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The complex genetics of cleft lip and palate

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SUMMARY Clefts of the lip and palate are a common craniofacial anomaly, requiring complex multidisciplinary treatment and having lifelong implications for affected individuals. The aetiology of both cleft lip with or without cleft palate (CLP) and isolated cleft palate (CP) is thought to be multifactorial, with both genetic and environmental factors playing a role. In recent years, a number of significant breakthroughs have occurred with respect to the genetics of these conditions, in particular, characterization of the underlying gene defects associated with several important clefting syndromes. These include the identification of mutations in the interferon regulatory factor-6 (*IRF6*) gene as the cause of van der Woude syndrome and the poliovirus receptor related-1 (*PVRL1*) gene as being responsible for an autosomal recessive ectodermal dysplasia syndrome associated with clefting. While no specific disease-causing gene mutations have been identified in non-syndromic clefting, a number of candidate genes have been isolated through both linkage and association studies. However, it is clear that environmental factors also play a role and an important area of future research will be to unravel interactions that occur between candidate genes and environmental factors during early development of the embryo.

Orthodontists are intimately involved in the therapeutic management of individuals affected by CLP and it is important that they keep abreast of current knowledge of the aetiology behind these conditions. This review aims to summarize some of the more significant advances in the genetics of CLP and highlight current thinking on the modes of inheritance and genetic loci that might be involved in this complex disorder.

Introduction

Clefts involving the lip and/or palate (CLP) or isolated clefts of the palate (CP) are a significant congenital anomaly, requiring complex long-term treatment and having lifelong implications for those individuals unfortunate enough to be affected. They represent a complex phenotype and reflect a breakdown in the normal mechanisms involved during early embryological development of the face (Figure 1). The incidence of these defects varies according to geographical location, ethnicity and socio-economic status, but in Caucasian populations it is reasonably uniform, with 1:800 to 1:1000 (CLP) and approximately 1:1000 (CP) live births affected (Fraser, 1970; Bonaiti-Pellie *et al.*, 1982; Gorlin *et al.*, 2001). The clinical manifestations of these defects are diverse, ranging from isolated clefts of the lip to complete bilateral clefts of the lip, alveolus and palate (Figure 2). Broadly speaking, approximately 70 per cent of CLP cases are non-syndromic, occurring as an isolated condition unassociated with any other recognizable anomalies, while the remaining 30 per cent of syndromic cases are present in association with deficits or structural abnormalities occurring outside the region of the cleft (Jones, 1988; Schutte and Murray, 1999).

Our understanding of the aetiology and pathogenesis of these conditions, particularly the non-syndromic variants, still remains relatively poor. This is a reflection of the complexity and diversity of the mechanisms involved at the molecular level during embryogenesis, with both genetic and environmental factors playing an important and influential role (Johnson and Bronsky, 1995; Schutte and Murray, 1999; Prescott *et al.*, 2001; Spritz, 2001; Wilkie and Morriss-Kay, 2001; Murray, 2002). Primary evidence for a genetic role has been available for some years; the sibling risk for CLP is approximately 30 times higher than that for the normal population prevalence, while the concordance rate in monozygotic twins is approximately 25–45 per cent as opposed to 3–6 per cent for dizygotic twins (Mitchell and Risch, 1992; Gorlin *et al.*, 2001). However, this lack of complete concordance in monozygotic twins also illustrates the importance of environmental factors in the aetiology of this condition. With recent advances in modern molecular biology and methods for the analysis of population genetics, progress has been made in identifying some of the genes associated with this anomaly and how they influence the embryonic development of the facial complex. This review aims to outline some of these mechanisms and highlight several key advances that have been made within this field over the last few years.

Syndromic CLP

Over 300 syndromes are known to have clefting of the lip or palate as an associated feature (Online Mendelian inheritance in man: http://www.ncbi.nlm.gov/omim). As with all clinically recognizable syndromes, cases of syndromic CLP or CP can be broadly subdivided into

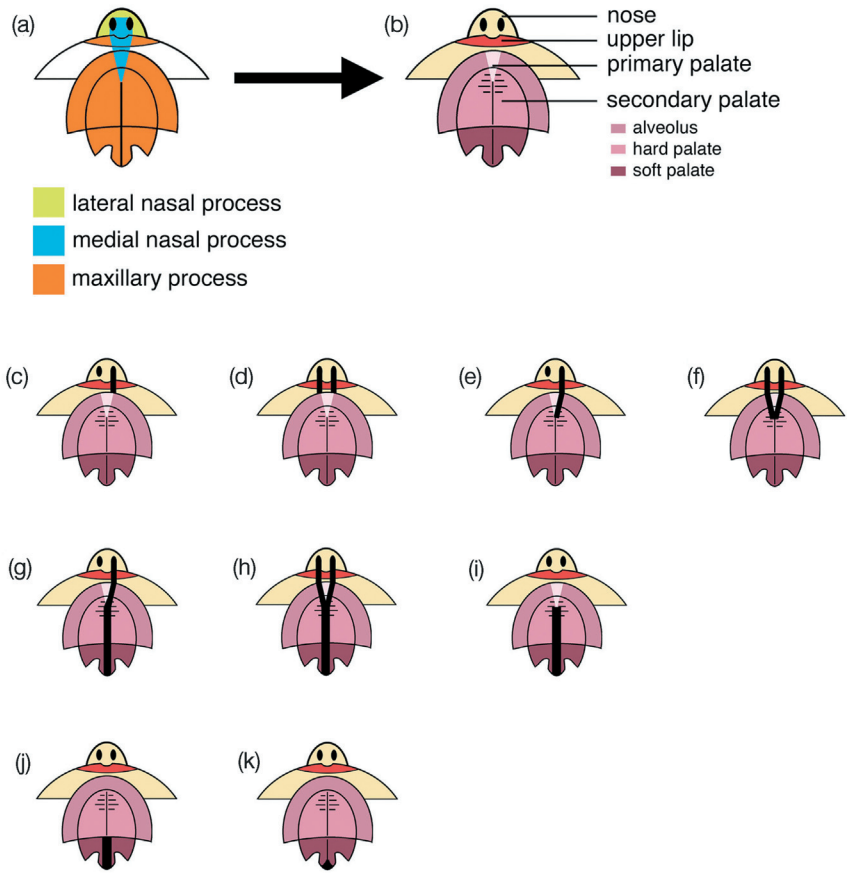

Figure 1 Embryological origins of the midline facial structures. (a, b) In the developing embryo, the lateral nasal processes form the alae and sides of the nose, while the medial nasal processes form the intermaxillary segment, composed of the upper lip philtrum, the primary palate and the four incisor teeth. The maxillary process forms the remainder of the upper lip and the secondary palate, consisting of the hard palate and associated dentition anteriorly and posteriorly, and the soft palate. Various types of orofacial clefting. (c) Unilateral cleft lip; (d) bilateral cleft lip; (e) unilateral cleft lip and primary palate; (f) bilateral cleft lip and primary palate; (g) complete unilateral cleft of the lip and palate; (h) complete bilateral cleft of the lip and palate; (i) isolated cleft of the secondary palate; (j) isolated cleft of the soft palate; (k) submucous cleft of the soft palate.

those that occur as part of a characterized Mendelian disorder (resulting from a single gene defect), those arising from structural abnormalities of the chromosomes, syndromes associated with known teratogens or those whose causation remains obscure and are therefore currently uncharacterized. Single gene disorders are the result of specific gene mutations on the autosomes or sex chromosomes and are inherited following Mendelian rules (autosomal dominant or recessive and X-linked dominant or recessive, respectively) with varying levels of penetrance and expressivity. Cytogenetics, or the study of chromosomal abnormalities, has revealed a wide range of physical chromosomal alterations, including variations in both number and structure, which can cause perturbations of gene function and congenital malformations. It has been estimated that 6 per cent of all congenital malformations are due to visible cytogenetic abnormalities (Kalter and Warkany, 1983) and approximately 5 per cent of both the autosomal deletions and duplications that produce congenital defects have CLP as a feature (Brewer *et al.*, 1998, 1999). It should be noted, however, that advances in molecular techniques now allow the identification of alterations that affect very small regions of the chromosome and, in some cases, specific genes responsible for cytogenetic syndromes are being isolated. Therefore, the distinction between chromosomal abnormalities *per se* and single gene disorders is rapidly becoming indistinct.

Of much recent excitement has been the identification of some candidate genes thought to be responsible for several major syndromic clefting disorders. One of the most common human autosomal dominant disorders associated with CLP is van der Woude syndrome (VWS), which contributes to around 1 per cent of syndromic CLP cases (van der Woude, 1954). This condition is associated with highly characteristic pitting of the lower lip mucosa and CLP. The locus for VWS has previously been identified as a region of chromosome 1 (1q32-q41)

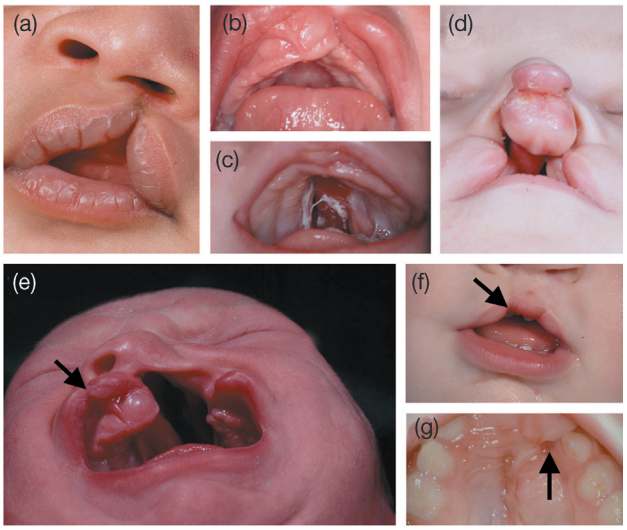

Figure 2 The clinical spectrum of orofacial clefting deformities. (a) Left-sided isolated unilateral cleft of the lip; (b) left-sided unilateral cleft of the lip and alveolus; (c) isolated bilateral cleft of the palate; (d) complete bilateral cleft of the lip and palate; (e) left-sided complete unilateral cleft of the lip and palate (the arrow indicates mild notching of the right lip); (f) the subject in (e) following lip repair (the arrow indicates persistence of the right lip notch); (g) the subject in (e) following palate repair (the arrow indicates the presence of a residual defect in the alveolus).

(Schutte *et al.*, 2000), but until now the identity of the offending gene had remained elusive. Recently, a unique approach has exploited the discovery of monozygotic twins who demonstrated VWS in one member of the pair, but not in the other twin or parents (Kondo *et al.*, 2002). This allowed the identification of a nonsense mutation in the interferon regulatory factor-6 (*IRF6*) gene in the affected twin. *IRF6* encodes a transcription factor belonging to a nine-member family involved in regulating the expression of Interferon-α and -β following viral infection. However, the exact role of *IRF6* during development is unknown. This point notwithstanding, in the developing mouse embryo, *Irf6* demonstrates high levels of expression in a variety of craniofacial structures, including the medial edges of the fusing palatal processes, tooth buds, hair follicles and skin. This expression pattern and the findings that haploinsufficiency of *IRF6* causes VWS suggest an important role during craniofacial development, with some suggestion that it mediates interactions between members of the transforming growth factor-β (TGFβ) superfamily of signalling peptides (Kondo *et al.*, 2002; Muenke, 2002). Indeed, 45 additional unrelated families affected by VWS have also been demonstrated to carry mutations in the *IRF6* gene (Kondo *et al.*, 2002).

Another recent breakthrough is the identification of a homozygous loss-of-function mutation in the poliovirus receptor related-1 (*PVRL1*) gene as being responsible for an autosomal recessive CLP-ectodermal dysplasia syndrome (CLPED-1) (Suzuki *et al.*, 2000). This discovery was made in an isolated population derived from Margarita Island, situated just north of Venezuela. Within this population, CLPED-1 is a relatively frequent condition because it results from homozygosity for a common nonsense mutation in *PVRL1* called W185X. Within the indigenous population of the island, approximately one in 26 normal people are heterozygous for W185X. *PVRL1* encodes a cell adhesion molecule called nectin-1, which in the mouse embryo is highly expressed in the medial edge epithelium of the developing palate (Suzuki *et al.*, 2000). These findings suggest that normal *PVRL1* function is important in mediating fusion of the palatal shelves during the later stages of palatogenesis.

A mixed clefting type is the rare occurrence of CLP and CP within members of the same pedigree. A number of the ectodermal dysplasia-type clefting syndromes (EDCS) demonstrate a mixed clefting type. Recently, mutational analysis of some of these syndromes has begun to unravel the complexities of the genetic interactions that underlie these disorders. The autosomal dominant ectrodactyly, ectodermal dysplasia, orofacial cleft (EEC) syndrome is characterized by central reduction defects in the hands and feet (ectrodactyly) often associated with syndactyly, ectodermal dysplasia manifesting as dry skin, sparse hair, dystrophic nails and hypoplastic teeth and CLP. It has been demonstrated that heterozygous mutations in the *P63* gene on chromosome 3q27 lead to the EEC syndrome (Celli *et al.*, 1999; Ianakiev *et al.*, 2000). *P63* is a homologue of the transcription factor-encoding *P53* tumour suppressor gene and many of the causative mutations result in amino acid substitutions that are predicted to abolish the DNA binding capacity of P63 (Celli *et al.*, 1999; Ianakiev *et al.*, 2000). In the mouse embryo, *p63* is highly expressed in ectodermal tissue, particularly that of the limb bud apical ectodermal ridge and the maxillary and mandibular processes of the first branchial arch (Mills *et al.*, 1999; Yang *et al.*, 1999). Ablation of *p63* function in homozygous mice results in craniofacial anomalies, limb truncation and an absence of epidermal appendages such as hair, sweat glands and tooth follicles (Mills *et al.*, 1999; Yang *et al.*, 1999). Other EEC-like syndromes within the EDCS umbrella have also been reported in association with mutations in *P63* and these include ankyloblepharon, ectodermal dysplasia, clefting syndrome (AEC or Hay–Wells syndrome) (McGrath *et al.*, 2001) and limb mammary syndrome (LMS) (van Bokhoven *et al.*, 2001). Some of the clinical differences between these various EDCS can be subtle, but they are important. AEC is characterized by ankyloblepharon (fused eyelids) in addition to features characteristic of ectodermal dysplasia and the presence of CLP. LMS is characterized by the presence of ectrodactyly, mammary gland/nipple hypoplasia and, importantly, isolated CP rather than CLP. Overall, the *P63* mutations identified

with EEC, AEC and LMS all demonstrate subtle differences and, therefore, exhibit clear genotype–phenotype correlations (McGrath *et al.*, 2001; van Bokhoven *et al.*, 2001). This high phenotypic variability observed for specific mutations in *P63* demonstrates the importance of modifying factors during development of the embryo, not least in the causation of CLP and isolated CP within members of the same pedigree.

Msx1 (formerly *Hox7*) (Hill *et al.*, 1989; Robert *et al.*, 1989; MacKenzie *et al.*, 1991a, b) is a member of a distinct subfamily of homeobox genes related to the *Drosophila msh* (muscle segment homeobox) gene (Holland, 1991). *Msx1* encodes a transcription factor and also demonstrates a regionally restricted expression pattern in the developing murine craniofacial complex, including the palate (MacKenzie *et al.*, 1991a, b). Mice lacking *Msx1* function exhibit a variety of craniofacial defects including clefting of the secondary palate, complete arrest of tooth development at the bud stage and anomalies of several facial bones (Satokata and Maas, 1994). A heterozygous *MSX1* nonsense mutation has recently been identified in a three-generation Dutch family exhibiting various combinations of CLP, CP and selective tooth agenesis (van den Boogaard *et al.*, 2000). Significant linkage disequilibrium has also been found between CLP and neutral polymorphisms within *MSX1* and *TGFβ3* (Lidral *et al.*, 1998). However, in the family described by van den Boogaard *et al.* (2001) there was marked variation in the expressivity of CLP and the additional presence of non-penetrant cases indicates that this is a comparatively weak phenotype, almost certainly modulated by additional genetic and environmental factors (Wilkie and Morriss-Kay, 2001).

Another malformation disorder associated with CLP but also characterized by disturbances in development of the midline facial structures is X-linked Opitz syndrome, a condition associated with mutations in the *MID1* gene on chromosome Xp22 (Quaderi *et al.*, 1997). *MID1* encodes a RING finger, B-box zinc finger and coiled–coiled protein, which is associated with cytoplasmic microtubules. A variety of mutations in *MID1* have been identified in individuals affected by Opitz syndrome, but the exact developmental role of the encoded protein remains unclear (Quaderi *et al.*, 1997; Gaudenz *et al.*, 1998; Schweiger *et al.*, 1999; Cox *et al.*, 2000). However, the murine *Mid1* gene does demonstrate high expression in the developing branchial arches, which is consistent with the craniofacial anomalies seen in patients with *MID1* mutations (Quaderi *et al.*, 1997).

Syndromic CP

In addition to syndromic CLP, progress has also been made in elucidating the genetic mechanisms behind several syndromic causes of isolated CP. X-linked CP (CPX) is a rare semi-dominant X-linked disorder characterized by CP and ankyloglossia (Lowry, 1970; Bjornsson *et al.*, 1989). The causative gene was originally localized to chromosome Xq21 (Moore *et al.*, 1987), but recently Braybrook *et al.* (2001) succeeded in pinpointing a variety of mutations in the *TBX22* gene (which encodes a member of the T-box family of transcription factors) in individuals from a number of separate families, as being responsible for CPX. These mutations, including missense, nonsense, splicesite and frameshift, were all predicted to result in a complete loss of function of *TBX22*. Interestingly, *TBX22* is also expressed in the developing palate and potential target genes for this transcription factor have been shown to include members of the fibroblast growth factor and TGFβ families, which are known to encode signalling molecules heavily implicated in early craniofacial development (Aldred, 2001; Braybrook *et al.*, 2001; Casci, 2001; Murray, 2001). *TBX22* is the first gene to be identified for a major CP syndrome and is particularly significant in view of the fact that targeted disruption of *Tbx1* in the mouse results in a wide range of developmental anomalies which encompass almost all of the common features of the DiGeorge/velocardiofacial syndromes (Jerome and Papaioannou, 2001; Lindsay *et al.*, 2001). These syndromes, which arise as manifestations of deletions in chromosome 22q11, are thought to be caused by a failure in function or migration of neural crest cells and predominantly affect derivatives of the third and fourth branchial arches and their associated pharyngeal pouches, but affected individuals can also have CP (Scambler, 2000). Heterozygous null mutant mice demonstrating haploinsufficiency of *Tbx1* have aortic arch defects, whereas homozygous null mutants exhibit a more severe phenotype that includes isolated CP (Lindsay *et al.*, 1998; Jerome and Papaioannou, 2001).

A wide variety of other syndromic disorders exhibit varying levels of CP as part of their phenotype (Gorlin *et al.*, 2001) and many of the causative genes have now been identified. Treacher Collins syndrome (TCS) is an autosomal dominant disorder of craniofacial development, which occurs with an incidence of around 1:50 000 live births (Gorlin *et al.*, 2001). The features of TCS are highly variable, but essentially consist of external and middle ear malformations, downsloping palpebral fissures with colobomas of the lower eyelids, zygomatic and mandibular hypoplasia and CP in 28–35 per cent of affected individuals (Franceschetti and Klein, 1949; Stovin *et al.*, 1960; Peterson-Falzone and Pruzansky, 1976). A large-scale collaborative effort has used positional cloning to localize the TCS gene (*TCOF1*) to human chromosome 5q32-q33.1 (Treacher Collins Syndrome Collaborative Group, 1996). *TCOF1* encodes a protein called treacle, which shows weak homology to a family of nucleolar phosphoproteins (Dixon *et al.*, 1997; Wise *et al.*, 1997). A number of largely family-specific mutations have been identified in

affected individuals, which have been predicted to result in the creation of a premature termination codon in the transcribed protein (Gladwin *et al.*, 1996; Edwards *et al.*, 1997). The exact function of treacle is currently unknown, but it has been hypothesized to be involved in the shuttling of proteins between the nucleolus and cytoplasm of the cell. This is based largely on its predicted sequence and the findings that mutations can lead to altered localization from the nucleolus to different compartments of the cell (Marsh *et al.*, 1998; Winokur and Shiang, 1998). How these functions relate specifically to the control of craniofacial development is at present unknown.

Holoprosencephaly (HPE) is a developmental disorder that encompasses a spectrum of defects ranging from mild anomalies of midline patterning to a complete failure of forebrain division, with associated cyclopia (Muenke *et al.*, 1994). HPE is comparatively rare in live births (around 1:15 000), but in early pregnancy this figure is much higher (around 1:250) with the majority of these foetuses being miscarried (Wilkie and Morriss-Kay, 2001). This makes HPE a significant congenital anomaly and foetuses that do go to term can exhibit CP. Mutations in the gene encoding the Sonic hedgehog (SHH) signalling peptide have been associated with a holoprosencephalic phenotype in both mice (Chiang *et al.*, 1996) and humans (Belloni *et al.*, 1996; Roessler *et al.*, 1996). Interestingly, there are dosage-dependent differences in susceptibility between mice and humans. Heterozygous *Shh* –/– mice are phenotypically normal, whereas heterozygous mutations in human families produce HPE with a range of phenotypes (Belloni *et al.*, 1996; Chiang *et al.*, 1996; Roessler *et al.*, 1996). These findings highlight the key role that the SHH signalling peptide plays in midline patterning of the human embryo.

Stickler syndrome (hereditary arthro-ophthalmopathy) is an autosomal dominant disorder of collagen connective tissue associated with ocular, auditory, articular and craniofacial manifestations (Herrman *et al.*, 1975). Stickler syndrome is subdivided into types 1 and 2 on the basis of the vitreoretinal phenotype in the eye, but the systemic features are essentially similar for both groups, with approximately 25 per cent of cases exhibiting some form of midline clefting, including CP (Snead and Yates, 1999). Approximately 75 per cent of subjects with Stickler syndrome are type 1 and demonstrate linkage to the *COL2A1* gene, which encodes type II collagen (Snead *et al.*, 1999). Type XI collagen is a more minor fibrillar collagen and mutations in the *COLIIA1* gene, which encodes the α1 chain of type XI procollagen, have been demonstrated in patients with the type 2 Stickler phenotype (Richards *et al.*, 1996). One of the effects of these varying defects in collagen biosynthesis is abnormal skeletal morphogenesis, which can then manifest as isolated CP.

Non-syndromic clefting

The study of causative factors in non-syndromic CLP/CP in humans has been considerably hampered by the nature of the condition. Non-syndromic orofacial clefting arises as a complex multifactorial trait, being a myriad of Mendelian patterns exhibiting varying levels of penetrance, sex differences and environmental overlays, with the result that gene identification is difficult (Murray, 1995).

Large-scale family linkage analysis has provided a statistical method of detecting the chromosomal location of possible loci within a population where gene defects might result in a predisposition to CLP. The first report of such an analysis suggested possible linkage between CLP and the blood clotting factor XIII gene (*F13A*) on chromosome 6p (Eiberg *et al.*, 1987). Unfortunately, further evidence for support of the *F13A* gene has been more equivocal (Hecht *et al.*, 1993; Vintiner *et al.*, 1993). However, successive linkage studies have provided further indications for the involvement in CLP of regions on the 6p chromosome. These include 6p23-24 (Prescott *et al.*, 2000), 6p24.3 (Davies *et al.*, 1995) and 6p23 (Carinci *et al.*, 1995). Together, these findings present a real possibility that a gene on human chromosome 6p may play a role in non-syndromic clefting (Murray, 1995). Linkage has also been reported for CLP to endothelin-1 (*ET1*), which encodes a vaso-active peptide expressed in vascular endothelial cells (Carinci *et al.*, 1995). ET1 is involved in the regulation of blood pressure and *ET1* –/– mice carrying a targeted disruption of this gene do have hypertension. However, they also exhibit craniofacial defects, including a marked reduction in tongue size, micrognathia and CP (Kurihara *et al.*, 1994). In addition, one of the G-protein coupled endothelin receptors, *ETA*, is known to be expressed in neural crest-derived ectomesenchyme of the branchial arches. Targeted disruption of *ETA* or *ET1* in mice produces craniofacial defects that resemble a broad human condition called CATCH-22 (cardiac defects, abnormal facies, thymic hypoplasia, CP, hypocalcaemia, associated with chromosome 22 microdeletion) (Wilson *et al.*, 1993). CATCH-22 represents a spectrum of human malformation syndromes resulting from abnormal development of the third and fourth branchial arches. It has recently been shown that the craniofacial defects in *ETA* –/– mice are, in part, due to an absence of the goosecoid transcription factor (Clouthier *et al.*, 1998).

In addition to *F13A* and *ET1*, a number of other candidate genes have also demonstrated linkage to CLP, but the results have been contradictory. These include the proto-oncogene *BCL3* on chromosome 19 (Stein *et al.*, 1995; Maestri *et al.*, 1997; Wyszynski *et al.*, 1997a) and the retinoic acid receptor alpha gene (*RARA*) on chromosome 17 (Shaw *et al.*, 1993; Vintiner *et al.*, 1993). A more recent genome-wide linkage study in families

with multiple cases of non-syndromic CLP concluded that no single major CLP locus exists and a multifactorial model was the most likely explanation of the genetic component of this disorder (Prescott *et al.*, 2000, 2001). A putative role for these candidate genes therefore remains to be firmly established, but it is not unreasonable to suggest that both together and individually they might have a modifying or additive role within the aetiology of non-syndromic CLP (Carinci *et al.*, 2000).

More recently, association studies using the candidate gene approach have become an important method of genetic analysis for non-syndromic CLP/CP (Hodge, 1993). The suggestion that abnormal function of a particular gene might play a role in the aetiology of these conditions can arise for a variety of reasons; the presence of a CLP/CP phenotype in knockout mice generated with targeted disruption of a particular gene (Table 1), the specific embryonic expression domain or chromosomal location of a newly cloned gene or the predicted functional properties of a known protein can all provide clues. Having selected a particular candidate gene, it is then possible to analyse epidemiologically the frequencies of different genetic variants of the gene or nearby chromosomal markers and provide evidence of association for their aetiological role in a particular condition, for example non-syndromic CLP/CP (Prescott *et al.*, 2001). This approach has provided some evidence to show an association between TGFα and non-syndromic CLP (Ardinger *et al.*, 1989; Chenevix-Trench *et al.*, 1992; Holder *et al.*, 1992; Stoll *et al.*, 1992; Feng *et al.*, 1994; Field *et al.*, 1994). TGFα is one member of a large group of developmentally important intercellular signalling molecules and in the mouse, Tgfα protein has been localized in the epithelium of the palatal shelves prior to fusion (Dixon *et al.*, 1991). However, while targeted disruption of *Tgfα* produces defects in the hair follicles and eyes of the mouse, it does not produce CLP (Luetteke *et al.*, 1993; Mann *et al.*, 1993). In addition, more classical linkage-based genetic studies, albeit carried out on relatively small numbers of families, have failed to detect an association between TGFα and CLP (Hecht *et al.*, 1991; Vintiner *et al.*, 1992). Together, all these investigations suggest that TGFα is not a major dominant gene for CLP, but probably acts as a modifier (Murray, 1995; Prescott *et al.*, 2001). Unfortunately, disagreement between different genetic studies carried out on populations of varying size and ethnic background has become a recurring theme in the investigation of non-syndromic CLP/CP over the years (Spritz, 2001). Another locus that has been identified in association with non-syndromic CLP encodes the *TGFβ3* gene on chromosome 14q24 (Maestri *et al.*, 1997; Lidral *et al.*, 1998). In the case of *Tgfβ3*, the mouse knockout does have a CP phenotype (Proetzel *et al.*, 1995) and the Tgfβ3 signalling protein would appear to have an important role during fusion of the secondary palate, directly controlling the differentiation of epithelium to mesenchyme in the midline seam between the adjacent palatal shelves in the mouse (Kaartinen *et al.*, 1997). In addition, exogenous TGFβ protein can correct the palatal fusion defect in *Tgfβ3* –/– embryos *in vitro* (Taya *et al.*, 1999) and promote scarless healing following surgical cleft lip repair in mice embryos *in utero* (Kohama *et al.*, 2002).

Table 1 Mouse knockouts associated with cleft palate.

Gene	Gene product	Reference
Gad67	γ-aminobutyric acid-producing enzyme	Condie *et al.* (1997)
Gabrβ3	γ-aminobutyric acid receptor	Culiat *et al.* (1995)
Tgfβ3	Signalling peptide	Proetzel *et al.* (1995)
ActivinβA	Signalling peptide	Matzuk *et al.* (1995)
ET1	Vasoactive peptide	Kurihara *et al.* (1994)
Hoxa2	Transcription factor	Gendron-Maguire *et al.* (1993) Rijli *et al.* (1993)
Dlx2	Transcription factor	Qiu *et al.* (1997)
Lhx8	Transcription factor	Zhao *et al.* (1999)
Msx1	Transcription factor	Satokata and Maas (1994)
Pax9	Transcription factor	Peters *et al.* (1998)
Pitx1	Transcription factor	Szeto *et al.* (1999)
Tbx1	Transcription factor	Lindsay *et al.* (2001) Jerome and Papaioannou (2001)
p63	Transcription factor	Mills *et al.* (1999) Yang *et al.* (1999)

The recent finding of a W185X *PVRL1* mutation being responsible for CLPED-1 on Margarita Island has been the starting point to determine whether this mutation might also be an aetiological factor in isolated CLP. The basis for this investigation is the fact that the incidence of CLP is also known to be high within this population (Suzuki *et al.*, 2000). While the high heterozygosity trait for W185X *PVRL1* and the limited population size on Margarita Island precluded the study of this mutation with respect to isolated CLP in these islanders, a larger geographically adjacent population in northern Venezuela did demonstrate significant increases in this mutation in individuals with isolated CLP (Sözen *et al.*, 2001). While this mutation is only a moderate risk factor for CLP (the majority of individuals with isolated CLP studied in the population did not carry the mutation), this study is important because it has provided the first evidence of a specific mutation of pathological significance as a genetic risk factor for non-syndromic CLP (Aldred, 2001; Casci, 2001; Spritz, 2001; Wilkie and Morriss-Kay, 2001).

Environmental influences

A number of observations also suggest a significant environmental contribution in the aetiology of CLP/CP;

the lack of total concordance in monozygotic twins, the relatively rare findings of non-syndromic cases being present throughout large family groups and the varying social, geographical and ethnic incidence of these malformations (Spritz, 2001). The majority of CLP cases are, therefore, multifactorial, and a variety of environmental factors have been implicated (Wyszynski and Beaty, 1996). It is logical to state that the true aetiology relevant to these conditions cannot be treated in isolation, but it should be remembered that intrauterine environmental factors will influence foetal development in combination with the individual genetic background of the embryo (Prescott *et al.*, 2001).

Maternal cigarette smoking, leading to embryonic hypoxia, has been associated with an increased incidence of non-syndromic CLP. However, the current evidence for an association is far from overwhelming. A relatively recent meta-analysis of relevant studies produced over the 20 years prior to 1996 suggested a small, but statistically significant, association between maternal cigarette smoking during the first trimester of gestation and an increased risk of having a child with CLP or CP (Wyszynski *et al.*, 1997b). Interestingly, there is some suggestion that the risk of clefting associated with maternal smoking can be increased in infants carrying the cleft-associated *TGFα* mutation (Shaw *et al.*, 1996), but potential synergism between these two factors has also been refuted (Christensen *et al.*, 1999). Some evidence also exists to suggest that altitude hypoxia during pregnancy might also be associated with an increased incidence of several birth defects, including CLP (Castilla *et al.*, 1999). Maternal alcohol (ethanol) ingestion (frequently associated with cigarette smoking) can result in an increased risk of CLP (Romitti *et al.*, 1999). Interestingly, this latter study also found evidence for gene–environment interactions in non-syndromic CLP aetiology, with a greater incidence of CLP in children carrying allelic variants at the *MSX1* site (Romitti *et al.*, 1999). Certainly, women who abuse alcohol during pregnancy are at significant risk of bearing a child with the manifestation termed foetal alcohol syndrome (FAS) (Jones *et al.*, 1973). Affected individuals exhibit pre- and post-natal growth retardation, craniofacial anomalies and dysfunction of the central nervous system. FAS represents the mild end of the HPE spectrum of anomalies, which can exhibit clefting, and the heavier the consumption, the more likely a CLP/CP phenotype will form a component of the craniofacial defect (Shaw and Lammer, 1999). It is alarming to think that approximately one in 30 women are thought to abuse alcohol during pregnancy and that around 6 per cent of these will have children with clinically recognizable FAS (Gorlin *et al.*, 2001).

Some attention has also been paid to the nutritional status of pregnant mothers with respect to incidences of clefting phenotypes in their offspring, in particular, a role for folic acid supplementation as a method of reducing CLP incidence. Certainly, there is conclusive evidence for maternal folate supplementation in the prevention of neural tube defects (Medical Research Council, 1991) and some epidemiological investigations have suggested that deficient maternal folic acid intake may predispose to orofacial clefting. However, other studies have failed to find an association. At the present time, a relationship would therefore appear to be inconclusive and it is currently not proven whether folate deficiency is a major contributor to non-syndromic CLP (Hartridge *et al.*, 1999).

Conclusions

It is likely that advances in our understanding of both the genetic and environmental aetiology of CLP will continue. With the recent draft sequencing of both the human and mouse genomes and the introduction of gene micro-array technology, further identification of the candidate genes and genetic pathways involved in syndromic clefting can be expected. More complex and widespread multifactorial genetic analyses are likely to be required to dissect further the aetiology of non-syndromic CLP and, in particular, the emergence of studies linking environmental influences with the genetic background of susceptible embryos. Ultimately, all of these advances will allow more accurate methods of genetic screening, the identification of high-risk individuals or family groups and improved pre-natal diagnosis. In turn, we may witness the introduction of both preventative and *in vivo* foetal therapy for these debilitating conditions.

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