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Orofacial clefting: recent insights into a complex trait

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Orofacial clefts are common birth defects of multifactorial etiology. Several novel approaches have recently been applied to investigate the causes of clefts. These include examining Mendelian forms of clefting to identify genes that might also be implicated in isolated clefting, analyzing chromosomal rearrangements in which clefting is part of the resultant phenotype, studying animal models in which clefts arise either spontaneously or as a result of mutagenesis experiments, exploring how expression patterns correlate with gene function and examining the effects of gene–environment interactions. Together, these complementary strategies are providing researchers with new clues as to what mechanisms underlie orofacial clefting.

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Introduction

Orofacial clefts comprise a large fraction of all human birth defects and are notable for their significant lifelong morbidity and complex etiology. On the basis of anatomical, genetic and embryological findings, orofacial clefts are commonly subdivided into those affecting the lip and/or palate (CL/P) and those involving the palate only (CPO) [1]. Clefts can be further categorized into syndromic (see Glossary) and isolated forms, according to whether affected individuals have other physical and developmental anomalies. Because the great majority of clefts appear to be isolated (~70% CL/P and ~50% CPO) [2], understanding the causes of these forms of clefts has long been a focus of research.

Many aspects of clefting, including epidemiology, clinical care, and genetic and environmental risks, have been recently reviewed [3]. In this overview, we focus on recent developments in genetics [4], animal models [5] and gene–environment interactions [6].

Development of the lip and palate

After conception, a precisely coordinated cascade of developmental processes involving cell migration, growth, differentiation and apoptosis results in the development of craniofacial structures from the originating oropharyngeal membrane [7]. Early in the sixth week, the medial nasal prominences merge with each other and the bilateral maxillary processes to form the primary palate and the upper lip. The lower lip and jaw are produced by the mandibular prominences, which merge across the midline. The secondary palate begins to develop early in the sixth week from the two palatal shelves, which extend from internal aspects of the maxillary prominences. During weeks 7–8, apoptosis and epithelial–mesenchymal transformation (EMT) at the medial edges enable the palatal shelves to fuse after the shelves have ascended to an appropriate position above the tongue. Proteins such as integrins, matrix metalloproteinases, microtubules and actin cytoskeletons are involved in the EMT process [8].

The molecular events that underlie the formation of orofacial structures are under the strict control of an array of genes that includes the fibroblast growth factors (Fgfs), sonic hedgehog (Shh), bone morphogenetic proteins (Bmps), members of the transforming growth factor β (Tgf-β) superfamily, and transcription factors such as Dlx, Pitx, Hox, Gli and T-box families [2]. Hydration of extracellular matrix components (principally hyaluronan) in the shelf mesenchyme is thought to provide the necessary intrinsic force to cause shelf elevation [9]. However, contraction of elastic fibers and/or skeletal muscle fibers, and an increase in vascularity of the developing palate have also been proposed as alternative mechanisms underpinning shelf elevation. Palatal fusion itself appears to be driven by several cell adhesion molecules, including nectin 1, desmosomes and type IX collagen, and growth factors, such as TGFα/EGFR and TGF-β_3 [8,9].

The search for candidate genes

A variety of genetic approaches have been used to identify candidate genes and loci responsible for clefting [4]. Compiled in Table 1 is a list of candidate genes derived from linkage and association studies, studies of the roles these genes play in animal development and the phenotypes they generate when disrupted in mouse knockouts [1,5]. Genome-wide linkage scans have also provided some important clues. To date, 13 genome-wide scans

Glossary

Breakpoint mapping – Identification and delineation of chromosomal breakpoints caused by deletions, insertions, inversions or translocations. Breakpoints are mapped using high-resolution techniques such as fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH; see below).

Case-parent triad approach – A study design in which a triad made up of the mother, father and affected offspring is used as the unit of analysis. Parental alleles not transmitted to the offspring are used as ethnically matched genetic controls in statistical analyses.

Comparative genomic hybridization (CGH) – CGH is a fluorescent molecular cytogenetic technique for detecting chromosomal imbalances. Two genomic DNA samples are simultaneously hybridized *in situ* to normal human metaphase spreads, and regions of increased or decreased copy number are located or mapped relative to the normal metaphase chromosomes.

Confidence interval – This is an interval calculated from a given set of sample data that has a specified probability of containing the parameter being estimated. If samples of the same size are drawn repeatedly from a population and a confidence interval is calculated from each sample then 95% of these intervals should contain the population parameter.

Haploinsufficiency – A locus shows haploinsufficiency if more gene product is required to produce a normal phenotype than the amount produced by a single copy. This situation might arise if an individual is heterozygous for a certain gene mutation or hemizygous at a particular locus owing to a deletion of the corresponding allele.

LOD score – A measure to assess the strength of the evidence in favour of linkage. A LOD of 3 indicates 10^3 odds in favour of linkage compared to no linkage. HLOD is the LOD score corresponding to the likelihood ratio for linkage given heterogeneity.

Meta-analysis – A statistical method that integrates the results of several existing independent studies in order to provide a larger sample size for evaluation, and to produce a stronger conclusion that can be provided by any single study.

Quantitative-PCR – A sensitive method that enables the quantification of the amount of either DNA or RNA products generated during each cycle of the PCR.

Syndromic – Refers to cleft cases with an accompanying physical and/or developmental anomaly.

for nonsyndromic CL/P have been performed, and a meta-analysis (see Glossary) of these individual scans revealed significant heterogeneity LOD scores (see Glossary) on chromosomes 1p, 6p, 6q, 14q and 15q, and a particularly strong signal on 9q [10•].

The past two years in particular have witnessed several exciting new advances in the mapping of genes for clefting. The latest data from mouse and human studies have helped identify several genes known to underlie Mendelian syndromic forms of CL/P as also playing a role in the etiology of isolated clefts. These include *IRF6* [11••], *MSX1* [12], *PVRL1* [13], *TBX22* [14] and *FGFR1* [15] (Table 1).

Clues from Mendelian forms of clefts

Mendelian forms of clefting with phenotypes closely mimicking those of isolated clefts can greatly facilitate the mapping of genes underlying the isolated forms [2]. The autosomal dominant Van der Woude syndrome (VWS) is the best model studied to date. In addition to clefts, pits in the lower lip and hypodontia are the only additional features in VWS patients. Recently, mutations in the interferon regulatory factor 6 (*IRF6*) gene were reported to underlie VWS [16], and, subsequently, variants in *IRF6* were found to be significantly associated with nonsyndromic clefting as well [11••,17]. In the mouse, *Irf6* transcripts are highly expressed in the palatal medial edge epithelium (MEE) immediately before and during fusion of the palatal shelves [16] (Figure 1). It has been speculated that mutations in *IRF6* might repress the TGF-β signaling pathway in a manner analogous to *IRF1*-mediated repression, leading to increased epithelial apoptosis before the bilateral processes have managed to fuse [8].

Knocking out a second gene, *Msx1* (*msh* homeobox homolog 1), in mice results in clefting [18]. The Msx proteins are known to play key roles in epithelial–mesenchymal tissue interactions during craniofacial development [19]. In humans, *MSX1* is deleted in cases of a 4p deletion syndrome that is frequently associated with clefting [20]. Moreover, a nonsense mutation in exon 1 of *MSX1* caused tooth agenesis and various combinations of clefts in a Dutch family [21]. In a follow-up study of 1000 unrelated individuals with CL/P, complete sequencing of the gene showed that mutations in *MSX1* alone could account for 2% of isolated CL/P [12,22].

A third gene, *FGFR1* (fibroblast growth factor receptor-1), encodes a transmembrane receptor tyrosine kinase that transduces signals from secreted FGFs [23]. Loss-of-function mutations in *FGFR1* cause the autosomal dominant form of Kallmann syndrome (KAL2), which is characterized by hypogonadism and anosmia, and clefting in around 5–10% of the cases [15]. The variable expression of *FGFR1* variants results in some affected individuals presenting with isolated CL/P alone (JC Murray, unpublished).

Mutations in *TP63* are implicated in five distinct human developmental disorders, characterized by limb abnormalities, ectodermal dysplasia and orofacial clefts [24]. Interestingly, the distribution of mutations over the different p63 protein domains shows a clear pattern of genotype–phenotype correlation. Other notable examples of clefting syndromes that might include phenocopies of isolated clefts are X-linked cleft palate with ankyloglossia, caused by mutations in *TBX22* [14,25], cleft lip and palate-ectodermal dysplasia syndrome (*PVRL1*) [13,26], and lymphedema-distichiasis syndrome (*FOXC2*) [27,28]. Other genes underlying additional clefting syndromes that are also excellent candidates for investigating the causes of isolated clefts include *FOXE1* in Bamforth-Lazarus syndrome [29] and *FLNA* in otopalatodigital syndromes types 1 and 2 [30].

Clues from genomic rearrangements

Genomic rearrangements can arise when interspersed repeat elements lying in tandem facilitate submicroscopic

Table 1

Genes implicated in orofacial clefting based on evidence from animal models, expression analyses, and human linkage and/or association studies.

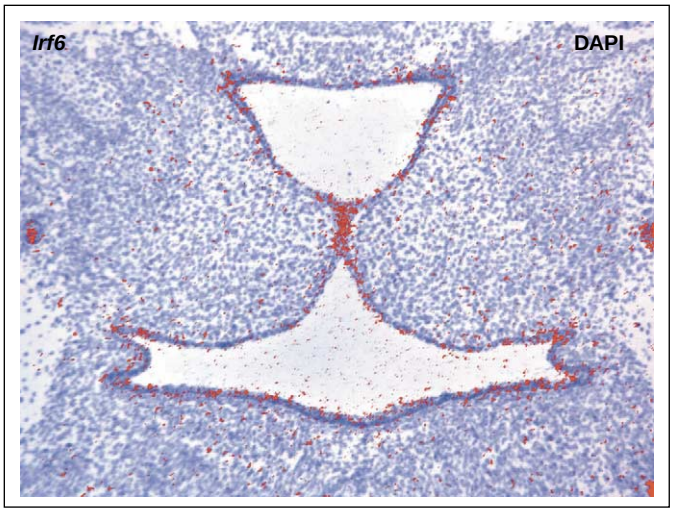
Gene	Cytogenetic location[a]	Gene function	Animal model phenotype[b]	Expression data	Linkage/association	Known syndrome
IRF6	1q32	TF	NA	+	+	Van der Woude
SKI1	1q32	GF	+	+	+	
MTHFR	1p36	CS	NA	+/−	+	
TGFA	2p13	GF	−	+/−	+	
TP63	3q27	TF	+	+	−	EEC
MSX1	4p16	TF	+	+	+	Witkop
EDN1	6p24.1	CAM	+	NA	+	
FGFR1	8p11.2–8p11.1	GFR	+	+	+	Kallmann
PPP3CC	8p21.3	CS	NA	+	+	
FOXE1	9q22	TF	+	+	+	Bamforth-Lazarus
PVRL1	11q23	CAM	NA	+/−	+	Margarita Island
TGFB3	14q24	GF	+	+	+	
GABRB3	15q11.2–15q12	CS	+	−	+	
FOXC2	16q22–16q24	TF	−	+	−	Lymphedema-distichiasis
RARA	17q21	CS	NA	+	+	
BCL3	19q13	TF	−	−	+	
TBX22	Xq21	TF	NA	+	+	CP and ankyloglossia

Abbreviations: CAM, cell adhesion molecule; CS, cell signaling; EEC, ectrodactyly ectodermal dysplasia; GF, growth factor; GFR, growth factor receptor; NA, not available TF, transcription factor; −, negative; +, positive; +/−, weak.
Table 1 is based in part on data compiled from references [1,6,8].
[a] Cytogenetic location according to Entrez Gene (http://www.ncbi.nih.gov/entrez/query.fcgi?db=gene).
[b] From the Mouse Genome Informatics database (MGI; http://www.informatics.jax.org/).

deletion and duplication events or translocations and/or inversions between or within chromosomes [31]. Genetic variants that result in a phenotype including clefting and that are found segregating with a genomic rearrangement in multiple members are best represented by the 22q deletion syndrome. Duplications of this same region have been associated with cleft palate [32], suggesting that genome-wide searches using comparative genomic hybridization (CGH; see Glossary), quantitative-PCR (see Glossary) or allele-loss might reveal additional clefting loci.

Recently, two relevant genes or gene clusters with balanced translocations and CL/P have been identified: the first gene at 19q13 [33] and the second gene at 2q32 [34•]. The candidate gene transected at 2q32 is *SATB2*. It

Figure 1



Irf6 expression in the E14.5 prefusion mouse palate. The figure depicts an *in situ* frontal section through the posterior palate. Nuclei were stained with DAPI for background staining and the silver grains were pseudo-colored red in the merged image. (Photograph kindly provided by Alexandra Knight and Professor Michael J Dixon).

is highly expressed in both the lip and the palate, making it an excellent candidate for isolated CL/P. Expression analyses in the mouse secondary palate reveal that the strongest expression of *Satb2* occurs before palatal shelf fusion (E13.5), with a dramatic down-regulation after the shelves have fused (E14.5) (Figure 2). Additional examples of clefts arising from displaced genomic material are the *Dancer* mutation [35] and clefts in the 22q and 1p36 deletion syndromes [36,37].

The transforming growth factors

Transforming growth factors are one of the most extensively studied gene families in relation to clefting. One member of this superfamily, transforming growth factor α (TGF-α), binds to the epidermal growth factor receptor (EGFR) and elicits responses similar to but more potent than EGF. The expression pattern of TGF-α in palatal tissues, especially in the midline seam and subjacent mesenchyme of the palatal shelves at the time of shelf fusion, supports a role for *TGFA* in clefting. Although inconclusive, data from association studies indicate that either *TGFA* itself or markers in its vicinity might play an important role in clefting [38].

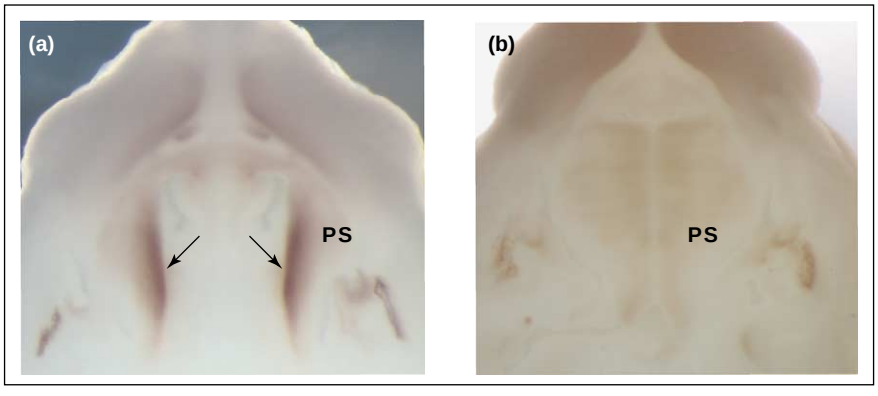
Studies of expression patterns have shown that, although each Tgf-β is temporally and spatially expressed in the developing palate, only the $Tgfb3^{-/-}$ knockout inhibited normal palatal shelf fusion in mice [39]. Moreover, the mechanism by which Tgf-β_3 affects palatal shelf fusion appears to be targeted and specific: the MEE in $Tgfb3^{-/-}$ mice fails to stop cellular proliferation [40], displays reduced apoptosis [41], fails to alter its morphology and adheres less well [42], fails to degrade the basement membrane and fails to undergo EMT [43]. Furthermore, exogenous TGF-β_3 can induce palatal fusion in the chicken through a process that requires physical contact of the MEE and formation of the midline seam [39]. Thus, Tgfb3 signaling is unequivocally a key pathway in palate development in the mouse. It also appears to be involved in palatal development in humans, because association studies have provided some corroborative data [38].

Animal models and expression data

Molecular studies in the mouse and chick have been pivotal in the identification of genes that regulate the dynamic cellular changes in the MEE. Although chick palatal shelves grow towards one another above the tongue and make contact, they do not actually fuse. The chick, therefore, has the advantage of mirroring the pathology seen in cleft palate, whereas the mouse provides an excellent model to study palatal shelf fusion. Indeed, studies in these animal models have helped to identify a battery of genes essential for palatal formation: *Tgfb3* [44]; *Bmp*s [45]; *Tbx22* [46]; *Fgfr*s [47]; *Pdgfc* [48]; *RhoA* [49]; the gene encoding PtdIns-3 kinase [43]; *Gabrb3* [50]; *Gad1* [51,52]; *Cspg* [53]; and *Mmp*s and *Timp2* [54].

Animal models with clefts arising spontaneously or as a result of mutagenesis experiments provide another exciting avenue for gene mapping [55]. The mouse is an excellent model for studying human clefting because the development of craniofacial structures in these two species is remarkably similar. Whereas cleft palate is a common phenotype in the mouse, cleft lip is rare. To date, four mutations have been reported with cleft lip and palate phenotypes in mice. These include two spontaneous mutations called *Twirler* and *Dancer*, a transgene insertion-induced deletion mutation called *Legless*, and a radiation-induced mutation called *Brachyphalangy* [56]. Both the *Dancer* and *Twirler* mutations are almost fully penetrant for CL/P in homozygotes. Furthermore, *Dancer* was shown to arise from a translocation of the *p23* gene

Figure 2



Satb2 expression during development of the murine secondary palate. Whole-mount *in situ* hybridization analysis of *Satb2* expression in mouse embryos at (a) E13.5 and (b) E14.5. **(a)** At E13.5, the strongest expression of *Satb2* is detected in the mesenchyme underlying the presumptive medial edge epithelia (arrows). **(b)** By the time of palatal shelf fusion at E14.5, the expression is dramatically down-regulated. Abbreviations: PS, palatal shelf. (Photographs kindly provided by Professor Michael J Dixon).

sequence into the *Tbx10* locus, resulting in ectopic expression of *Tbx10* under the influence of the *p23* promoter [35].

In addition to these mutant strains, cleft lip also occurs spontaneously in around 5–30% of embryos and neonates in a well-studied family of inbred mouse strains (the 'A' strains) [56]. A genome-wide screen for cleft susceptibility loci in the *A/WySn* strain identified two epistatically interacting loci, *clf1* and *clf2*, that contribute to the cleft lip phenotype [57]. The *clf1* locus contains two *Wnt* genes, *Wnt3* and *Wnt9b*, suggesting a potential role for the Wnt signaling pathway in orofacial development [58••].

As to expression analysis, the strongest candidate genes are likely to be those whose normal expressions encompass the critical time and tissue for lip and palate development. Three global approaches are currently available for gene expression analysis in craniofacial structures: (i) the ongoing studies of the Craniofacial and Oral Gene Expression Network (COGENE), which provides public web access to genome-wide expression analysis data of craniofacial tissues isolated from human embryos (http://humgen.wustl.edu/COGENE/); (ii) Optical Projection Tomography (OPT), which enables the visualization of the relative expression of genes both temporally and spatially [59]; and finally, (iii) the mouse N-ethyl-N-nitrosourea (ENU) mutagenesis projects [60], which in addition to helping identify potential candidates for craniofacial development also serve as a means of verifying whether the expression patterns of existing candidate genes are consistent with hypotheses about function. A recent study [61] of the effects of ENU mutagenesis on the offspring of male mice suggested that genes related to isolated cleft palate might be recessive in phenotype, whereas point mutations appeared to be more relevant to the pathogenesis of cleft lip and palate. See Box 1 for additional resources on the internet.

A role for environmental risk factors

Birth defects are likely to recur in families not only because of shared genetic factors but also as a result of

Box 1 Additional resources on the internet.

Center for Craniofacial Development and Disorders (CCDD)
http://www.hopkinsmedicine.org/craniofacial/Home/Index.cfm
OMIM is a curated database of human genes and genetic disorders. It enables rapid and direct linking between disease, gene sequence and chromosomal locus.

Craniofacial and Oral Gene Expression Network (COGENE)
http://humgen.wustl.edu/COGENE/
COGENE represents a consortium of investigators involved in describing human gene expression changes that occur during early stages of development, with particular emphasis on craniofacial development.

Developmental Genome Anatomy Project (DGAP)
http://www.bwhpathology.org/dgap/
DGAP looks for apparently balanced chromosomal rearrangements in patients with multiple congenital anomalies, and uses this information to map and identify genes that are disrupted or dysregulated at critical stages of human development.

Entrez Gene
http://www.ncbi.nih.gov/entrez/query.fcgi?db=gene
Entrez Gene provides a unified query interface for gene-oriented searches. It provides information on official nomenclature, aliases, sequence accessions, phenotypes, homology, map locations, and related websites.

Mouse Genome Informatics (MGI)
http://www.informatics.jax.org/
MGI provides integrated access to data on the genetics, genomics and biology of the laboratory mouse.

Murray laboratory website
http://genetics.uiowa.edu/
This is JCM's laboratory website. The web pages provide information on review protocols currently used in the lab, access to both published and unpublished data regarding genes and ongoing studies. Also included are extensive descriptions of each major project currently underway and options for obtaining additional information about them.

National Institute of Dental and Craniofacial Research (NIDCR)
http://www.nidcr.nih.gov/
NIDCR focuses on improving oral, dental and craniofacial health through research, research-training and the dissemination of health information. The site has numerous links to NIDCR clinical trials, funding opportunities for research and training, health information, and news and reports, among others.

N-ethyl-N-nitrosourea (ENU) mutagenesis projects
http://www.mouse-genome.bcm.tmc.edu/Home.asp
The ENU mutagenesis projects aim at determining the function of genes on the mouse chromosome 11 by saturating the wild type chromosomes with point mutations using the chemical N-ethyl-N-nitrosourea (ENU). Many of the new mutants thus created might represent models of human diseases such as birth defects, patterning defects, etc.

Online Mendelian Inheritance in Man (OMIM)
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
OMIM is a curated database of human genes and genetic disorders. It enables rapid and direct linking between disease, gene sequence and chromosomal locus.

Optical Projection Tomography (OPT)
http://genex.hgu.mrc.ac.uk/OPT_Microscopy/optwebsite/frontpage/index.htm
This site provides extensive data on optical projection tomography microscopy, which is a new technique that enables 3D imaging of biological specimens.

Single Nucleotide Polymorphism database (dbSNP)
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp
dbSNP is a central repository for a broad collection of simple genetic polymorphisms.

Society of Craniofacial Genetics
http://www.craniofacialgenetics.org/
This is the official website of the Society of Craniofacial Genetics, developed to promote education, research and communication in normal and abnormal development of the craniofacies.

shared environmental factors [62]. Cigarette smoking during pregnancy, with the attendant hypoxia, is associated with several adverse reproductive outcomes. The most recent meta-analysis on the effects of smoking indicates a moderately increased risk of orofacial clefts [63]. Specifically, estimates of relative risks were 1.34 (95% confidence interval (see Glossary) (CI); 1.25–1.44) for CL/P and 1.22 (95% CI; 1.10–1.35) for CPO.

Maternal nutrition during pregnancy also appears to play an important role. For example, low dietary intake of B-complex vitamins, in addition to exposure to deficient or excessive amounts of vitamin A, have been linked to increased risks of clefts [64,65]. Increased risks from exposures can suggest metabolic pathways whose disruption might trigger the development of clefts. Several studies have shown that folic acid and other B-complex vitamins might have a beneficial effect on reducing the risk of orofacial clefts [66–69].

The role of cholesterol-lowering drugs (e.g. statins) in prenatal development has been recently discussed [70]. Statins that reach the embryo through maternal intake of the drug might inhibit cholesterol biosynthesis and, consequently, affect the sterol-dependent Hedgehog family of morphogens, which is critical for the proper development of a range of structures, including the face. In a study of the adverse effects of gestational exposure to statins, two cases had cleft lip and two others had cleft palate among 31 adverse birth outcomes [71]. Other drugs, such as corticoids, have also received some attention, although the effects are modest in size [72].

Of particular importance to a complex trait such as clefts is the study of the likely impact of both genetic and environmental factors. Several studies have investigated interactions of a range of common environmental factors, such as cigarette smoking, alcohol intake, multivitamin/folic acid supplementation and the use of medication, with variant alleles in several genes that include *TGFA, TGFB3, MSX1, BCL3, RARA, MTHFR, CYP1A1, NAT1, NAT2, GSTT1* and *EPHX1*. These have been reviewed elsewhere [1,73,74].

In assessing disease risk, most previous studies have typically focused on the affected child as the unit of analysis. Recent works in clefts, however, have started to focus on parental contributions too, particularly for the assessment of maternally mediated effects and the effects of imprinting [75,76,77•]. Another recent extension of the case–parent triad approach (see Glossary) consists of using information from grandparents to explore the joint effects of maternal and offspring genotypes and to provide a direct estimation of relative risks [78]. These new analytical approaches ensure improved power for the detection of an effect, if present, and the judicious use of all the available data from the families.

Conclusions

Great strides have been made in recent years in our understanding of how orofacial clefts arise at a molecular level. Contributions from the single genes *IRF6*, *MSX1* and *FGFR1* now seem to explain approximately 15% of isolated clefts. Cigarette smoking and, possibly, disruptions in the folate biosynthetic pathway represent potential environmental risks. Given the now large sample sets available for study, linkage scans will hopefully have sufficient power to identify gene–environment interactions. Coupled with new discoveries in gene expression and animal models, researchers are finally starting to unravel the causes of orofacial clefts and, hopefully, new opportunities for improvements in diagnosis and treatment of this complex genetic can soon be made available to cleft patients and their families.

Update

A genome-wide scan for loci involved in CPO was recently conducted in a group of Finnish multiplex families [79]. Finland has one of the highest rates of isolated CPO among Caucasian populations, and even more intriguing is the higher observed prevalence of CPO compared to CL/P. Finland is therefore especially attractive for the study of isolated cleft palate. This study reported suggestive linkage at 1p34, 2p24-p25, and 12q21. The authors also screened nine unrelated affected individuals for mutations in *IRF6*, but no mutation was found.

Lately, Loeys *et al.* [80] reported that mutations in *TGFBR1* or *TGFBR2* were the cause behind a novel syndrome that is characterized by altered cardiovascular, neurocognitive, skeletal and craniofacial development. Tissues from affected individuals showed increased TGF-β signaling, reflected by nuclear enrichment of phosphorylated Smad2. In a related paper, Cui and coworkers [81] demonstrated that over-expression of Smad2 could rescue the cleft palate phenotype in $Tgf\text{-}\beta_3^{-/-}$ mutant mice. These reports provide further evidence that aberrant TGF-β signaling plays a prominent role in the pathogenesis of many common human malformations, including cleft palate.

Data from a recent study on Bmp-signaling in lip and palate fusion in mice uncovered a *Bmp4–Bmpr1a* genetic pathway involved in lip fusion, and revealed distinct roles of Bmp-signaling in lip and palate development [82]. Whereas *Bmpr1a* mutants had fully penetrant bilateral CL/P with tooth agenesis, most likely as a result of defective proliferation, *Bmp4* mutants had isolated cleft lip, possibly caused by premature apoptosis in the medial nasal processes. This suggests that Bmp-signaling plays distinct roles in lip fusion and secondary palate development. Interestingly, signaling through Bmpr1a appeared to affect the expression of transcriptional regulators such as *Barx1* and *Pax9*, but not of *Msx1*, *Tbx22* or *Osr2*.

As to studies of gene–environment interactions in relation to clefts, a recent meta-analysis examined the association between maternal cigarette smoking and infant's genotype at the *TaqI* site in *TGFA* [83]. Although maternal smoking was a consistent risk factor for both CL/P and CPO across all studies, the modest effects of interaction seemed to be restricted to cleft palate only.

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- •• of outstanding interest

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Marazita *et al.* combined the results of all previously performed genome-wide scans of isolated clefts with new data from their own analyses. More than 500 families are represented in this large dataset and one locus on 9q showed a heterogeneity LOD score of over 6.5, providing very strong support for a cleft locus or loci in this region.

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