Shkair, H., Karam, A., Alashkar, J., Holmes, T.N., Pugh, E.W., Doheny, K.F. *et al.* (2003) A genome-wide scan for loci predisposing to non-syndromic cleft lip with or without cleft palate in two large Syrian families. *Am. J. Med. Genet.*, **123A**, 140–147.
- Marazita, M.L., Murray, J.M., Cooper, M., Bailey-Wilson, T.J., Albacha-Hejazi, H., Lidral, A., Moreno, L., Arcos-Bargos, M. and Beaty, T. (2003) Meta-analysis of 11 genome scans for cleft lip with or without cleft palate. *Am. J. Hum. Genet.*, **73**, A79.
- Vieira, A.R. and Orioli, I.M. (2001) Candidate genes for nonsyndromic cleft lip and palate. *ASDCJ Dent. Child.*, **68**, 272–279.
- Lowry, R.B. (1970) Sex linked cleft palate in a British Columbian Indian family. *Pediatrics*; **46**, 123–128.
- Rushton, A.R. (1979) Sex linked inheritance of cleft palate. *Hum. Genet.*, **48**, 179–181.
- Rollnick, B.R. and Kaye, C.I. (1986) Mendelian inheritance of isolated nonsyndromic cleft palate. *Am. J. Med. Genet.*, **24**, 465–473.
- Bixler, D. (1987) Letter to the editor: X-linked cleft palate. *Am. J. Med. Genet.*, **28**, 503–505.
- Hall, B.D. (1987) Letter to the editor: A further X-linked isolated nonsyndromic cleft palate family with a nonexpressing obligate affected male. *Am. J. Med. Genet.*, **26**, 239–240.

45. Moore, G.E., Ivens, A., Chambers, J., Farrall, M., Williamson, R., Bjornsson, A., Arnason, A. and Jensson, O. (1987) Linkage of an X-chromosome cleft palate gene. *Nature*, **326**, 91–92.
46. Stanier, P., Forbes, S.A., Arnason, A., Bjornsson, A., Sveinbjornsdottir, E., Williamson, R. and Moore, G.E. (1993) The localisation of a gene causing X-linked cleft palate and ankyloglossia (CPX) in an Icelandic kindred is between DXS326 and DXYS1X. *Genomics*, **17**, 549–555.
47. Bjornsson, A., Arnason, A. and Tippet, P. (1989) X-linked midline defect in an Icelandic family. *Cleft Palate J.*, **26**, 3–8.
48. Gorski, S.M., Adams, K.J., Birch, P.H., Friedman, J.M. and Goodfellow, P.J. (1992) The gene responsible for X-linked cleft palate (CPX) in a British Columbian native kindred is localized between *PGK1* and *DXYS1*. *Am. J. Hum. Genet.*, **50**, 1129–1136.
49. Marçano, A.C.B., Ming, J.E., Du, Y.Z., George, R.A., Ryan, S.G., Richieri-Costa, A. and Muenke, M. (2000) X-linked cleft palate and ankyloglossia: refinement of the minimal critical region in Xq21.3. *Am. J. Hum. Genet.*, **67**, A1802.
50. Braybrook, C., Doudney, K., Marçano, A.C.B., Arnason, A., Bjornsson, A., Patton, M.A., Goodfellow, P.J., Moore, G.E. and Stanier, P. (2001) The T-box transcription factor gene *TBX22* is mutated in X-linked cleft palate and ankyloglossia. *Nat. Genet.*, **29**, 179–183.
51. Dobrovolskaia-Zavadskaia, N. (1927) Sur la mortification spontanee de la queue chez la souris nouveau-née et sur l'existence d'un caractere hereditaire 'non-viable'. *C.R. Soc. Biol.*, **97**, 114–116.
52. Wilkinson, D.G., Bhatt, S. and Herrmann, B.G. (1990) Expression pattern of the mouse *T* gene and its role in mesoderm formation. *Nature*, **343**, 567–659.
53. Bamshad, M., Lin, R.C., Law, D.J., Watkins, W.S., Krakowiak, P.A., Moore, M.E., Franceschini, B., Lala, R., Holmes, L.B., Gebuhr, T.C. *et al.* (1997) Mutations in human *TBX3* alter limb, apocrine and genital development in ulnar-mammary syndrome. *Nat. Genet.*, **16**, 311–315.
54. Basson, C.T., Backinsky, D.R., Lin, R.C., Levi, T., Elkins, J.A., Soultis, J., Grayzel, D., Kroumpousou, K., Trail, T.A., Leblanc-Straceski, J. *et al.* (1997) Mutations in human *TBX5* cause limb and cardiac malformations in Holt–Oram syndrome. *Nat. Genet.*, **15**, 30–35.
55. Jerome, L.A. and Papaioannou, V.E. (2001) DiGeorge syndrome phenotype in mice mutant for the T-box gene, *Tbx1*. *Nat. Genet.*, **27**, 286–291.
56. Lindsey, E.A., Vitelli, F., Su, H., Morishima, M., Huynh, T., Pramparo, T., Jurevic, V., Ogunrinu, G., Sutherland, H.F., Scambler, P.J. *et al.* (2001) *Tbx1* haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature*, **410**, 97–101.
57. Lamolet, B., Pulichino, A.M., Lamonerie, T., Gauthier, Y., Brue, T., Enjalbert, A. and Drouin, J. (2001) A pituitary cell-restricted T box factor, *Tpit*, activates POMC transcription in cooperation with Pitx homeoproteins. *Cell*, **104**, 849–859.
58. Marçano, A.C.B., Doudney, K., Braybrook, C., Squires, R., Patton, M.A., Lees, M., Richieri-Costa, A., Lidal, A.C., Murray, J.C., Moore, G.E. and Stanier, P. (2004) *TBX22* mutations are a frequent cause of cleft palate. *J. Med. Genet.*, **41**, 68–74.
59. Gorski, S.M., Adams, K.J., Birch, P.H., Chodirker, B.N., Greenberg, C.R. and Goodfellow, P.J. (1994) Linkage analysis of X-linked cleft palate and ankyloglossia in Manitoba Menonite and British Columbia Native kindreds. *Hum. Genet.*, **94**, 141–148.
60. Basson, C.T., Huang, T., Lin, R.C., Bachinsky, D.R., Weremowicz, S., Vaglio, A., Bruzzone, R., Quadrelli, R., Lerone, M., Romeo, G. *et al.* (1999) Different *TBX5* interactions in heart and limb defined by Holt–Oram syndrome mutations. *Proc. Natl Acad. Sci. USA*, **96**, 2919–2924.
61. Ghosh, T.K., Packham, E.A., Bonser, A.J., Robinson, T.E., Cross, S.J. and Brook, J.D. (2001) Characterization of the *TBX5* binding site and analysis of mutations that cause Holt–Oram syndrome. *Hum. Mol. Genet.*, **10**, 1983–1994.
62. Fan, C., Liu, M. and Wang, Q. (2003) Functional analysis of *TBX5* missense mutations associated with holt-oram syndrome. *J. Biol. Chem.*, **278**, 8780–8785.
63. Braybrook, C., Lisgo, S., Doudney, K., Henderson, D., Marçano, A.C.B., Strachan, T., Patton, M.A., Villard, L., Moore, G.E., Stanier, P. and Lindsay, S. (2002) Craniofacial expression of human and murine *TBX22* correlates with the cleft palate and ankyloglossia phenotype observed in CPX patients. *Hum. Mol. Genet.*, **11**, 2793–2804.
64. Bush, J.O., Lan, Y., Maltby, K.M. and Jiang, R. (2002) Isolation and developmental expression analysis of *Tbx22*, the mouse homolog of the human X-linked cleft palate gene. *Dev. Dyn.*, **225**, 322–326.
65. Haenig, B., Schmidt, C., Kraus, F., Pfordt, M. and Kispert, A. (2002) Cloning and expression analysis of the chick ortholog of *TBX22*, the gene mutated in X-linked cleft palate and ankyloglossia. *Mech. Dev.*, **117**, 321–325.
66. Suzuki, K., Hu, D., Bustos, T., Zlotogora, J., Richieri-Costa, A., Helms, J.A. and Spritz, R.A. (2000) Mutations of *PVRL1*, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. *Nat. Genet.*, **25**, 427–430.
67. Suzuki, K., Hu, D., Bustos, T., Zlotogora, J., Richieri-Costa, A., Helms, J.A. and Spritz, R.A. (2000) Mutations of *PVRL1*, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. *Nat. Genet.*, **25**, 427–430.
68. Geraghty, R.J., Krummenacher, C., Cohen, G.H., Eisenberg, R.J. and Spear, P.G. (1998) Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor. *Science*, **280**, 1618–1620.
69. Sozen, M.A., Suzuki, K., Talavora, M.M., Bustos, T., Fernandez Iglesias, J.E. and Spritz, R.A. (2001) Mutation of *PVRL1* is associated with sporadic, non-syndromic cleft lip/palate in northern Venezuela. *Nat. Genet.*, **29**, 141–142.
70. Kondo, S., Schutte, B.C., Richardson, R.J., Bjork, B.C., Knight, A.S., Watanabe, Y., Howard, E., de Lima, R.L., Daack-Hirsch, S., Sander, A. *et al.* (2002) Mutations in *IRF6* cause Van der Woude and popliteal pterygium syndromes. *Nat. Genet.*, **32**, 285–289.
71. Zuccherro, T., Cooper, M., Caprau, D., Ribero, L., Suzuki, Y., Yoshiura, K., Christensen, K., Moreno, L., Johnson, M., Field, L. *et al.* (2003) *IRF6* is a major modifier for nonsyndromic cleft with or without cleft palate. *Am. J. Hum. Genet.*, **73**, A4.
72. Prescott, N., Garcia-Rodriguez, C., Lees, M. and Winter, R.M. (2003) Candidate genes in nonsyndromic cleft lip and palate. *Am. J. Hum. Genet.*, **73**, A727.
73. Celli, J., Duijf, P., Hamel, B.C., Bamshad, M., Kramer, B., Smits, A.P., Newbury-Ecob, R., Hennekam, R.C., Van Buggenhout, G., van Haeringen, A. *et al.* (1999) Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. *Cell*, **99**, 143–153.
74. Barrow, L.L., van Bokhoven, H., Daack-Hirsch, S., Andersen, T., van Beersum, S.E., Gorlin, R. and Murray, J.C. (2002) Analysis of the p63 gene in classical EEC syndrome, related syndromes, and non-syndromic orofacial clefts. *J. Med. Genet.*, **39**, 559–566.
75. Jiang, R., Lan, Y., Chapman, H.D., Shawber, C., Norton, C.R., Serreze, D.V., Weinmaster, G. and Gridley, T. Defects in limb, craniofacial, and thymic development in Jagged2 mutant mice. *Genes Dev.*, **12**, 1046–1057.
76. Lidal, A.C., Romitti, P.A., Basart, A.M., Doetschman, T., Leysens, N.J., Daack-Hirsch, S., Semina, E.V., Johnson, L.R., Machida, J., Burds, A. *et al.* (1998) Association of *MSX1* and *TGFB3* with nonsyndromic clefting in humans. *Am. J. Hum. Genet.*, **63**, 557–568.
77. Van den Boogard, M.-J.H., Dorland, M., Beemer, F.A. and Ploos van Amstel, H.K. (2000) *MSX1* mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat. Genet.*, **24**, 342–343.
78. Hecht, J.T., Mulliken, J.B. and Blanton, S.H. (2002) Evidence for a cleft palate only locus on chromosome 4 near *MSX1*. *Am. J. Med. Genet.*, **110**, 406–407.
79. Vieira, A.R., Orioli, I.M., Castilla, E.E., Cooper, M.E., Marazita, M.L. and Murray, J.C. (2003) *MSX1* and *TGFB3* contribute to clefting in South America. *J. Dent. Res.*, **82**, 289–292.
80. Jugessur, A., Lie, R.T., Wilcox, A.J., Murray, J.C., Taylor, J.A., Saugstad, O.D., Vindenes, H.A. and Abyholm, F. (2003) Variants of developmental genes (*TGFA*, *TGFB3*, and *MSX1*) and their associations with orofacial clefts: a case-parent triad analysis. *Genet. Epidemiol.*, **24**, 230–239.
81. Fallin, M.D., Hetmanski, J.B., Park, J., Scott, A.F., Ingersoll, R., Fuernkranz, H.A., McIntosh, I. and Beaty, T.H. (2003) Family-based analysis of *MSX1* haplotypes for association with oral clefts. *Genet. Epidemiol.*, **25**, 168–175.
82. Mitchell, L.E., Murray, J.C., O'Brien, S. and Christensen, K. (2001) Evaluation of two putative susceptibility loci for oral clefts in the Danish population. *Am. J. Epidemiol.*, **153**, 1007–1015.
83. Koillinen, H., Ollikainen, V., Rautio, J., Hukki, J. and Kere, J. (2003) Linkage disequilibrium searched for between non-syndromic cleft palate and four candidate loci. *J. Med. Genet.*, **40**, 464–468.
84. Jezewski, P., Vieira, A., Schultz, R., Machida, J., Suzuki, Y., Ludwig, B., Daack-Hirsch, S., O'Brien, S., Nishimura, C., Johnson, M. and Murray, J.C. (2003) Mutations in *MSX1* are associated with non-syndromic orofacial clefting. *J. Med. Genet.*, **40**, 399–407.

85. Dode, C., Levilliers, J., Dupont, J.M., De Paepe, A., Le Du, N., Soussi-Yanicostas, N., Coimbra, R.S., Delmaghani, S., Compain-Nouaille, S., Baverel, F. *et al.* (2003) Loss-of-function mutations in *FGFR1* cause autosomal dominant Kallmann syndrome. *Nat. Genet.*, **33**, 463–465.
86. Clifton-Bligh, R.J., Wentworth, J.M., Heinz, P., Crisp, M.S., John, R., Lazarus, J.H., Ludgate, M. and Chatterjee, V.K. (1998) Mutation of the gene encoding human *TTF-2* associated with thyroid agenesis, cleft palate and choanal atresia. *Nat. Genet.*, **19**, 399–401.
87. Fang, J., Dagenais, S.L., Erickson, R.P., Arlt, M.F., Glynn, M.W., Gorski, J.L., Seaver, L.H. and Glover, T.W. (2000) Mutations in *FOXC2* (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema–distichiasis syndrome. *Am. J. Hum. Genet.*, **67**, 1382–1388.
88. FitzPatrick, D.R., Carr, I.M., McLaren, L., Leek, J.P., Wightman, P., Williamson, K., Gautier, P., McGill, N., Hayward, C., Firth, H. *et al.* (2003) Identification of *SATB2* as the cleft palate gene on 2q32–q33. *Hum. Mol. Genet.*, **12**, 2491–2501.
89. Beiraghi, S., Zhou, M., Talmadge, C.B., Went-Sumegi, N., Davis, J.R., Huang, D., Saal, H., Seemayer, T.A. and Sumegi, J. (2003) Identification and characterization of a novel gene disrupted by a pericentric inversion inv(4)(p13.1q21.1) in a family with cleft lip. *Gene*, **309**, 11–21.
90. Brown, N.L., Knott, L., Halligan, E., Yarram, S.J., Mansell, J.P. and Sandy, J.R. (2003) Microarray analysis of murine palatogenesis: Temporal expression of genes during normal palate development. *Dev. Growth Differ.*, **45**, 153–165.
91. Fowles, L.F., Bennetts, J.S., Berkman, J.L., Williams, E., Koopman, P., Teasdale, R.D. and Wicking, C. (2003) Genomic screen for genes involved in mammalian craniofacial development. *Genesis*, **35**, 73–87.