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# Cleft lip and palate: synthesizing genetic and environmental influences

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## Abstract

Clefts of the lip and/or palate (CLP) are common birth defects of complex etiology. CLP can occur in isolation or as part of a broad range of chromosomal, Mendelian, or teratogenic syndromes. Although there has been marked progress in identifying genetic and environmental triggers for syndromic CLP, the etiology of the more common non-syndromic (isolated) forms remains poorly characterized. Recently, using a combination of epidemiology, careful phenotyping, genome-wide association studies and analysis of animal models, several distinct genetic and environmental risk factors have been identified and confirmed for non-syndromic CLP. These findings have advanced our understanding of developmental biology and created new opportunities for clinical translation research.

## Introduction

Clefts of the lip and/or palate (CLP) are immediately recognizable disruptions of normal facial structure. Although not a major cause of mortality in developed countries, CLP does cause considerable morbidity to affected children and imposes a substantial financial risk for families with a concomitant societal burden[1]. Individuals with CLP may experience problems with feeding, speaking, hearing and social integration that can be corrected to varying degrees by surgery, dental treatment, speech therapy and psychosocial intervention. CLP is etiologically heterogeneous and this has critical implications for understanding the biology of facial development, how environmental risks interact with genetic factors and how we can incorporate known etiologic variables to improve clinical care. Recent successes in genome-wide linkage and association studies have identified novel loci significantly associated with CLP[2–6]. Researchers are currently striving to identify the etiologic variants at these novel loci to understand the developmental disturbances leading to CLP, and this knowledge should eventually result in improved prevention, treatment and prognosis for individuals with these conditions.

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Development of the lip and palate is outlined in Figure 1. The common forms of CLP involve disruption of tissue planes above the lip extending into the nares and/or the palate (hard and/or soft) (Figure 2a, 2b). Fogh-Andersen and Fraser noted clefts involving the anterior structures (lip and primary palate) could be separated on both genetic and embryologic grounds from those involving only the secondary palate[7, 8]. While there are many disruptions affecting the craniofacial complex, the overwhelming majority involve only the upper lip and/or palate. Further, approximately 70% of cases of CLP occur as isolated entities with no other apparent cognitive or structural abnormalities; commonly termed "isolated, non-syndromic CLP". Because the defects arise early in embryological development, have a complex etiology with both genetic and environmental contributions and modest recurrence rates, it has proven difficult to identify specific etiologic factors. A combination of epidemiologic, candidate gene and genome-wide studies, plus analysis of animal models, has recently provided deeper insights into the causes of non-syndromic CLP.

With the advent of the genomics era, there have been major advances in identifying the causative genetic mutations underlying syndromic forms of CLP (http://www.ncbi.nlm.nih.gov/omim). In contrast, owing to its genetic heterogeneity, departure from Mendelian inheritance patterns, the lack of (and expense of) genomic tools and the necessity for very large datasets, there has been less progress in advancing our understanding of the genetic etiology of non-syndromic CLP. However, the recent development of innovative approaches to phenotyping and powerful genomic tools, together with extrapolation from studies of syndromic forms of CLP, has increased our understanding of non-syndromic CLP. Because of its particular challenges, in this review we focus on non-syndromic CLP and we summarize syndromic forms (which are genetically tractable) only briefly. We discuss important epidemiologic clues, environmental contributions, genetic architecture and issues of phenotyping, gene discovery and insights into molecular pathogenesis. We also speculate about the clinical implications of these findings for recurrence and new clinical associations building on advances in imaging and large databases to examine long-term outcomes.

## Challenges in Studying CLP

### Epidemiology

CLP affects approximately 1/700 live births, with wide variability across geographic origin, racial and ethnic groups, as well as environmental exposures and socioeconomic status. In general, Asian and Amerindian populations have the highest reported birth prevalence rates, often as high as 1/500, European-derived populations have intermediate prevalence rates at about 1/1000, and African-derived populations have the lowest prevalence rates at about 1/2500. These observations suggest the relative contribution of individual susceptibility genes may vary across different populations[6, 9, 10]. The frequency of CLP also differs by sex and laterality: there is a 2:1 male to female ratio for clefts involving the lip and approximately a 1:2 male to female ratio for clefts of the palate only; and there is a 2:1 ratio of left to right sided clefts among unilateral cleft lip cases.

Historically, CLP has been divided into cleft palate only and cleft lip with or without cleft palate (CL/P).[7, 8] However, recent epidemiologic data suggest that cleft lip only may have unique etiologic features, including strong genetic associations, while some individuals with cleft palate only show evidence of sub-clinical cleft lip[11–14]. Nevertheless, this broad sub-division of anatomical defects is consistent with the distinct developmental origins of the lip/primary palate and the secondary palate. Furthermore, separate cellular and genetic etiologies for CL/P and cleft palate only are consistent with the general observation that these two conditions do not segregate in the same family, although exceptions have been reported for families with etiologic mutations in specific genes (for example, tumor protein

p63 (*TP63*), msh homeobox 1 (*MSX1*),interferon regulatory factor 6 (*IRF6*) and fibroblast growth factor receptor 1 (*FGFR1*)).[15–19] Approximately 70% of all cases of CL/P and 50% of cases of cleft palate only are considered to be non-syndromic.[20–22] The remaining cases are composed of a wide range of malformation syndromes, including over 500 Mendelian syndromes (see Online Mendelian Inheritance in Man (OMIM) for further information www.ncbi.nlm.nih.gov/omim/), as well as those arising secondary to chromosomal or teratogenic effects. These syndromic forms are somewhat more tractable to genetic analysis, and Table 1 provides a summary of a subset in which the underlying genetic mutation has been identified.

### Genetic Architecture and Phenotyping

While twin studies and familial clustering studies have provided compelling evidence for a genetic component to non-syndromic CLP,[23] few pedigrees show clear-cut Mendelian inheritance and most cases appear sporadic.[24] Moreover, CLP is known to be influenced by environmental risk factors;[25, 26] consequently, a multifactorial model of inheritance is favored in which genetic risk factors of small individual impact may interact with environmental covariates.[12] These combined factors complicate genetic analysis of non-syndromic forms of CLP.

Accurate phenotyping is crucial to understanding both the epidemiology and etiology of any congenital malformation, because the power to detect effects is weakened when heterogeneous groups are treated as a single entity. Although clefts of the lip and palate show a range of phenotypic expression (Figure 2A, 2B), they are generally defined as qualitative traits (i.e. affected or unaffected). Dividing CLP in this simplistic way has the potential to lose important information. For example, different patterns of genome-wide linkage are observed when multiplex families are divided into subgroups depending on the overt CLP phenotypes present in affected individuals, which suggests careful attention to phenotypes will be an important tool in furthering our understanding of the genetic heterogeneity underlying non-syndromic CLP.[2] Furthermore, numerous lines of evidence now suggest the spectrum is more complex and should include a variety of sub-clinical phenotypic features observed in either an individual with CLP and/or their “unaffected” relatives.[27]

Sub-clinical phenotypes can include minor structural variants including lip pits/prints,[28] dental anomalies,[29] defects of the orbicularis oris muscle,[30, 31] 3D facial image measurement,[27] brain variants as assessed by MRI[32, 33] or by surrogate measures[34, 35], and speech or cognitive differences such as velopharyngeal insufficiency, reading disability and IQ. Palatal subphenotypes have been less explored but also include bifid uvula, submucous cleft palate, the differentiation of clefts of the hard and soft palate and possibly ankyloglossia. A better understanding of palatal subdivisions by phenotype and pathway will benefit from both human and mouse models. Defects of the orbicularis oris muscle show particular promise for enhancing the search for causative genetic variants and for contributing to clinical risk assessment.[30, 36–39] Orbicularis oris defects can be assessed using high-resolution ultrasound of the upper lip (Figure 3A, 3B). Sub-clinical phenotyping therefore holds great promise to enhance the power of family studies and may lead to opportunities for translational research relevant for both clinical care of patients and clinical genetics as a science.

## Gene Discovery in Non-Syndromic CLP

To date, genetic approaches to non-syndromic CLP have included: linkage analysis using large, multiplex families or smaller but inbred families, or analysis of affected relative pairs; association studies using case/parent trios or case-control samples; identification of

chromosomal anomalies or micro-deletions in cases; and direct sequencing of affected individuals. These methods can be applied to candidate genes or genome-wide strategies can be used. Each approach has its own advantages and disadvantages, some of which will depend on the underlying genetic architecture of the disease, as well as the realities of economics and technology. We briefly summarize successes using a range of approaches, followed with more detail on the results of recent genome-wide association (GWA) studies. Most studies of non-syndromic clefts to date have focused on cleft lip with or without cleft palate rather than isolated cleft palate. This has been biased perhaps by the larger numbers, easier ascertainment and less confusion from confounding syndromes. Future studies will need to address this gap and also the somewhat counterintuitive observation that more mouse models are available for cleft palate than cleft lip.

## Candidate genes, chromosomal anomalies, linkage and sequencing

Candidate gene studies have been at the core of cleft research since Ardinger and colleagues[40] suggested a role for *TGFA* (transforming growth factor, alpha) variants in risk for non-syndromic CL/P. The identification of candidate genes has traditionally relied on gene expression and developmental analyses performed in model organisms, particularly the mouse, either to first identify the candidate genes or to provide biological plausibility for the association. More recently, extrapolation from the study of syndromic forms of CL/P has proven to be a useful adjunct to this approach. As with candidate gene studies of many complex disorders, rigorous confirmatory replication is not common, with only variants in *IRF6* (interferon regulatory factor 6) yielding consistent evidence of association across multiple studies[13, 41–44] (discussed further below). Analysis of chromosomal anomalies in patients has proven to be a productive route for identification or confirmation of CL/P loci, with recent successes for *FGFR2* (fibroblast growth factor receptor 2)[45] and *SUMO1* (a member of the small ubiquitin-like modifier family).[46–48] Candidate gene-based association studies and analysis of chromosomal anomalies have recently been reviewed in detail[26, 49].

There have been many attempts to use linkage analysis to identify regions of the genome likely to carry genes controlling pathogenesis of CLP, and the region surrounding the *FOXE1* (forkhead box E1) gene reached genome-wide levels of significance with subsequent fine-mapping and replication.[2, 50] There have been several resequencing studies of candidate genes to identify specific variants that might underlie statistical associations with clefting, and the best current evidence has been reported for mutations in *MSX1*,[17, 51] *FGFR1* and *FGF8*,[52] and *BMP4* (bone morphogenetic protein 4).[36] Whole exome sequencing has recently been successful in identifying causative genetic variants for Mendelian traits,[53, 54] including Miller syndrome[55], (which is an autosomal recessive syndrome that can include cleft palate[55]) and Kabuki syndrome[56], (a dominant disorder than can include cleft palate[56]), but is yet to be successful for complex and heterogeneous traits such as non-syndromic CLP.

## Genome-Wide Association Studies

As is now apparent for many common complex disorders, GWA studies have provided recent major advances in our understanding of genes and pathways that play a role in the etiology of CLP. To date, there are three published GWA studies for CL/P using the case-control design,[3–5] and one case-parent trio study from an international consortium that is part of GENEVA (the gene environment association studies consortium).[6, 57] These studies have mostly excluded cases with cleft palate only, based on likely etiologic heterogeneity. Birnbaum and colleagues[3] confirmed the impact of *IRF6*, which had previously been identified in candidate gene studies,[13, 41] and discovered a new region on chromosome 8q24 that gave extremely strong evidence of association in their European case-control sample. Grant and colleagues[458] independently confirmed that this “gene desert” region on

chromosome 8q24 was strongly associated with CL/P in a sample of European American cases and controls. Mangold and colleagues[5] subsequently used an expanded dataset from Europe and identified additional loci at chromosomes 10q25 (*VAX1*, ventral anterior homeobox 1) and 17q22 (*NOG*, noggin) that achieved genome-wide significance.

The GENEVA Cleft Consortium study[6] used case-parent trios from multiple populations and reconfirmed the *IRF6* findings, as well as replicating the chromosome 8q24 and 10q25 (*VAX1*) findings. Interestingly, in this consortium study the level of statistical evidence from markers within chromosome 8q24 was much stronger among case-parent trios of European ancestry, whereas the evidence for linkage and association for markers in *IRF6* was much stronger in trios of Asian ancestry. This GENEVA study identified at least two new loci (*MAFB* and *ABCA4*) not previously associated with CL/P as significant at the genome-wide significance level, with stronger signals in Asian compared to European populations.[6] The signals and this population difference were replicated using independent families from multiple populations (see further details below.

These observations suggest that not only are there multiple genetic variants influencing risk of CL/P, but that some of these genes may be differentially tagged by polymorphic markers in a population-specific manner. For example, in the chromosome 8q24 region the most significant SNP (rs987525) showed similar patterns of over-transmission to the affected child but had a higher minor allele frequency among parents of European ancestry compared to parents of Asian ancestry (0.26 vs. 0.07).[6] In fact, the entire region of signal on chromosome 8q24 showed higher rates of heterozygosity among parents of European ancestry compared to those of Asian ancestry, which means that European trios would be far more informative than Asian trios for this region. Therefore, it may be more difficult to identify causal genetic variants in some populations compared to others. Some putative causal genes have been identified through polymorphic markers in most populations (for example, *IRF6*), while others (for example, 8q24, *MAFB, ABCA4*) seem to be more population-specific, which could reflect variable coverage by available marker panels or true allelic heterogeneity. True allelic heterogeneity, in which multiple mutations occurred on different background haplotypes, would make it much more difficult to identify causal genes through association studies; however, Dickson and colleagues[59] noted there may be mixtures of multiple rare alleles on common haplotypes within a single causal gene for complex and heterogeneous disorders such as CLP.

Below, we provide a short summary of each of the genes confirmed or identified through GWA studies together with insights into the molecular pathogenesis derived from analysis of animal models. In Table 2, we summarize genes with a confirmed role in non-syndromic CLP, those that seem likely to be involved, and some that have been intensively studied but where the supporting data remain less convincing.

## Insights into molecular pathogenesis

While GWA studies will increase the number of CLP loci identified, the move from a GWA study signal to a causative variant will still be demanding. Animal models and gene expression data are powerful tools for identifying candidate genes for complex traits; importantly, they also contribute to our knowledge of normal facial development and the molecular pathogenesis of CLP. The mouse is the pre-eminent model organism for studies of this type as facial development in this species mirrors human craniofacial development, and mouse strains with high rates of CLP are available. A number of excellent reviews have described the cellular and molecular mechanisms underlying normal and abnormal development,[60, 61]; here we provide examples of how the mouse has impacted our understanding of the molecular pathogenesis of CLP in humans.

### IRF6

Mutations in *IRF6* were first identified as etiologic in the autosomal dominant Van der Woude syndrome, which can include CL/P and/or cleft palate only along with dental anomalies and lip fistulas.[18] Subsequent research showed common alleles in *IRF6* were associated with non-syndromic CL/P.[41] This association has been independently replicated in GWA studies as well as in many candidate gene studies[3–6, 13, 41–43, 62] with some failures of replication possibly due to population differences.[63] Recently, an approach that integrated the identification of *cis*-regulatory elements using sequence conservation across multiple species, analysis of animal models and biochemical analyses resulted in the identification of one specific sequence variant (rs642961) located within an enhancer ~10 kb upstream of the *IRF6* transcription start site that is significantly over-transmitted in non-syndromic cleft lip only.[13] Importantly, this apparent risk allele was found to disrupt a binding site for transcription factor AP-2α, which is mutated in the autosomal dominant CLP disorder branchio-oculo-facial syndrome[64], therefore strongly suggesting it is a contributory variant.[13]

A role of *IRF6* in CLP is further supported by analysis of animal models. Recent research has shown that *Irf6* mutant mice exhibit a hyper-proliferative epidermis that fails to undergo terminal differentiation, which leads to multiple epithelial adhesions that can occlude the oral cavity and result in cleft palate.[65, 66] These results demonstrated that IRF6 is a key determinant of the keratinocyte proliferation/differentiation switch and subsequent research indicated that IRF6 also plays a key role in the formation of oral periderm, spatio-temporal regulation of which is essential in ensuring appropriate palatal adhesion.[67] Recently, a combination of mouse genetics, expression analyses, chromatin immunoprecipitation and luciferase reporter assays has shown *IRF6* is a direct target of p63, which underlies several malformation syndromes that include CLP as a hallmark feature[15, 16]. p63 activates *IRF6* transcription through an enhancer element, variation within which increases susceptibility to cleft lip only.[68]

### MAFB

The *MAFB* gene encodes a basic leucine zipper transcription factor. Markers near *MAFB* achieved genome-wide significance in the GENEVA Cleft Consortium study[6], with trios of Asian descent providing much stronger statistical evidence. In independent replication samples, 1149 pedigrees of European ancestry showed evidence of linkage and association with a SNP (rs13041247, p=0.0007) located 260 bp from the SNP yielding the strongest signal among Asian families (rs11696257, p=0.0009 in 331 independent pedigrees). A missense mutation, H131Q, in *MAFB* was found in 3.5% of Filipinos with CL/P but only 0.7% of controls (P<0.0001). This variant occurs in a region of strongly conserved sequence, suggesting there may be a rare variant in *MAFB* that contributes to the observed GWAS signal. It is noteworthy the gene-poor regions on either side of *MAFB* include numerous binding sites for transcription factors known to play a role in palate development (including transcription factors in the MSX, IRF, SOX and BACH gene families). In the mouse, *Mafb* expression was shown to be strong in the epithelium of the palatal shelves and in the medial edge epithelium during palatal fusion.[6]

### ABCA4

*ABCA4* encodes an ATP-binding cassette transporter. Multiple markers in *ABCA4* and in the 3' end of the gene gave evidence of linkage and association at the genome-wide significance level in the GENEVA Cleft Consortium GWA study[6], again with stronger evidence among Asian samples. Two of the SNPs with strongest signals were replicated in independent family samples, and one of these SNPs (rs560426) gave a far stronger signal in Asian families (p=0.0003 in 331 pedigrees) compared to European families (p=0.005 in 1149

pedigrees). This difference in the strength of statistical evidence again raises the possibility of either an allele common to both groups but with differing frequencies, or multiple risk alleles occurring on different haplotype backgrounds. *ABCA4* is known to cause the autosomal recessive retinal degenerative disease Stargardt's disease and sequencing of the 50 exons of *ABCA4* in 190 CL/P cases identified 27 different missense mutations, many of which have been previously reported in Stargardt's or other ocular disorders (http://www.ncbi.nlm.nih.gov/omim). Since *ABCA4* is surrounded by many other genes, the peak signal in *ABCA4* may be a surrogate for etiologic variants in another gene nearby. Furthermore, no *Abca4* expression has been seen in mouse palatal shelves around the time of palatal fusion.[6]

## VAX1

In the studies by Mangold et al.[5] and the GENEVA Cleft Consortium6, markers in or near the *VAX1* gene at chromosome 10q25 yielded evidence approaching genome-wide significance; the same two SNPs in *VAX1* (rs7078160 and rs4752028) were over-represented in CL/P cases in both studies.[5, 6] *VAX1* encodes a transcriptional regulator with a DNA-binding homeobox domain. Mouse knockouts for *Vax1* develop cleft palate and this gene is expressed widely in developing craniofacial structures;[69] thus variants in *VAX1* itself are strong candidates for contributing to CLP.

## Wnt signaling

Although not as yet implicated by GWA studies, variants within WNT genes have been reported to be associated with non-syndromic CL/P[70] and mutations in *WNT3* underlie autosomal recessive tetra-amelia with cleft lip and palate[71]. Although the evidence for the involvement of WNT signaling in non-syndromic CL/P is not strong, these findings have led to further analysis of genes in the Wnt signalling pathway as candidates for normal development of the lip and palate. Targeted mutation of *Wnt9b* in mice leads to CLP; and the A/WySn strain of mice, which have increased incidence of spontaneous CLP, have insertion of a retrotransposon 6.6 kb downstream of the *Wnt9b* gene (a site known as the *clf1* locus)[72] These findings suggest Wnt9b plays a key role in development of the lip.[72–74] Further support for this hypothesis arises from the observation that canonical Wnt signaling is activated during midfacial morphogenesis in mice[75], and genetic inactivation of low density lipoprotein receptor-related protein 6 (*Lrp6*), a co-receptor of the Wnt/β-catenin signaling pathway, causes CLP[76]. Intriguingly, *Msx1* and *Msx2* (see below) have been shown to be downstream targets of this Wnt/β-catenin signaling pathway during lip formation and fusion.[76]

## MSX1 and BMP signaling

As in humans, loss-of-function mutations in the homeobox gene *Msx1* result in cleft palate in mice.[77] *Msx1* is a downstream target of BMP signaling in a number of embryonic sites and *Msx1* is necessary for expression of *Bmp4* and/or *Bmp2*.[78] In mice, loss-of-function of type I Bmp receptor (*Bmpr1a*) in the craniofacial primordia resulted in **CL/P**, while deficiency of *Bmp4* resulted in cleft lip only[79]; this shows that Bmp signaling has distinct functions in development of the lip versus the secondary palate. In the context of *Bmp4* deficiency, all *Bmp4* mutant embryos exhibited bilateral cleft lip at E12, but only 22% still displayed cleft lip at E14 suggesting some *in utero* repair mechanism.[79] These observations parallel the findings that mutations in *BMP4* may underlie a subset of cases of subepithelial, microform and overt cleft lip in humans.[36]

## Environmental Factors and Gene-Environment Interaction

Identification of environmental components of clefting and studies of gene by environment interaction require large (ideally prospective) cohort studies and access to genetic material to be optimally effective. While a few such resources are available (Denmark, Norway, the National Birth Defects Prevention Study in the US)[11,14, GENISCA ref] they are still primarily in the analysis phase. Nonetheless there are a few studies that have begun to provide data on environmental risks. Since the environment is more malleable identification of environmental risks, particularly if they can be personalized with genetic covariates, afford the best short term opportunities to be applied to prevention.

Maternal smoking has been associated repeatedly with increased risk of CLP and meta-analysis strongly supports an overall odds ratio (OR) for having CLP of ~1.3 among offspring of mothers who smoke.[80–82] Increased risks from exposure to maternal smoking during the peri-conceptual period raises the possibility that genes in certain metabolic pathways may play a role in the development of CLP. Specifically, markers in the *GSTT1* (glutathione S-transferase theta) or *NOS3* (nitric oxide synthase 3) genes appear to influence risk of CL/P in the presence of maternal smoking.[81, 83–85] The *GSTT1* markers are gene deletion variants, which suggests deficiencies in detoxification pathways may underlie some of this susceptibility. Smoking has also been recently associated with a joint risk with variants in the *IRF6* gene[86] and the same study reported interactions between multivitamins and *IRF6* variants. These findings provide evidence that gene-environment interactions are important in CLP. In addition, some specific teratogens[25,26 ADD ABBOTT HERE], for example valproic acid, have yielded evidence of association with cleft palate.[87]

Exposure to maternal alcohol consumption has also been suggested as a risk factor, but the evidence has been more inconsistent.[26]. Studies also suggest that 'binge' drinking patterns (high doses of alcohol in short periods of time) increase risk[88], and this is supported by associations with variation in the *ADH1C* alcohol dehydrogenase gene.[89] However, these links to alcohol consumption remain to be confirmed. Nutritional factors, such as folate deficiency, have also been suggested to influence risk of CL/P, based on both observational studies and interventional trials using folate supplementation to prevent recurrences of CL/P in families.[90] However, the studies of vitamin supplementation with folate remain controversial[1, 91] and recent studies of levels of folate receptor antibodies did not find an association with CL/P[92]. Furthermore, food fortification programs using folic acid have shown detectable decreases in the rates of clefting in some[93, 94] but not all[95, 96] studies. In the future, other nutrient and micronutrient studies will need to be expanded to look for evidence of effects. For example, there are some data to support roles for zinc deficiency in risk of oral clefts in populations in which zinc status is highly compromised[97], for cholesterol deficiency in facial clefting,[98] as well for as multivitamins in general in cleft prevention.[94]

Besides nutrients and toxins other environmental exposures have been, and should continue to be, assessed for possible roles in clefting. These exposures include hyperthermia,[99] stress, maternal obesity, occupational exposures, ionizing radiation and infection[10]. Pregnancy planning has been shown to have a protective effect and the basis of this observation needs to be more deeply explored.[100] Nonetheless there is no consensus yet on the harmful effects of these factors and prospective cohort studies large enough to measure effects on a relatively rare disorder such as clefting may be required. A particular challenge will be to determine the specificity of the role of an exposure in contributing to clefting, as many exposures will have both identifiable but also unidentifiable coincident risks. Analytic approaches such as Mendelian randomization will be helpful in making these determinations.[101]

## Integrating evidence into clinical care

Despite the recent identification of genes likely to influence the risk of non-syndromic CLP, these results have yet to have any direct impact on genetic counseling or clinical management. Improved epidemiologic information does, however, allow for better point estimates for familial recurrence risks[14], and it seems likely that genotypic information for apparent risk alleles associated with higher risk of oral clefts could be useful in clinical assessment (once we have a better definition of the full number of causal genes and their potential interactions with one another and with environmental risk factors). The next critical phase of statistical analyses will be to examine the heterogeneity underlying the etiology of oral clefts and to investigate the gene-gene and gene-environment interactions that control risk. A range of study designs will be needed to achieve this level of documentation, including family studies, case-control studies and eventually prospective cohort data. Importantly, incorporating information from sub-clinical phenotypes, such as orbicularis oris defects or dental anomalies, may also allow us to identify etiologically homogeneous sub-groups of cleft cases, and thus should enhance family studies and estimates of recurrence risk.[39] New array-based copy number variant analysis and whole exome or even whole genome resequencing could also afford future opportunities for improved molecular diagnostics, as does the increasingly better ultrasound analysis of the fetus for the presence and severity of cleft type prior to birth.

### Gene expression in time and space

Global approaches to expression analysis of genes in craniofacial structures have already provided a broad view of gene expression. For example, the COGENE project http://humgen.wustl.edu/COGENE/ provides public web access to human gene expression data for 24 craniofacial specific human tissues isolated from day 26 to day 60 human embryos. In zebrafish, mRNA sequencing and microRNA analysis have been informative for understanding palate development, so it would be useful to build on this knowledge[102]. Similarly, the ability to analyze tissues in their correct three-dimensional orientation is central to understanding biological processes, particularly when tissues undergo a complex and intricate series of movements relative to each other as occurs in the developing craniofacial region. The mapping of gene and protein expression patterns within these complex shapes can provide important clues about their biological functions and also indicates which genes and/or proteins may interact with one another. The expression of genes relative to each other in both time and space can be visually represented using Optical Projection Tomography (OPT)[103] and an atlas of craniofacial gene expression patterns is available online http://genex.hgu.mrc.ac.uk/emage/home.php.

### Cis-regulatory element identification

Much of the genetic variation underlying complex disorders (such as non-syndromic CLP) is likely to occur in regulatory elements outside coding sequences of genes. These elements are challenging to identify as they often regulate genes across substantial genomic distances. Although evolutionary sequence conservation can facilitate discovery of regulatory elements, this technique does not predict their spatio-temporal pattern of activity *in vivo*.[104] Recently, chromatin immunoprecipitation followed by Next Generation Sequence analysis (ChIP-seq) for the enhancer-associated protein p300 has been demonstrated to be a highly sensitive method to accurately identify enhancer elements and their associated activities.[105] Clearly, detailed mapping of regulatory elements will provide additional (and functionally relevant) targets for sequence analysis, particularly where they fall within regions of the genome implicated by GWA studies or other approaches. The power of integrating association studies in well-characterized patient populations with identification of *cis*-

regulatory elements, analysis of animal models and biochemical analyses is amply illustrated by the example of *IRF6* noted above.

### Wider implications

Biological roles outside the craniofacial complex are known for some of the candidate genes associated with CLP, increasing the importance of CLP gene-finding endeavors. One recent publication on a small dataset suggests a role for *IRF6* in wound healing, at least in the autosomal dominant Van der Woude syndrome.[106] Long-term outcomes of individuals born with clefts may include risks for higher overall mortality rates, mental health problems,[107] a higher risk of cancer (particularly breast cancer) in affected individuals[108] and their family members[109] plus alterations in child bearing patterns.[110] Identifying long-term adverse outcomes (e.g. cancer and psychiatric disorders) that are seemingly unrelated to a common birth defect may eventually result in decreasing lifelong health burden by recognizing risks at their early, pre-symptomatic stages. Studies into the etiology of clefts may well enhance our understanding of other common, complex traits and allow us to move beyond the attitude that CLP is only a structural birth defect, but instead is a lifelong disorder for which therapies and prevention can promise a fuller and healthier lifespan.

### Future approaches

Future advances in our understanding of the molecular pathogenesis of CLP will require strategies that increasingly integrate genetic analysis of precisely phenotyped cohorts of patients, global approaches for the identification and ranking of candidate genes, and improved methods for delineating and analyzing functional elements controlling gene expression. Integration of genetic and environmental risk using epigenetics, systems biology, gene expression and epidemiology all will be required to generate a synthesis that will both better characterize etiologies, as well as provide access to better clinical care and prevention.

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## References

1. Wehby G, Cassell CH. The impact of orofacial clefts on quality of life and healthcare use and costs. Oral Dis. 2010; 16:3–10. [PubMed: 19656316]
2. Marazita M, Lidral AC, Murray JC, Field LL, Maher BS, McHenry TG, Cooper ME, Govil M, Daack-Hirsch S, Riley B, Jugessur A, Felix T, Moreno L, Mansilla MA, Vieira AR, Doheny K, Pugh E, Valencia-Ramirez C, Arcos-Burgos M. Genome scan, fine-mapping, and candidate gene analysis of non-syndromic cleft lip with or without cleft palate reveals phenotype specific differences in linkage and association results. Hum Hered. 2009; 68:151–170. [PubMed: 19521098]
3. Birnbaum SLK, Reutter H, Herms S, Steffens M, Rubini M, Baluardo C, Ferrian M, Almeida de Assis N, Alblas MA, Barth S, Freudenberg J, Lauster C, Schmidt G, Scheer M, Braumann B, Bergé SJ, Reich RH, Schiefke F, Hemprich A, Pötzsch S, Steegers-Theunissen RP, Pötzsch B, Moebus S, Horsthemke B, Kramer FJ, Wienker TF, Mossey PA, Propping P, Cichon S, Hoffmann P, Knapp M, Nöthen MM, Mangold E. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. Nat Genet. 2009; 41:473–477. [PubMed: 19270707] This is the first

successful genome wide association study in clefting and identified a very significant and previously unsuspected locus for clefts at 8q24 in a large gene dessert.

4. Grant S, Wang K, Zhang H, Glaberson W, Annaiah K, Kim CE, Bradfield JP, Glessner JT, Thomas KA, Garris M, Frackelton EC, Otieno FG, Chiavacci RM, Nah HD, Kirschner RE, Hakonarson H. A genome-wide association study identifies a locus for nonsyndromic cleft lip with or without cleft palate on 8q24. J Pediatr. 2009; 155:909–913. [PubMed: 19656524]
5. Mangold E, Ludwig KU, Birnbaum S, Baluardo C, Ferrian M, Herms S, Reutter H, de Assis NA, Chawa TA, Mattheisen M, Steffens M, Barth S, Kluck N, Paul A, Becker J, Lauster C, Schmidt G, Braumann B, Scheer M, Reich RH, Hemprich A, Pötzsch S, Blaumeiser B, Moebus S, Krawczak M, Schreiber S, Meitinger T, Wichmann HE, Steegers-Theunissen RP, Kramer FJ, Cichon S, Propping P, Wienker TF, Knapp M, Rubini M, Mossey PA, Hoffmann P, Nöthen MM. Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. Nat Genet. 2010; 42:24–26. [PubMed: 20023658]
6. Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB, Liang KY, Wu T, Murray T, Fallin MD, Redett RA, Raymond G, Schwender H, Jin SC, Cooper ME, Dunnwald M, Mansilla MA, Leslie E, Bullard S, Lidral AC, Moreno LM, Menezes R, Vieira AR, Petrin A, Wilcox AJ, Lie RT, Jabs EW, Wu-Chou YH, Chen PK, Wang H, Ye X, Huang S, Yeow V, Chong SS, Jee SH, Shi B, Christensen K, Melbye M, Doheny KF, Pugh EW, Ling H, Castilla EE, Czeizel AE, Ma L, Field LL, Brody L, Pangilinan F, Mills JL, Molloy AM, Kirke PN, Scott JM, Arcos-Burgos M, Scott AF. A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. Nat Genet. 2010; 42:525–529. [PubMed: 20436469] This GWAS used the case/parent trio approach and identified at least two new loci (near the *ABCA4* and *MAFB* genes) for clefts. It further demonstrated that population heterogeneity is an important consideration in GWAS studies.
7. Fogh-Andersen, P. Inheritance of Harelip and Cleft Palate. Copenhagen: Munksgaard; 1942.
8. Fraser FC. Thoughts on the etiology of clefts of the palate and lip. Acta Genet Stat Med. 1955; 5:358–369. [PubMed: 13339079]
9. Christensen K, Mitchell LE. Familial recurrence-pattern analysis of nonsyndromic isolated cleft palate--a Danish Registry study. Am J Hum Genet. 1996; 58:182–190. [PubMed: 8554055]
10. Mossey P, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. Lancet. 2009; 374:1773–1785. [PubMed: 19747722]
11. Harville EW, Wilcox AJ, Lie RT, Vindenes H, Abyholm F. Cleft lip and palate versus cleft lip only: are they distinct defects? Am J Epidemiol. 2005; 162:448–453. [PubMed: 16076837]
12. Rahimov F, Marazita ML, Visel A, Cooper ME, Hitchler MJ, Rubini M, Domann FE, Govil M, Christensen K, Bille C, Melbye M, Jugessur A, Lie RT, Wilcox AJ, Fitzpatrick DR, Green ED, Mossey PA, Little J, Steegers-Theunissen RP, Pennacchio LA, Schutte BC, Murray JC. Disruption of an AP-2 alpha binding site in an IRF6 enhancer is associated with cleft lip. Nat Genet. 2008; 40:1341–1347. [PubMed: 18836445] A still rare demonstration of moving from an associated SNP to finding one of the likely etiologic SNPs for clefts. It also brought into play a new gene (*TFAP2A*) and pathway to cleft studies.
13. Weinberg S, Brandon CA, McHenry TH, Neiswanger K, Deleyiannis FWB, de Salamanca JE, Castilla EE, Czeizel AE, Vieira AR, Marazita ML. Rethinking isolated cleft palate: Evidence of occult lip defects in a subset of cases. Am J Med Genet Part A. 2008; 146A:1670–1675. [PubMed: 18536047]
14. Grosen D, Skytthe A, Bille C, Molsted K, Sivertsen A, Murray JC, Christensen K. A cohort study of recurrence patterns among more than 54,000 relatives of oral cleft cases in Denmark: support for the multifactorial threshold model of inheritance. J Med Genet. 2010; 47:162–168. [PubMed: 19752161] The most extensive study to date of recurrence risks for clefts in first, second and third degree relatives.
15. Celli J, Duijf P, Hamel BC, Bamshad M, Kramer B, Smits AP, Newbury-Ecob R, Hennekam RC, Van Buggenhout G, van Haeringen A, Woods CG, van Essen AJ, de Waal R, Vriend G, Haber DA, Yang A, McKeon F, Brunner HG, van Bokhoven H. Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. Cell. 1999; 99:143–153. [PubMed: 10535733]
16. McGrath JA, Duijf PH, Doetsch V, Irvine AD, de Waal R, Vanmolkot KR, Wessagowit V, Kelly A, Atherton DJ, Griffiths WA, Orlow SJ, van Haeringen A, Ausems MG, Yang A, McKeon F,

Bamshad MA, Brunner HG, Hamel BC, van Bokhoven H. Hay-Wells syndrome is caused by heterozygous missense mutations in the SAM domain of p63. Hum Mol Genet. 2001; 10:221–229. [PubMed: 11159940]

17. van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. Nat Genet. 2000; 24:342–343. [PubMed: 10742093]
18. Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, Howard E, de Lima RL, Daack-Hirsch S, Sander A, McDonald-McGinn DM, Zackai EH, Lammer EJ, Aylsworth AS, Ardinger HH, Lidral AC, Pober BR, Moreno L, Arcos-Burgos M, Valencia C, Houdayer C, Bahuau M, Moretti-Ferreira D, Richieri-Costa A, Dixon MJ, Murray JC. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nat Genet. 2002; 32:285–289. [PubMed: 12219090]
19. Dodé C, Levilliers J, Dupont JM, De Paepe A, Le Dû N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pêcheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler ML, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C, Hardelin JP. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet. 2003; 33:463–465. [PubMed: 12627230]
20. Jones MC. Etiology of facial clefts: prospective evaluation of 428 patients. Cleft Palate J. 1988; 25:16–20. [PubMed: 3422594]
21. FitzPatrick D, Farrall M. An estimation of the number of susceptibility loci for isolated cleft palate. J Craniofac Genet Dev Biol. 1993; 13:230–235. [PubMed: 8288730]
22. Marazita ML, Field LL, Cooper ME, Tobias R, Maher BS, Peanchitlertkajorn S, Liu YE. Nonsyndromic cleft lip with or without cleft palate in China: assessment of candidate regions. Cleft Palate Craniofac J. 2002; 39:149–156. [PubMed: 11879070]
23. Mitchell, LE. Mode of inheritance of oral clefts. In: Wyszyski, DF., editor. Cleft Lip and Palate: From Origin to Treatment. Oxford University Press; 2002. p. 234-239.
24. Jugessur A, Shi M, Gjessing HK, Lie RT, Wilcox AJ, Weinberg CR, Christensen K, Daack-Hirsch S, Trung TN, Bille C, Lidral AC, Murray JC. Genetic determinants of facial clefting: analysis of 357 candidate genes using two national cleft studies from Scandinavia. PLoS One. 2009; 4(4):e5358. [PubMed: 19396358]
25. Murray JC. Gene/environment causes of cleft lip and/or palate. Clin Genet. 2002; 61(4):248–256. [PubMed: 12030886]
26. Mossey P, Little J. Addressing the challenges of cleft lip and palate research in India. Indian J Plast Surg. 2009; 42:9–18.
27. Weinberg S, Naidoo SD, Bardi KM, Brandon CA, Neiswanger K, Resick JM, Martin RA, Marazita ML. Face shape of unaffected parents with cleft affected offspring: combining three-dimensional surface imaging and geometric morphometrics. Orthod Craniofac Res. 2009; 12(4):271–281. [PubMed: 19840279]
28. Neiswanger K, Chirigos KW, Klotz CM, Cooper ME, Bardi KM, Brandon CA, Weinberg SM, Vieira AR, Martin RA, Czeizel AE, Castilla EE, Poletta FA, Marazita ML. Whorl patterns on the lower lip are associated with nonsyndromic cleft lip with or without cleft palate. Am J Med Genet Part A. 2009; 149A(23):2673–2679. [PubMed: 19921634]
29. Vieira AR, McHenry TG, Daack-Hirsch S, Murray JC, Marazita ML. Candidate gene/loci studies in cleft lip/palate and dental anomalies finds novel susceptibility genes for clefts. Genet Med. 2008; 10(9):668–674. [PubMed: 18978678]
30. Neiswanger K, Weinberg SM, Rogers CR, Brandon CA, Cooper ME, Bardi KM, Deleyiannis FWB, Resick JR, Bowen A, Mooney MP, de Salamanca JE, Gonzalez B, Maher BS, Martin RA, Marazita ML. Orbicularis Oris Muscle Defects as an Expanded Phenotypic Feature in Nonsyndromic Cleft Lip with or without Cleft Palate. Am J Med Genet Part A. 2007; 143A(11): 1143–1149. [PubMed: 17497721] Opened the door for subphenotyping as a critical variable in cleft studies. It also provided an opportunity to use a clinical test in determining risks for recurrence of clefts in families.
31. Weinberg SM, Neiswanger K, Richtsmeier JT, Maher BS, Mooney MP, Siegel MI, Marazita ML. Three-dimensional morphometric analysis of craniofacial shape in the unaffected relatives of

individuals with nonsyndromic orofacial clefts: a possible marker for genetic susceptibility. Am J Med Genet Part A. 2008; 146A(4):409–420. [PubMed: 18203157]

32. Nopoulos P, Berg S, Canady J, Richman L, Van Demark D, Andreasen NC. Structural brain abnormalities in adult males with clefts of the lip and/or palate. Genet Med. 2002 Jan–Feb; 4(1):1–9. [PubMed: 11839951]
33. Conrad ADS, Richman L, Canady J, Karnell M, Axelson E, Nopoulos P. Cerebellum Structure Differences and Relationship to Speech in Boys and Girls with Non-Syndromic Cleft of the Lip and/or Palate. Cleft Palate-Cran J. 2010; 47(5):469–475.
34. Wentzlaff K, Cooper ME, Yang P, Aston C, Liu YE, Melnick M, Marazita ML. Associations between non-righthandedness and cleft lip with or without cleft palate in a Chinese population. J Craniofac Genet Dev Bio. 1997; 17:141–147. [PubMed: 9338857]
35. Scott NM, Weinberg SM, Neiswanger K, Brandon CA, Marazita ML. Hair whorls and handedness: informative phenotypic markers in nonsyndromic cleft lip with or without cleft palate (NS CL/P) cases and their unaffected relatives. Am J Med Genet Part A. 2005; 136(2):158–161. [PubMed: 15940700]
36. Suzuki S, Marazita ML, Cooper ME, Miwa N, Hing A, Jugessur A, Natsume N, Shimozato K, Ohbayashi N, Suzuki Y, Niimi T, Minami K, Yamamoto M, Altannamar TJ, Erkhembaatar T, Furukawa H, Daack-Hirsch S, L'heureux J, Brandon CA, Weinberg SM, Neiswanger K, Deleyiannis FW, de Salamanca JE, Vieira AR, Lidral AC, Martin JF, Murray JC. Mutations in BMP4 are associated with subepithelial, microform, and overt cleft lip. Am J Hum Genet. 2009; 84(3):406–411. [PubMed: 19249007]
37. Marazita M. Subclinical features in non-syndromic cleft lip with or without cleft palate (CL/P): review of the evidence that subepithelial orbicularis oris muscle defects are part of an expanded phenotype for CL/P. Craniofac Res. 2007; 10(2):82–87.
38. Rogers CRWS, Smith TD, Deleyiannis FWB, Mooney MP, Marazita ML. basis for apparent subepithelial cleft lip: histological and ultrasonographic survey of the Orbicularis Oris muscle. Palate-Cran J. 2008; 45(5):518–524.
39. Klotz CM, Wang X, Desensi RS, Grubs RE, Costello BJ, Marazita ML. Revisiting the Recurrence Risk of Nonsyndromic Cleft Lip with or without Cleft Palate. Am J Med Genet A. 2010; 152A(11):2697–2702. [PubMed: 20949506]
40. Ardinger HH, Buetow KH, Bell GI, Bardach J, VanDemark DR, Murray JC. Association of genetic variation of the transforming growth factor-alpha gene with cleft lip and palate. Am J Hum Genet. 1989; 45(3):348–353. [PubMed: 2570526]
41. Zucchero TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, Caprau D, Christensen K, Suzuki Y, Machida J, Natsume N, Yoshiura K, Vieira AR, Orioli IM, Castilla EE, Moreno L, Arcos-Burgos M, Lidral AC, Field LL, Liu YE, Ray A, Goldstein TH, Schultz RE, Shi M, Johnson MK, Kondo S, Schutte BC, Marazita ML, Murray JC. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. N Engl J Med. 2004; 351(8):769–780. [PubMed: 15317890]
42. Ghassibé M, Bayet B, Revencu N, Verellen-Dumoulin C, Gillerot Y, Vanwijck R, Vikkula M. Interferon regulatory factor-6: a gene predisposing to isolated cleft lip with or without cleft palate in the Belgian population. Eur J Hum Genet. 2005; 35(11):1239–1242.
43. Park J, McIntosh I, Hetmanski JB, Jabs EW, Vander Kolk CA, Wu-Chou YH, Chen PK, Chong SS, Yeow V, Jee SH, Park BY, Fallin MD, Ingersoll R, Scott AF, Beaty TH. Association between IRF6 and nonsyndromic cleft lip with or without cleft palate in four populations. Genet Med. 2007; 9(4):219–227. [PubMed: 17438386]
44. Scapoli L, Martinelli M, Pezzetti F, Carinci P, Tognon M, Carinci F. Strong evidence of linkage disequilibrium between polymorphisms at the IRF6 locus and nonsyndromic cleft lip with or without cleft palate, in an Italian population. Am J Hum Genet. 2005; 76(1):180–183. [PubMed: 15558496]
45. Osoegawa K, Vessere GM, Utami KH, Mansilla MA, Johnson MK, Riley BM, L'Heureux J, Pfundt R, Staaf J, van der Vliet WA, Lidral AC, Schoenmakers EF, Borg A, Schutte BC, Lammer EJ, Murray JC, de Jong PJ. Identification of novel candidate genes associated with cleft lip and palate using array comparative genomic hybridization. J Med Genet. 2008; 45(2):81–86. [PubMed: 17873121]

46. Alkuraya FS, Saadi I, Lund JJ, Turbe-Doan A, Morton CC, Maas RL. SUMO1 haploinsufficiency leads to cleft lip and palate. Science. 2006; 313(5794):1751. [PubMed: 16990542]
47. Shi M, Mostowska A, Jugessur A, Johnson MK, Mansilla MA, Christensen K, Lie RT, Wilcox AJ, Murray JC. Identification of microdeletions in candidate genes for cleft lip and/or palate. Birth Defects Res A Clin Mol Teratol. 2009; 85(1):42–51. [PubMed: 19137569]
48. Mostowska AHK, Wojcicki P, Biedziak B, Paradowska P, Jagodzinski PP. Association between genetic variants of reported candidate genes or regions and risk of cleft lip with or without cleft palate in the polish population. Birth Defects Res A Clin Mol Teratol. 2010; 88(7):538–545. [PubMed: 20544801]
49. Jugessur A, Farlie PG, Kilpatrick N. The Genetics of Isolated Orofacial Clefts: From Genotypes to Subphenotypes. Oral Dis. 2009; 15(7):437–453. [PubMed: 19583827]
50. Moreno L, Mansilla MA, Bullard SA, Cooper ME, Busch TD, Machida J, Johnson MK, Brauer D, Krahn K, Daack-Hirsch S, L'Heureux J, Valencia-Ramirez C, Rivera D, Lopez AM, Moreno MA, Hing A, Lammer EJ, Jones M, Christensen K, Lie RT, Jugessur A, Wilcox AJ, Chines P, Pugh E, Doheny K, Arcos-Burgos M, Marazita ML, Murray JC, Lidral AC. FOXE1 Association with both Isolated Cleft Lip with or without Cleft Palate; and Isolated Cleft Palate. Hum Mol Gen. 2009; 18(24):4879–4896. [PubMed: 19779022] This study moved from a linkage localization for clefts as a complex trait to finding the likely specific gene (*FOXE1*) that is involved. The path from linkage to gene identification has been relatively unsuccessful but this study showed that large populations can be used to identify both rare and common variants contributing to a phenotype.
51. Jezewski PA, Vieira AR, Nishimura C, Ludwig B, Johnson M, O'Brien SE, Daack-Hirsch S, Schultz RE, Weber A, Nepomucena B, Romitti PA, Christensen K, Orioli IM, Castilla EE, Machida J, Natsume N, Murray JC. Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. J Med Genet. 2003; 40(6):399–407. [PubMed: 12807959]
52. Riley BM, Murray JC. Sequence evaluation of FGF and FGFR gene conserved non-coding elements in non-syndromic cleft lip and palate cases. Am J Med Genet A. 2007a; 143A(24):3228–3234. [PubMed: 17963255]
53. Lupski JR, Reid JG, Gonzaga-Jauregui C, Rio Deiros D, Chen DC, Nazareth L, Bainbridge M, Dinh H, Jing C, Wheeler DA, McGuire AL, Zhang F, Stankiewicz P, Halperin JJ, Yang C, Gehman C, Guo D, Irikat RK, Tom W, Fantin NJ, Muzny DM, Gibbs RA. Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. New Eng J Med. 2010; 362:1181–1911. [PubMed: 20220177]
54. Roach JC, Glusman G, Smit AF, Huff CD, Hubley R, Shannon PT, Rowen L, Pant KP, Goodman N, Bamshad M, Shendure J, Drmanac R, Jorde LB, Hood L, Galas DJ. Analysis of genetic inheritance in a family quartet by whole-genome sequencing. Science. 2010; 328:636–639. [PubMed: 20220176]
55. Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ. Exome sequencing identifies the cause of a mendelian disorder. Nat Genet. 2010; 42:30–35. [PubMed: 19915526]
56. Ng SB, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, Lee C, Turner EH, Smith JD, Rieder MJ, Yoshiura K, Matsumoto N, Ohta T, Niikawa N, Nickerson DA, Bamshad MJ, Shendure J. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Genet. 2010; 42(9):790–793. The first application of whole exome sequencing to a dominant disorder that includes craniofacial features. This success opens up a whole new approach to Mendelian craniofacial disorders and suggests the it may be useful in complex traits as well.
57. Cornelis MC, Cole JW, Hansel NN, Barnes KC, Beaty TH, Bennett SN, Bierut LJ, Boerwinkle E, Doheny KF, Feenstra B, Feingold E, Fornage M, Haiman CA, Harris EL, Hayes MG, Heit JA, Hu FB, Kang JH, Laurie CC, Ling H, Manolio TA, Marazita ML, Mathias RA, Mirel DB, Paschall J, Pasquale LR, Pugh EW, Rice JP, Udren J, van Dam RM, Wang X, Wiggs JL, Williams K, Yu K. GENEVA Consortium. The Gene, Environment Association Studies consortium (GENEVA): maximizing the knowledge obtained from GWAS by collaboration across studies of multiple conditions. Genet Epidemiol. 2010; 34(4):364–372. [PubMed: 20091798]
58. Grant SF, Wang K, Zhang H, Glaberson W, Annaiah K, Kim CE, Bradfield JP, Glessner JT, Thomas KA, Garris M, Frackelton EC, Otieno FG, Chiavacci RM, Nah HD, Kirschner RE,

Hakonarson H. A genome-wide association study identifies a locus for nonsyndromic cleft lip with or without cleft palate on 8q24. J Pediatr. 2009; 155:909–913. [PubMed: 19656524]

59. Dickson S, Wang K, Krantz I, Hakonarson H, Goldstein DB. Rare variants create synthetic genome-wide associations. PLoS Biol. 2010; 8(1) e1000294.
60. Jiang R, Bush JO, Lidral AC. Development of the upper lip: morphogenetic and molecular mechanisms. Dev Dyn. 2006; 235:1152–1166. [PubMed: 16292776]
61. Gritli-Linde A. Molecular control of secondary palate development. Dev Biol. 2007; 301(2):309–326. [PubMed: 16942766]
62. Blanton SH, Cortez A, Stal S, Mulliken JB, Finnell RH, Hecht JT. Variation in IRF6 contributes to nonsyndromic cleft lip and palate. Am J Med Genet A. 2005; 137A(3):259–262. [PubMed: 16096995]
63. Blanton SH, Garcia E, Mulliken JB, Stal S, Hecht JT. Ethnic Heterogeneity of IRF6 AP-2a Binding Site Promoter SNP Association With Nonsyndromic Cleft Lip and Palate. Cleft Palate-Cran J. 2010; 47(6):574–577.
64. Milunsky JM, Maher TA, Zhao G, Roberts AE, Stalker HJ, Zori RT, Burch MN, Clemens M, Mulliken JB, Smith R, Lin AE. TFAP2A mutations result in branchio-oculo-facial syndrome. Am J Hum Genet. 2008; 82(5):1171–1177. [PubMed: 18423521]
65. Richardson RJ, Dixon J, Malhotra S, Hardman MJ, Knowles L, Boot-Handford RP, Shore P, Whitmarsh A, Dixon MJ. Irf6 is a key determinant of the keratinocyte proliferation-differentiation switch. Nat Genet. 2006; 38(11):1329–1334. [PubMed: 17041603] The establishment of a critical mouse model for isolated clefts. It also demonstrates the role of the first gene associated with clefting with certainty (*IRF6*) in keratinocyte differentiation.
66. Ingraham CR, Kinoshita A, Kondo S, Yang B, Sajan S, Trout KJ, Malik MI, Dunnwald M, Goudy SL, Lovett M, Murray JC, Schutte BC. Abnormal skin, limb and craniofacial morphogenesis in mice deficient for interferon regulatory factor 6 (Irf6). Nat Genet. 2006; 38(11):1335–1340. [PubMed: 17041601] The companion paper to Richardson et al showing the same effect on keratinocytes.
67. Richardson R, Dixon J, Jiang R, Dixon MJ. Integration of IRF6 and Jagged2 signalling is essential for controlling palatal adhesion and fusion competence. Hum Mol Gen. 2009; 18:2632–2642. [PubMed: 19439425]
68. Thomason, H.; Dixon, M. Encyclopaedia of Life Sciences. John Wiley and Sons Inc; 2008. Craniofacial defects and cleft lip and palate.
69. Hallonet M, Hollemann T, Pieler T, Gruss P. Vax1, a novel homeobox-containing gene, directs development of the basal forebrain and visual system. Genes Dev. 1999; 13(23):3106–3114. [PubMed: 10601036]
70. Chiquet B, Blanton SH, Burt A, Ma D, Stal S, Mulliken JB, Hecht JT. Variation in WNT genes is associated with non-syndromic cleft lip with or without cleft palate. Hum Mol Gen. 2008; 17:2212–2218. [PubMed: 18413325]
71. Niemann S, Zhao C, Pascu F, Stahl U, Aulepp U, Niswander L, Weber JL, Müller U. Homozygous WNT3 mutation causes tetra-amelia in a large consanguineous family. Am J Hum Genet. 2004; 74:558–563. [PubMed: 14872406]
72. Juriloff DM, Harris MJ, McMahon AP, Carroll TJ, Lidral AC. Wnt9b is the mutated gene involved in multifactorial nonsyndromic cleft lip with or without cleft palate in A/WySn mice, as confirmed by a genetic complementation test. Birth Defects Res A Clin Mol Teratol. 2006; 76(8):574–579. [PubMed: 16998816]
73. Carroll T, Park JS, Hayashi S, Majumdar A, McMahon AP. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. Dev Cell. 2005; 9(283–92)
74. Juriloff DM, Harris MJ, Dewell SL, Brown CJ, Mager DL, Gagnier L, Mah DG. Investigations of the genomic region that contains the clf1 mutation, a causal gene in multifactorial cleft lip and palate in mice. Birth Defects Res A Clin Mol Teratol. 2005; 73(2):103–113. [PubMed: 15690355]
75. Lan Y, Ryan RC, Zhang Z, Bullard SA, Bush JO, Maltby KM, Lidral AC, Jiang R. Expression of Wnt9b and activation of canonical Wnt signaling during midfacial morphogenesis in mice. Devel Dyn. 2006; 235:1448–1454. [PubMed: 16496313]

76. Song L, Li Y, Wang K, Wang YZ, Molotkov A, Gao L, Zhao T, Yamagami T, Wang Y, Gan Q, Pleasure DE, Zhou CJ. Lrp6-mediated canonical Wnt signaling is required for lip formation and fusion. Development. 2009; 136:3161–3171. [PubMed: 19700620]
77. Satokata I, Maas R. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. Nat Genet. 1994; 6(4):348–356. [PubMed: 7914451]
78. Zhang Z, Song Y, Zhao X, Zhang X, Fermin C, Chen Y. 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. 2002; 129(17):4135–4146. [PubMed: 12163415]
79. Liu W, Sun X, Braut A, Mishina Y, Behringer RR, Mina M, Martin JF. Distinct functions for Bmp signaling in lip and palate fusion in mice. Development. 2005; 132(6):1453–1461. [PubMed: 15716346]
80. Little J, Cardy A, Munger RG. Tobacco smoking and oral clefts: a meta-analysis. Bull World Health Organ. 2002; 82(3):213–218. [PubMed: 15112010] An excellent meta-analysis providing overwhelming support for a role of maternal tobacco use in contributing to clefting.
81. Shi M, Christensen K, Weinberg CR, Romitti P, Bathum L, Lozada A, Morris RW, Lovett M, Murray JC. Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. Am J Hum Genet. 2007; 80(1):76–90. [PubMed: 17160896]
82. Shi M, Wehby GL, Murray JC. Review on genetic variants and maternal smoking in the etiology of oral clefts and other birth defects. Birth Defects Res C Embryo Today. 2008; 84(1):16–29. [PubMed: 18383123]
83. van Rooij A, Wegerif MJ, Roelofs HM, Peters WH, Kuijpers-Jagtman AM, Zielhuis GA, Merkus HM, Steegers-Theunissen RP. Smoking, genetic polymorphisms in biotransformation enzymes, and nonsyndromic oral clefting: a gene-environment interaction. Epidemiology. 2001; 12(5):502–507. [PubMed: 11505167]
84. Lammer EJ, Shaw GM, Iovannisci DM, Van Waes J, Finnell RH. Maternal smoking and the risk of orofacial clefts: Susceptibility with NAT1 and NAT2 polymorphisms. Epidemiology. 2004; 15(2): 150–156. [PubMed: 15127906]
85. Zhu H, Kartiko S, Finnell RH. Importance of gene-environment interactions in the etiology of selected birth defects. Clin Genet. 2009; 75(5):409–423. [PubMed: 19459879]
86. Wu T, Liang KY, Hetmanski JB, Ruczinski I, Fallin MD, Ingersoll RG, Wang H, Huang S, Ye X, Wu-Chou YH, Chen PK, Jabs EW, Shi B, Redett R, Scott AF, Beaty TH. Evidence of gene-environment interaction for the IRF6 gene and maternal multivitamin supplementation in controlling the risk of cleft lip with/without cleft palate. Hum Genet. 2010; 128(4):401–410. [PubMed: 20652317]
87. Jentink J, Loane MA, Dolk H, Barisic I, Garne E, Morris JK, de Jong-van den Berg LT. EUROCAT. Antiepileptic Study Working Group. Valproic acid monotherapy in pregnancy and major congenital malformations. N Engl J Med. 2010; 362(23):2185–2193. [PubMed: 20558369]
88. Deroo LA, Wilcox AJ. First-Trimester Maternal Alcohol Consumption and the Risk of Infant Oral Clefts in Norway: A Population-based Case-Control Study. Am I Epidemiol. 2008; 168(6):638–646.
89. Boyles AL, Wilcox AJ, Taylor JA, Shi M, Weinberg CR, Meyer K, Fredriksen A, Ueland PM, Johansen AM, Drevon CA, Jugessur A, Trung TN, Gjessing HK, Vollset SE, Murray JC, Christensen K, Lie RT. Oral facial clefts and gene polymorphisms in metabolism of folate/one-carbon and vitamin A: a pathway-wide association study. Genet Epidemiol. 2009; 33(3):247–255. [PubMed: 19048631]
90. Wehby GL, Murray JC. Folic acid and orofacial clefts: a review of the evidence. Oral Dis. 2010; 16(1):11–19. [PubMed: 20331806]
91. Wilcox A, Lie RT, Solvoll K, Taylor J, McConnaughey DR, Abyholm F, Vindenes H, Vollset SE, Drevon CA. Folic acid supplements and risk of facial clefts: national population based case-control study. BMJ. 2007; 334(7591):464. [PubMed: 17259187]
92. Bille C, Pedersen DA, Andersen AM, Mansilla MA, Murray JC, Christensen K, Ballard JL, Gorman EB, Cabrera RM, Finnell RH. Autoantibodies to folate receptor alpha during early pregnancy and risk of oral clefts in Denmark. Pediatric Res. 2010; 67(3):274–279.

93. Yazdy M, Honein MA, Xing J. Reduction in orofacial clefts following folic acid fortification of the U.S. grain supply. Birth Defects Res A Clin Mol Teratol. 2007; 79(1):16–23. [PubMed: 17177274]
94. Johnson C, Little J. Folate intake, markers of folate status and oral clefts: is the evidence converging? Int J Epidemiol. 2008; 37(5):1041–1058. [PubMed: 18583393]
95. Ray JG, Vermeulen MJ, Wyatt PR, Cole DE. Association between folic acid food fortification and congenital orofacial clefts. J Pediatr. 2003; 143(6):805–807. [PubMed: 14657833]
96. López-Camelo JS, Castilla EE, Orioli IM. INAGEMP (Instituto Nacional de Genética Médica Populacional); ECLAMC (Estudio Colaborativo Latino Americano de Malformaciones Congénitas). Folic acid flour fortification: impact on the frequencies of 52 congenital anomaly types in three South American countries. Am J Hum Genet A. 2010; 152A(10):2444–2458.
97. Munger RG, Tamura T, Johnston KE, Feldkamp ML, Pfister R, Carey JC. Plasma zinc concentrations of mothers and the risk of oral clefts in their children in Utah. Birth Defects Res A Clin Mol Teratol. 2009; 85(2):151–155. [PubMed: 19067407]
98. Porter F. Cholesterol precursors and facial clefting. J Clin Invest. 2006; 116(9):2322–2325. [PubMed: 16955133]
99. Shahrukh Hashmi S, Gallaway MS, Waller DK, Langlois PH, Hecht JT. National Birth Defects Prevention Study. Maternal fever during early pregnancy and the risk of oral clefts. Birth Defects Res A Clin Mol Teratol. 2010; 288(3):186–194. [PubMed: 20099315]
100. Mossey P, Davies JA, Little J. Prevention of orofacial clefts: does pregnancy planning have a role? Cleft Palate-Cran J. 2007; 434(3):244–250.
101. Wehby GL, Ohsfeldt RL, Murray JC. 'Mendelian randomization' equals instrumental variable analysis with genetic instruments. Stat Med. 2008; 27(15):2745–2749. [PubMed: 18344186]
102. Eberhart J, He X, Swartz ME, Yan YL, Song H, Boling TC, Kunerth AK, Walker MB, Kimmel CB, Postlethwait JH. MicroRNA Mirn140 modulates Pdgf signaling during palatogenesis. Nat Genet. 2008; 40(3):290–298. [PubMed: 18264099]
103. Sharpe J, Ahlgren U, Perry P, Hill B, Ross A, Hecksher-Sørensen J, Baldock R, Davidson D. Optical projection tomography as a tool for 3D microscopy and gene expression studies. Science. 2002; 296(5567):541–545. [PubMed: 11964482]
104. Visel A, Prabhakar S, Akiyama JA, Shoukry M, Lewis KD, Holt A, Plajzer-Frick I, Afzal V, Rubin EM, Pennacchio LA. Ultraconservation identifies a small subset of extremely constrained developmental enhancers. Nat Genet. 2008; 40(2):158–160. [PubMed: 18176564]
105. Visel A, Rubin EM, Pennacchio LA. Genomic views of distant-acting enhancers. Nature. 2009; 461(7261):199–205. [PubMed: 19741700]
106. Jones JL, Canady JW, Brookes JT, Wehby GL, L'Heureux J, Schutte BC, Murray JC, Dunnwald M. Wound complications following cleft repair in children with Van der Woude syndrome. J Craniofac. Surg. 2010; 21(5):1350–1353. [PubMed: 20856020]
107. Christensen K, Juel K, Herskind AM, Murray JC. Long term follow up study of survival associated with cleft lip and palate at birth. BMJ. 2004; 328(7453):1405. [PubMed: 15145797]
108. Bille C, Knudsen B. Changing lifestyles and oral clefts occurerence in Denmark. Cleft Palate-Cran J. 2005; 42(3):255–259.
109. Menezes R, Marazita ML, McHenry T, Cooper ME, Bardi K, Brandon C, Letra A, Martin RA, Vieira AR. AXIS inhibition protein 2, orofacial clefts and family history for cancer. J Am Dent Assoc. 2009; 140(1):80–84. [PubMed: 19119171]
110. Yttri JE, Christensen K, Knudsen L, Bille C. Reproductive patterns among Danish women with oral clefts. Cleft Palate-Cran J. 2010 Sept 8. (Epub ahead of print).
111. Muenke M. The pit, the cleft and the web. Nat Genet. 2002; 32(2):219–220. [PubMed: 12355077]

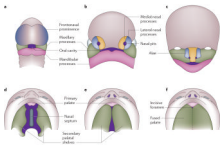

**Figure 1. Development of the lip and palate**

Schematic diagrams of the development of the lip and palate in humans. (A) The developing frontonasal prominence, paired maxillary processes and paired mandibular processes surround the primitive oral cavity by the fourth week of embryonic development. (B) By the fifth week, the nasal pits have formed, which leads to formation of the paired medial and lateral nasal processes. (C) The medial nasal processes have merged with the maxillary processes to form the upper lip and primary palate by the end of the sixth week. The lateral nasal processes form the nasal alae. Similarly, the mandibular processes fuse to form the lower jaw. (D) During the sixth week of embryogenesis, the secondary palate develops as bilateral outgrowths from the maxillary processes which grow vertically down the side of the tongue. (E) Subsequently, the palatal shelves elevate to a horizontal position above the tongue, contact one another and commence fusion. (F) Fusion of the palatal shelves ultimately divides the oronasal space into separate oral and nasal cavities. Figure is modified with permission from REF 68 Copyright permission**

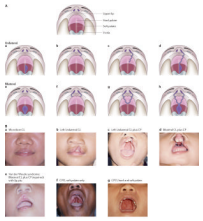

**Figure 2. Types of cleft**

**A**: A collection of images of different types of clefts, some with associated anomalies such as lip pits.[111] a–c, Van der Woude syndrome cases with associated lip pits; d, isolated cleft palate only; e, isolated unilateral cleft lip and palate; f–m, syndromic forms of clefting (f, CLP in Smith-Lemli-Opitz syndrome; g, midline cleft in holoproencephaly; h, bilateral CLP in *TGIF* mutation case and I, bilateral CLP in *SHH* variant; j, midline notch in OFD type 1; k, repaired cleft in *MID1* mutation; l, repaired unilateral CLP and; m, pseudo cleft lip). **[Legend to be modified depending on images provided. Include image permission details.]**

**B**: A set of illustrative drawings of CLP types.[111] a and e show unilateral and bilateral clefts of the soft palate; b, c and d show degrees of unilateral cleft lip and palate; f, g and h show degrees of bilateral cleft lip and palate. This figure is modified from REF. 111, with permission. Copyright Macmillan 2002.

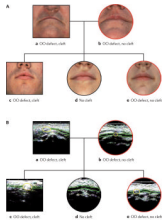

**Figure 3. Sub-clinical phenotypes**

**a**: Photographs of the upper lip region for each member of a nuclear family with two family members affected with nonsyndromic CLP (surgically repaired). The other three family members do not have externally visible defects, but two of them have sub-clinical defects of the *orbicularis oris* muscle (pedigree symbols circled in red).

**b**: The upper lip ultrasounds of each member of the family shown in panel a. Note the disruptions in the *orbicularis oris* muscle in the two people with CLP in the family, plus in two people with no external manifestation (pedigree symbols circled in red).

## Table 1

Clefting syndromes in which the mutated gene has been identified

| Cleft Type | Syndrome | Gene | Reference |
|---|---|---|---|
| Cleft lip +/− cleft palate | Autosomal dominant developmental malformations, deafness, and dystonia | *ACTB* | *1* |
| | Familial gastric cancer and CLP | *CDH1* | *2* |
| | Craniofrontonasal | *EFNB1* | *3* |
| | Roberts | *ESCO2* | *4* |
| | Holoprosencephaly | *GLI2* | *5* |
| | “Oro-facial-digital” | *GLI3* | *6* |
| | Hydrolethalus | *HYLS1* | *7* |
| | Van der Woude/popliteal pterygium | *IRF6* | *8* |
| | X-linked mental retardation and CL/P | *PHF8* | *9* |
| | Gorlin | *PTCH1* | *10,11* |
| | CLP – ectodermal dysplasia | *PVRL1* | *12* |
| | Holoprosencephaly | *SHH* | *13* |
| | Holoprosencephaly | *SIX3* | *14* |
| | Branchio-oculo-facial | *TFAP2A* | *15* |
| | Holoprosencephaly | *TGIF* | *16* |
| | Ectrodactyly-ectodermal dysplasia-clefting | *TP73L* | *17* |
| | Ankyloblepharon-ectodermal dysplasia-clefting | *TP73L* | *18* |
| | Tetra-amelia with CLP | *WNT3* | *19* |
| Cleft palate only | Oculofaciocardiodental | *BCOR* | *20* |
| | CHARGE | *CHD7* | *21* |
| | Lethal and Escobar multiple pterygium | *CHRNG* | *22* |
| | Stickler type 1 | *COL2A1* | *23* |
| | Stickler type 2 | *COL11A1* | *23* |
| | Stickler type 3 | *COL11A2* | *23* |
| | Desmosterolosis | *DHCR24* | *24* |
| | Smith-Lemli-Opitz | *DHCR7* | *25* |
| | Miller | *DHODH* | *26* |
| | Craniofrontonasal | *EFNB1* | *3* |
| | Kallmann | *FGFR1* | *27* |
| | Crouzon | *FGFR2* | *28* |
| | Apert | *FGFR2* | *29* |
| | Otopalatodigital types 1 and 2 | *FLNA* | *30* |
| | Larsen syndrome; atelosteogenesis | *FLNB* | *31* |
| | Hereditary lymphedema-distichiasis | *FOXC2* | *32* |
| | Bamforth-Lazarus | *FOXE1* | *33* |
| | “Oro-facial-digital” | *GLI3* | *6* |

| Cleft Type | Syndrome | Gene | Reference |
|---|---|---|---|
| | Van der Woude/popliteal pterygium | *IRF6* | *8* |
| | Andersen | *KCNJ2* | *34* |
| | Kabuki | *MLL2* | *35* |
| | Cornelia de Lange | *NIPBL* | *36,37* |
| | X-linked mental retardation | *PQBP1* | *38* |
| | Isolated cleft palate | *SATB2* | *39* |
| | Diastrophic dysplasia | *SLC26A2* | *40* |
| | Campomelic dysplasia | *SOX9* | *41,42* |
| | Pierre Robin | *SOX9* | *43* |
| | DiGeorge | *TBX1* | *44* |
| | X-linked cleft palate and ankyloglossia | *TBX22* | *45* |
| | Treacher Collins | *TCOF1* | *46* |
| | Loeys-Dietz | *TGFBR1* | *47* |
| | Loeys-Dietz | *TGFBR2* | *47* |
| | Saethre-Chotzen | *TWIST1* | *48,49* |
| Midline cleft lip | Opitz G/BBB | *MID1* | *50* |
| | Oro-facial-digital type I | *OFD1* | *51* |

*1*Procaccio, V. *et al*. A mutation of beta -actin that alters depolymerization dynamics is associated with autosomal dominant developmental malformations, deafness, and dystonia. *Am. J. Hum. Genet.* **78**, 947–960 (2006).

*2*Frebourg, T. *et al*. Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer. *J. Med. Genet.* **43**, 138–142 (2006).

*3*Twigg, S.R. *et al*. Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome. *Proc. Natl. Acad. Sci. USA.* **101**, 8652–8657 (2004).

*4*Vega, H. *et al*. Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion. *Nature Genet*. **37**, 468–470 (2005).

*5*Roessler, E. *et al*. Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proc. Natl. Acad. Sci. USA* **100**, 13424–13429 (2003).

*6*Johnston, J.J. *et al*. Molecular analysis expands the spectrum of phenotypes associated with GLI3 mutations. *Hum. Mutat.* **31**, 1142–1154 (2010).

*7*Mee, L. *et al*. Hydrolethalus syndrome is caused by a missense mutation in a novel gene HYLS1. *Hum. Mol. Genet*. **14**, 1475–1488 (2005).

*8*Kondo, S. *et al*. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nature Genet*. **32**, 285–289 (2002).

*9*Laumonnier, F. *et al*. Mutations in PHF8 are associated with X linked mental retardation and cleft lip/cleft palate. *J. Med. Genet*. **42**, 780–786 (2005).

*10*Hahn, H. *et al*. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell* **85**, 841–851 (1996).

*11*Johnson, R.L. *et al*. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* **272**, 1668–1671 (1996).

*12*Suzuki, K. *et al*. Mutations of PVRL1, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. *Nature Genet.* **25**, 427–430 (2000).

*13*Roessler, E. *et al*. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nature Genet.* **14**, 357–360 (1996).

*14*Wallis, D.E. *et al*. Mutations in the homeodomain of the human SIX3 gene cause holoprosencephaly. *Nature Genet*. **22**, 196–198 (1999).

[15] Milunsky, J.M. *et al*. TFAP2A mutations result in branchio-oculo-facial syndrome. *Am. J. Hum. Genet.* **82**, 1171–1177 (2008).

[16] Gripp, K.W. *et al*. Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. *Nature Genet.* **25**, 205–208 (2000).

[17] Celli, J. et al. Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. *Cell* **99**, 143–153 (1999).

[18] McGrath, J.A. *et al*. Hay-Wells syndrome is caused by heterozygous missense mutations in the SAM domain of p63. *Hum. Mol. Genet.* **10**, 221–229 (2001).

[19] Niemann, S. *et al*. Homozygous WNT3 mutation causes tetra-amelia in a large consanguineous family. *Am. J. Hum. Genet.* **74**, 558–563 (2004).

[20] Ng, D. *et al*. Oculofaciocardiodental and Lenz microphthalmia syndromes result from distinct classes of mutations in BCOR. *Nature Genet*. **36**, 411–416 (2004).

[21] Vissers, L.E. *et al*. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nature Genet*. **36**, 955–957 (2004).

[22] Morgan, N.V. *et al*. Mutations in the embryonal subunit of the acetylcholine receptor (CHRNG) cause lethal and Escobar variants of multiple pterygium syndrome. *Am. J. Hum. Genet.* **79**, 390–395 (2006).

[23] Snead, M.P. & Yates, J.R. Clinical and Molecular genetics of Stickler syndrome. *J. Med. Genet*. **36**, 353–359 (1999).

[24] Waterham, H.R. et al. Mutations in the 3beta-hydroxysterol Delta24-reductase gene cause desmosterolosis, an autosomal recessive disorder of cholesterol biosynthesis. *Am. J. Hum. Genet.* **69**, 685–694 (2001).

[25] Wassif, C.A. *et al*. Mutations in the human sterol delta7-reductase gene at 11q12-13 cause Smith-Lemli-Opitz syndrome. *Am. J. Hum. Genet*. **63**, 55–62 (1998).

[26] Ng, S.B. *et al*. Exome sequencing identifies the cause of a mendelian disorder. *Nature Genet.* **42**, 30–35 (2010).

[27] Dodé, C. *et al*. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nature Genet*. **33**, 463–465 (2003).

[28] Reardon, W. et al. Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. *Nature Genet*. 8, 98–103 (1994).

[29] Wilkie, A.O. *et al*. Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome. *Nature Genet*. **9**, 165–172 (1995).

[30] Robertson, S.P. *et al*. Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. *Nature Genet*. **33**, 487–491 (2003).

[31] Krakow, D. *et al*. Mutations in the gene encoding filamin B disrupt vertebral segmentation, joint formation and skeletogenesis. *Nature Genet*. **36**, 405–410 (2004).

[32] Fang, J. *et al*. 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 (2000).

[33] Clifton-Bligh, R.J. *et al*. Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. *Nature Genet*. 19, 399–401 (1998).

[34] Andelfinger, G. *et al*. KCNJ2 mutation results in Andersen syndrome with sex-specific cardiac and skeletal muscle phenotypes. *Am. J. Hum. Genet*. **71**, 663–668 (2002).

[35] Ng, D. *et al*. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nature Genet*. **42**, 790–793 (2010).

[36] Krantz, I.D. *et al*. Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of Drosophila melanogaster Nipped-B. *Nature Genet*. **36**, 631–635 (2004).

[37] Tonkin, E.T., Wang, T.J., Lisgo, S., Bamshad, M.J. & Strachan, T. NIPBL, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome. *Nature Genet*. **36**, 636–641 (2004).

[38] Kalscheuer, V.M. *et al*. Mutations in the polyglutamine binding protein 1 gene cause X-linked mental retardation. *Nature Genet*. **35**, 313–315 (2003).

[39] FitzPatrick, D.R. *et al*. Identification of SATB2 as the cleft palate gene on 2q32-q33. *Hum. Mol. Genet.* **12**, 2491–2501 (2003).

[40] Hästbacka, J. *et al*. The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. *Cell* **78**, 1073–1087 (1994).

[41] Foster, J.W. *et al*. (1994) Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature* **372**, 525–530 (1994).

[42] Wagner, T. *et al*. Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. *Cell* **79**, 1111–1120 (1994).

[43] Benko, S. *et al*. Highly conserved non-coding elements on either side of SOX9 associated with Pierre Robin sequence. *Nature Genet.* **41**, 359–364 (2009).

[44] Packham, E.A. & Brook, J.D. T-box genes in human disorders. *Hum. Mol. Genet*. **12**, R37–R44 (2003).

[45] Braybrook, C. *et al*. The T-box transcription factor gene TBX22 is mutated in X-linked cleft palate and ankyloglossia. *Nature Genet*. **29**, 179–183 (2001).

[46] Treacher Collins Syndrome Collaborative Group. Positional cloning of a gene involved in the pathogenesis of Treacher Collins syndrome. *Nature Genet*. **12**, 130–136 (1996).

[47] Loeys, B.L. *et al*. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nature Genet*. **37**, 275–81 (2005).

[48] Howard, T.D. et al. Mutations in TWIST, a basic helix-loop-helix transcription factor, in Saethre-Chotzen syndrome. *Nature Genet*. **15**, 36–41 (1997).

[49] el Ghouzzi, V. *et al*. Mutations of the TWIST gene in the Saethre-Chotzen syndrome. *Nature Genet*. **15**, 42–46 (1997).

[50] Quaderi, N.A. et al. Opitz G/BBB syndrome, a defect of midline development, is due to mutations in a new RING finger gene on Xp22. *Nature Genet*. **17**, 285–291 (1997).

[51] Ferrante, M.I. *et al*. Identification of the gene for oral-facial-digital type I syndrome. *Am. J. Hum. Genet*. **68**, 569–576 (2001).

## Table 2

Genes with a role in non-syndromic CLP

| Class/Gene | Evidence○ | Refs |
|---|---|---|
| Confirmed* | | |
| *IRF6* | GWA, LD, L, M | Zucchero et al., 2004 (41); Rahimov et al., 2008 (12); Birnbaum et al., 2009 (3) |
| 8q24 locus | GWA, LD | Birnbaum et al. 2009 (3); Grant *et al*., 2009 (4); Beaty et al. 2010 (6) |
| *VAX1* | GWA, LD | Mangold et al., 2010 (5); Beaty et al., 2010 (6) |
| Likely** | | |
| *MSX1* | LD, M | Lidral et al., 1998 (); Van den Boogaard et al. 2000 (17); Jezewski et al., 2003 (51); Vieira et al., 2004 (); Suzuki et al., 2004 () |
| *FOXE1* | L, LD, M | Vieira et al., 2005 (); Moreno et al., 2009 (50); Venza et al., 2006 () |
| *MYH9* | LD | Martinelli et al., 2007; Chiquet et al., 2009; Birnbaum et al., 2009 (3); Jia et al., 2010 |
| *MAFB* | GWA | Beaty et al. 2010 (6) |
| *ABCA4 (locus only)* | GWA | Beaty et al. 2010 (6) |
| *17q22 locus* | GWA | Mangold et al., 2010 (5); Beaty et al. 2010 (6) |
| *BMP4* | M | Suzuki et al., 2009 (36); Jianyan et al, 2010 |
| *FGFR2* | M | Riley et al., 2007; Riley and Murray, 2007 (52); Osoegawa et al, 2008 (45) |
| Intensively Studied*** | | |
| *TGFA* | LD | Ardinger et al., 1989 (40); Vieira, 2006; Carter et al, 2010 |
| *TGFB3* | LD, M | Lidral et al., 1998; Beaty et al., 2002; Vieira et al., 2003; Suazo et al, 2010 |
| *MTHFR* | LD | Mills et al., 2008; Jagomagi et al, 2010 |
| *GSTT1* | LD | Shi et al., 2007 (81) |
| *PDGFC* | LD, M | Ding et al., 2004; Choi et al., 2009; Jugessur *et al*., 2009 (24) |
| *FGF8* | M | Riley et al, 2007; Riley and Murray, 2007 |
| *PVRL1* | M, LD | Sozen et al., 2001; Avila et al., 2006; Sozen et al., 2009. |
| *SUMO1* | M | Alkurayra et al., 2005; Shi et al., 2009 (47); Mostowska et al., 2010; Carter et al, 2010 |
| *CRISPLD2* | LD | Chiquet et al, 2007; Letra et al, 2010 |

*At least two independent studies reaching conservative levels of significance

**At least one study with conservation/compelling data and other supportive studies.

***Multiple studies, no consensus or convincing meta-analysis

○GWA= Genome-wide association, LD=Candidate Gene Association, L = Linkage, M = Mutation Detection