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Genetics of Nonsyndromic Orofacial Clefts

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Abstract

With an average worldwide prevalence of approximately 1.2/1000 live births, orofacial clefts are the most common craniofacial birth defects in humans. Like other complex disorders, these birth defects are thought to result from the complex interplay of multiple genes and environmental factors. Significant progress in the identification of underlying genes and pathways has benefited from large populations available for study, increased international collaboration, rapid advances in genotyping technology, and major improvements in analytic approaches. Here we review recent advances in genetic epidemiological approaches to complex traits and their applications to studies of nonsyndromic orofacial clefts. Our main aim is to bring together a discussion of new and previously identified candidate genes to create a more cohesive picture of interacting pathways that shape the human craniofacial region. In future directions, we highlight the need to search for copy number variants that affect gene dosage and rare variants that are possibly associated with a higher disease penetrance. In addition, sequencing of protein-coding regions in candidate genes and screening for genetic variation in non-coding regulatory elements will help advance this important area of research.

Keywords

Nonsyndromic orofacial clefts; candidate gene; sequencing; single nucleotide polymorphism; association study; genome-wide association study

Orofacial clefts include cleft lip only (CLO), cleft lip and palate (CLP) and cleft palate only (CPO). Collectively, these are the most common craniofacial birth defects in humans, affecting approximately 1/800 live births worldwide. Extensive medical and behavioral interventions are needed to treat these common structural birth defects and they impose substantial economic and personal health burden (Wehby and Cassell, 2010) which can persist from infancy to childhood and throughout life. Mounting evidence suggests that multiple genes and environmental factors influence the risk of orofacial clefts, either

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individually or through their interactions in complex biological pathways. Technological advances and collaborative efforts have led to major advances in gene-mapping for clefts, with the first wave of genome-wide association (GWA) studies identifying several key candidate genes and loci (Birnbaum et al., 2009; Grant et al., 2009; Beaty et al., 2010; Mangold et al., 2010). By contrast, efforts to identify gene-environment (GxE) interactions have not been as successful, most likely because of a combination of insufficient sample size, study heterogeneity, differential assessment of environmental exposures, and a lack of robust methodology to detect these higher-order interactions.

Several reviews have recently been published on general aspects of craniofacial development and disorders affecting the craniofacial complex (Jugessur and Murray, 2005; Lidral and Moreno, 2005; Jiang et al., 2006; Gritli-Linde, 2007; Jugessur et al., 2009b; Meng et al., 2009; Mossey et al., 2009; Dixon et al., 2011). Rapid advances in high-throughput genetic technologies, coupled with more accurate phenotypic ascertainment and increased international collaboration, have led to significant progress in mapping genes for orofacial clefts. We review here key developments in candidate gene identification, new animal models, gene-expression studies, functional characterization of candidate genes, and GWA studies of clefts. We start by outlining classic genetic approaches that have mostly been used to identify candidate genes, followed by the application of new genomic technologies in the field. Finally, we discuss some of the more established environmental risk factors known to influence the risk of clefts and their interactions with specific genetic variants.

Overview of orofacial development

Orofacial clefts are caused by a failure of complete fusion between any of the independently derived facial primordia that collectively form the orofacial complex. Insights into the developmental circuitry controlling cell migration, proliferation, differentiation and apoptosis are necessary to understand how a particular cleft arises. Development of the human face begins with the migration of cranial neural crest cells from the dorsal region of the anterior neural tube into the facial region where they establish five distinct facial primordia during the 4^{th} week of gestation. These primordia consist of the unpaired frontonasal prominence that gives rise to a pair of lateral and medial nasal processes during the 5^{th} week, the paired bilateral maxillary prominences that form the upper jaw, and the paired mandibular prominences that develop into the lower jaw (Figure 1). During the following two weeks, the medial nasal processes enlarge and merge with each other to form the intermaxillary segment, providing the basis for the primary palate. Subsequently, the intermaxillary segment fuses with the flanking maxillary prominences, completing the formation of the upper lip [(Sadler, 2006); Figure 1]. Early development of these structures is mediated by epithelial-mesenchymal interactions and depends on a wide range of signaling molecules, including fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), and transforming growth factors (TGFs). Also involved are various developmental transcription factors belonging to the msh homeobox (MSX), distal-less homeobox (DLX), paired box (PAX), and T-Box (TBX) gene families.

The palatal shelves consist of neural crest-derived mesenchymal cells. They grow vertically from the bilateral maxillary processes during the 6^{th} week and elevate to a horizontal position above the tongue during the 7^{th} week of gestation. The palatal shelves continue to grow towards each other during the 8^{th} week until the medial edge epithelia (MEE) covering the opposing palatal shelves fuse at the midline to form a continuous epithelial seam which subsequently disintegrates (Sadler, 2006). There has been some controversy over the mechanism by which the palatal seam disappears; epithelial-mesenchymal transformation (EMT) initially received widespread acceptance as the mechanism for palatal fusion (Sun et

al., 2000; Nawshad and Hay, 2003), but others have later argued that programmed cell death is the major mechanism for shelf fusion (Cuervo and Covarrubias, 2004; Vaziri Sani et al., 2005). More recently, the work of Ahmed and colleagues showed that both migration and apoptosis are necessary for palatal confluence (Ahmed et al., 2007). What is incontrovertible, however, is that orofacial development is a rigidly coordinated spatio-temporal sequence of events involving cell migration, proliferation, differentiation, fusion and apoptosis. Disruption of any of these processes can result in an orofacial cleft.

Epidemiology of cleft lip and palate

Because lip formation precedes palatogenesis, failure of proper lip fusion has been suggested to interfere with palatal shelf contact. Genetic and embryologic studies suggest that distinct etiologic mechanisms underlie clefts of the lip with or without the primary palate (CL/P) and clefts of the secondary palate only (CPO) (Fraser, 1955; Sivertsen et al., 2008b). In some instances, both CL/P and CPO can be seen to segregate in a single family in which a single allele of an autosomal dominant form of clefting is present, suggesting some overlap in etiologies. This observation, termed ‘mixed clefting’, is best described in syndromic forms of clefts, such as those caused by mutations in *IRF6* (Kondo et al., 2002), *MSX1* (van den Boogaard et al., 2000), and *FGFR1* (Dode et al., 2003).

Orofacial clefts are categorized as syndromic if they are accompanied by additional structural and/or developmental abnormalities; nonsyndromic if they occur in isolation without other apparent abnormalities. The majority of CL/P cases are nonsyndromic (NS) (~70%), as are about half of CPO cases (Tolarova and Cervenka, 1998). The prevalence of NS CL/P varies according to ancestral origin (Vanderas, 1987) and socioeconomic status (Murray et al., 1997), ranging from ~1/500 in individuals of Asian and Amerindian origin to ~1/1000 in European and ~1/2500 in African populations (Mossey and Little, 2002). Some exceptions are observed in isolated geographic regions where the prevalence differs from that of the surrounding ancestral background—a discrepancy generally attributed to founder effects or specific environmental factors. Scandinavian populations, for example, tend to have a higher prevalence of cleft lip defects than most other European populations (Christensen, 1999; Mossey and Little, 2002; Harville et al., 2005).

A recent epidemiological assessment of a Norwegian cleft cohort suggested that at least a subset of CLO cases may have a separate etiology than CLP (Harville et al., 2005). When hospital and national birth registry data were combined, 17% of infants with CLP had at least one other non-cleft defect compared with only 9% of those with CLO. Although this supports the notion that CLP is simply a more severe version of CLO, the data also highlighted several qualitative differences not easily explained by disease severity alone. There was for instance a stronger male predominance among CLP infants compared with CLO infants. Furthermore, twins and cases born to consanguineous parents had a stronger risk of CLO than CLP. A study based on the US National Birth Defects Prevention Study (NBDPS) also showed that the association of CLO with associated anomalies was far lower than for cases of CLP with other anomalies (Genisca et al., 2009).

Molecular data also support etiologic differences between a subset of CLO and CLP. Rahimov and colleagues identified a common SNP (rs642961) within a highly conserved enhancer element for the interferon regulatory factor 6 (*IRF6*) gene that is strongly associated with NS CL/P (Rahimov et al., 2008). In that study, there was a clear separation of risk and transmission pattern with this SNP for NS CLO compared with NS CLP. Of note, the results for rs642961 were most significant for families in which affected individuals had NS CLO. A similar phenotype specificity was observed in a recent genome-wide linkage scan, where results for the *IRF6* region were most significant for NS CLO

(Marazita et al., 2009), and in a comprehensive search for fetal genetic effects among NS CLO and NS CLP trios in two Scandinavian population-based cleft cohorts (Jugessur et al., 2011).

Massive efforts have been undertaken to identify genes and loci underlying the isolated forms of clefting. This has largely been driven by several lines of evidence pointing to a strong genetic component to isolated clefts. Heritability estimates for both CL/P and CPO are above 90% (Grosen et al., 2010), and the risk of recurrence is 30–40 times higher among those with an affected first-degree relative compared to the population prevalence (Sivertsen et al., 2008a; Grosen et al., 2010). In the next section, we provide an overview of classic genetic approaches used to identify candidate genes/loci for clefts, followed by a review of recent applications of new genomic technologies that have significantly advanced the field.

Genetic approaches to identifying genes for orofacial clefts

Since the initial recognition of a strong genetic contribution to orofacial clefting (Fogh-Andersen, 1942), a variety of genetic approaches have been employed to identify genes and loci implicated in clefting. Animal models, chromosomal rearrangements, linkage studies, candidate gene based association studies, candidate gene sequencing and GWA studies have all had successful applications.

Linkage studies

Linkage studies assess the cosegregation of marker alleles with a disease phenotype in extended families with multiple affected individuals or in affected relative pairs. Because markers closest to the disease-causing mutation tend to cosegregate with the disease more often than expected by chance, the chromosomal location of the responsible gene can be refined to a narrow critical interval by tracking recombination events or assessing increased allele-sharing in affected relatives (Dawn Teare and Barrett, 2005). This approach has been particularly successful in mapping genes for rare, monogenic disorders, including syndromes with orofacial clefting such as Treacher Collins (The Treacher Collins Syndrome Collaborative Group, 1996), Van der Woude (Kondo et al., 2002), and cleft palate with ankyloglossia (Braybrook et al., 2001) syndromes.

In contrast, linkage studies for complex disorders such as NS CL/P have met with limited success due to the high degree of genetic and phenotypic heterogeneity commonly observed in these disorders. Whole genome-wide linkage scans using polymorphic microsatellite markers spread uniformly across the entire genome have identified possible susceptibility loci for NS CL/P on nearly every human chromosome in families from different populations (Mitchell et al., 1995; Prescott et al., 2000; Marazita et al., 2002; Radhakrishna et al., 2006; Beiraghi et al., 2007). As most of these findings have not been replicated in subsequent studies, it is plausible that no major gene is responsible for this condition, or that the genes observed have small, population-specific effects. Alternatively, these studies may have had insufficient power and/or marker resolution to detect variants of more modest effects (e.g. those increasing clefting risk by 10–50%). A meta-analysis of 13 genome-wide linkage scans revealed significant heterogeneity LOD scores for several loci on chromosome 1p, 6p, 6q, 14q, 15q, and 9q (Marazita et al., 2004). One candidate gene, *FOXE1* on 9q, has been identified (Moreno et al., 2009a), while fine-mapping studies for others are currently underway.

A major limitation of linkage studies of isolated clefts is the presence of extensive genetic and phenotypic heterogeneity. The phenotypic spectrum of orofacial clefting ranges from microforms and minimal clefts of the upper lip to complete bilateral clefts of the lip and palate. To date, most studies have assigned affected status only to those individuals who

exhibit overt features of clefting. However, there is growing consensus in the field that a number of subtle, subclinical features may be part of an 'extended' isolated cleft phenotype. For example, occult defects in the superior *orbicularis oris* (OO) muscle of the upper lip (also termed "subepithelial cleft lip") may represent the mildest form of an isolated cleft (Martin et al., 2000). Indeed, unaffected relatives of individuals with overt CL/P were twice as likely to have defects in the OO muscle as individuals with no family history of orofacial clefting (Neiswanger et al., 2007). Interestingly, a clear pattern of Mendelian inheritance emerged when an extended family member with OO muscle defects, initially diagnosed as unaffected, was later included in the pedigree as affected (Marazita, 2007; Neiswanger et al., 2007). These findings are already having an impact on clinical risk assessments (Klotz et al., 2010), and underscore the need for more accurate phenotypic ascertainment in future studies of clefts. Besides OO muscle defects, the role of dental anomalies (Letra et al., 2007; Vieira et al., 2008), structural brain abnormalities (Nopoulos et al., 2002; Conrad et al., 2010), craniofacial morphology (Weinberg et al., 2008; Weinberg et al., 2009), and whorl patterns on the lower lip (Neiswanger et al., 2009) as proxies for an underlying genetic risk shows great promise in refining linkage analysis. Broadening the phenotypic spectrum of isolated clefts to include these subphenotypes (Figure 2) may help explain incomplete penetrance issues that have been observed in several families where a seemingly etiologic mutation is transmitted from a parent without overt CL/P (Weinberg et al., 2006; Jugessur et al., 2009b).

Candidate gene association studies

Until recently, limitations in technology and a lack of extended families resulted in candidate gene association studies being the primary approach for genetic dissection of NS CL/P. In contrast to linkage analysis, association studies can be carried out on the large number of sporadic cleft cases that occur in isolation without affected relatives to detect alleles with minor effects. (As noted above, however, these apparently 'unaffected' relatives may be harboring a range of subclinical features that are yet to be diagnosed). In essence, association studies look for significantly altered frequency of an allele or haplotype in affected individuals as differing from what would be expected by chance if there were no association between the marker(s) and the phenotype of interest (Martin, 2006). The associated marker, currently with an emphasis on single nucleotide polymorphisms (SNPs) and copy number variants (CNVs), may either be causative itself or in strong linkage disequilibrium (LD) with the true etiologic variant. Association studies of isolated orofacial clefts have been carried out on cohorts of affected individuals and unrelated matched controls, or collections of offspring-parent trios. Linkage studies often locate large genomic intervals that house many candidate genes, requiring population-based fine-mapping studies to pinpoint the exact location of the responsible genes. The *FOXE1* gene was identified by using this combination of initial broad linkage-mapping with subsequent fine-mapping by association (Moreno et al., 2009a). Candidate genes have also been suggested by animal models that exhibit a clefting phenotype due to a targeted gene deletion or spontaneous mutation, even before the functional relevance of these genes in humans is known. For example, mice null for the homeobox msh-like 1 (*Msx1*) gene exhibit cleft palate (Satokata and Maas, 1994), which was subsequently shown to be involved in human nonsyndromic clefting as well (Lidral et al., 1998; Jezewski et al., 2003).

Gene-expression studies that demonstrate strong and restricted expression in the orofacial structures are also valuable for candidate gene selection. One important resource for prioritizing candidate genes, based on their expression patterns during early stages of craniofacial development, is the Craniofacial and Oral Gene Expression NEtwork (COGENE) database (http://hg.wustl.edu/cogene). COGENE catalogs expression profiles of a large number of genes in the craniofacial region (Cai et al., 2005). EMAGE (http://genex.hgu.mrc.ac.uk/Emage/database) is another example of a curated database of

gene-expression patterns in the developing mouse embryo. It provides standardized spatial representations of the sites of gene-expression, including those that are expressed in the craniofacial region, and is thus a valuable tool for selecting candidate genes for further evaluation (Christiansen et al., 2006). Expression data are particularly useful for follow-up studies of genes in critical linkage intervals and genes that cause clefts in transgenic mouse models. Chromosomal rearrangements that result in clefting, such as those that disrupt *IRF6* (Sander et al., 1994), *SATB2* (FitzPatrick et al., 2003) and *SUMO1* (Alkuraya et al., 2006), can be used as a primary source to determine the association of these genes with NS CL/P.

Perhaps the most fruitful approach that has led to the identification of two key genes for NS CL/P is the use of cleft syndromes as a model for isolated clefts [*IRF6* for Van der Woude syndrome (Zucchero et al., 2004) and *FOXE1* for Bamforth-Lazarus syndrome (Clifton-Bligh et al., 1998)]. Genes that cause Mendelian disorders with orofacial clefting have been widely assessed as potential candidates for NS CL/P based on the rationale that less deleterious common variants in these genes would contribute to the pathogenesis of relatively less severe isolated forms of clefting (Stanier and Moore, 2004). However, most studies to date that have shown significant associations with NS CL/P have failed to demonstrate obvious pathogenic changes in the coding sequences of these genes, leaving open the possibility that common etiologic variants in the regulatory elements of these genes might confer susceptibility to NS CL/P by altering the expression level of the gene in combination with additional genetic and/or environmental risk factors, consistent with the proposed etiologic mechanism underlying common, complex diseases (Lander and Schork, 1994).

Direct sequencing

Attempts to identify common causative variants in associated candidate genes have often led to the detection of multiple rare variants in individual families. Direct sequencing of protein-coding and regulatory regions has become a standard strategy to identify causal variants in candidate genes for NS CL/P (Jezewski et al., 2003; Marcano et al., 2004; Vieira et al., 2005; Watanabe et al., 2006; Riley et al., 2007; Riley and Murray, 2007; Suzuki et al., 2009). A mutation is considered pathogenic if it occurs *de novo* in the parental germline cells or if it is nonsense and absent in unaffected individuals. Only a handful of mutations detected in NS CL/P patients have met these stringent criteria (Riley et al., 2007). Many identified variants are either silent or missense, and are usually inherited from unaffected parents and sometimes shared with the unaffected siblings. This emphasizes one of the complicated issues that has yet to be resolved in complex disease genetics—the non-penetrance and expression variability of amino acid-changing mutations (Hindorff et al., 2009). Bioinformatic programs such as PolyPhen (Ramensky et al., 2002) and SIFT (Ng and Henikoff, 2003) have been informative in predicting the likely effects of missense variants based on their evolutionary conservation and their effects on the 3D structure of the protein. Given the vast majority of associated variants in GWA studies are not within protein-coding regions (Manolio et al., 2009), it is important to consider both coding and non-coding regions when searching for causal variants. Recent developments in massively parallel sequencing now make it practical to do whole-exome (the protein-coding part of the genome) or even whole-genome sequencing for Mendelian disorders (Ng et al., 2009; Lupski et al., 2010; Ng et al., 2010a), providing impetus to apply this strategy to complex traits such as NS CL/P, particularly in large and/or inbred families where linkage can assist in gene prioritization.

Array comparative genomic hybridization

Submicroscopic deletions and duplications are associated with numerous congenital anomalies (Mefford et al., 2007). Array comparative genomic hybridization (CGH)

measures DNA copy number differences between a reference genome from an unaffected individual and a patient's DNA. Over the past several years, array-CGH has proven to be a powerful approach to identify genes involved in various congenital anomalies (Vissers et al., 2005). Array-CGH based deletion analyses have recently been performed on NS CL/P subjects using whole-genome BAC clone arrays (Osoegawa et al., 2008). In a combined sample of 104 NS CL/P and NS CPO cases, one subject was identified with a 3.2 Mb deletion at chromosome 6q25.1-25.2 and another with a 2.2 Mb deletion at 10q26.11-26.13. These regions contain the genes for estrogen receptor 1 (*ESR1*) and fibroblast growth factor receptor 2 (*FGFR2*), respectively, which were subsequently identified as likely causative genes using a gene prioritization software. In another study, a low resolution scan for Mendelian allele loss in case-parent trios identified a 3.2 Mb *de novo* deletion at 6q25.1-25.2 and a 2.2 Mb deletion at 10q26.11-26.13 inherited from an affected mother (Shi et al., 2009). In addition, deletions involving the genes for transcription factor AP-2α (*TFAP2A*) and SMT3 suppressor of mif two 3 homolog 1 (*SUMO1*) were detected in four unrelated individuals with NS CL/P. These findings further prove the applicability of the array-CGH approach and the use of Mendelian allele loss in discovering novel genes affecting complex birth defects such as NS CL/P. These same approaches can be applied to candidate genes in order to detect deletions/duplications in the coding and non-coding regulatory elements that may affect a subset of NS CL/P cases.

Genome-wide association (GWA) studies

With recent advances in high-density SNP genotyping arrays and statistical methodology, GWA studies have heralded a new era of gene-discovery for complex diseases. Unlike the hypothesis-driven candidate gene approach, GWA studies do not have an *a priori* hypothesis and can thus identify novel genes and loci contributing to the trait of interest. More importantly, despite the relatively small increments in risk often identified, these studies can be instrumental in discovering new biological pathways involved in the disease etiology (Christensen and Murray, 2007; Hirschhorn, 2009). On the downside, the large number of tests and small odds ratios associated with risk alleles require very large sample sizes, often necessitating collaboration between different groups and the establishment of a reliable platform for genotype/phenotype harmonization (Manolio et al., 2007). To date, many GWA studies have been applied to complex diseases with a high prevalence in the population, such as cancer, diabetes, cardiovascular disorders, and obesity (Pearson and Manolio, 2008). But, like the candidate gene-based efforts, most of the identified common variants tend to confer relatively small increases in risk (1.1–1.5-fold), explaining only a small fraction of the overall phenotypic variance attributable to additive genetic factors (Manolio et al., 2009).

The first GWA study on NS CL/P was performed in individuals of Central European ancestry and identified a susceptibility locus on chromosome 8q24 (Birnbaum et al., 2009), which was subsequently replicated in three independent GWA studies (Grant et al., 2009; Beaty et al., 2010; Mangold et al., 2010). Table 1 summarizes the most salient findings from these four GWA studies. The most significant genes/loci demonstrated by these studies are: interferon regulatory factor 6 (*IRF6*); v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian) (*MAFB*); ATP-binding cassette, sub-family A (ABC1), member 4 (*ABCA4*); noggin (*NOG*); ventral anterior homeobox 1 (*VAX1*); Pvt1 oncogene (*PVT1*); gasdermin C (*GSDMC*); coiled-coil domain containing 26 (*CCDC26*); paired box 7 (*PAX7*); netrin 1 (*NTN1*); and *KIAA1598* (undefined). A detailed description of these genes is beyond the scope of this review, but more information is available in the references cited in Table 1.

Genes and pathways implicated in orofacial clefting

As with other complex traits, multiple genes and genetic pathways are likely to be implicated in craniofacial development and NS CL/P. In this section, we review several genes and pathways that are recognized as key players in NS CL/P based on their association in multiple studies and from evidence in animal model and *in vitro* studies.

Interferon Regulatory Factor 6 (IRF6)

Of the large number of candidate genes thought to contribute to orofacial clefting, *IRF6* is in a class of its own in that it is the only gene that has shown a convincing degree of consistency across studies. Mutations in *IRF6* cause two allelic autosomal-dominant clefting disorders known as Van der Woude (VWS) and popliteal pterygium (PPS) syndromes (Kondo et al., 2002). *IRF6* is strongly expressed in the ectoderm covering the developing facial primordia. Mice deficient for both *Irf6* alleles develop abnormally thick skin with severe limb and craniofacial abnormalities, including cleft of the secondary palate (Kondo et al., 2002; Knight et al., 2006). The lack of a normally stratified epidermis in *Irf6*-null mice, due to a defect in keratinocyte proliferation and differentiation, confirms an important role for *Irf6* in epidermal development (Ingraham et al., 2006; Richardson et al., 2006). Significant associations between *IRF6* and NS CL/P have been reported in multiple populations (Zucchero et al., 2004; Scapoli et al., 2005; Blanton et al., 2005; Ghassibe et al., 2005; Srichomthong et al., 2005; Park et al., 2007; Jugessur et al., 2008; Huang et al., 2009). In a follow-up study, a common etiologic variant (rs642961) in a highly conserved *IRF6* enhancer element was responsible for 18% of cleft lip occurrence in Northern European populations (Rahimov et al., 2008). The strongest observed association with this SNP was with NS CLO, with a relative risk of 2.4 for the homozygous genotype. However, no association was found with NS CPO. The associated A-allele of this SNP disrupts an AP-2α binding motif—a transcription factor required for craniofacial development (Schorle et al., 1996). Mutations in *TFAP2A* (the gene encoding AP-2α) cause branchio-oculo-facial syndrome (Milunsky et al., 2008) which is characterized by some of the same features observed in VWS (occasional lip pits and orofacial clefts), potentially linking these important pathways during craniofacial development.

More recently, Little and colleagues determined the DNA sequence to which wild-type IRF6 binds and used this sequence to show that IRF6 functions as a co-operative transcriptional activator (Little et al., 2009). Furthermore, VWS-causing mutations in the protein interaction domain of IRF6 disrupted this activity. Finally, in the GWA study by Beaty et al. (2010) (Table 1), four SNPs in *IRF6* showed genome-wide significance (P<5×10e-8) with clefting.

Forkhead box E1 (FOXE1)

FOXE1 is a member of the forkhead/winged helix-domain transcription factor family whose members are primarily involved in embryonic development. Targeted disruption of mouse *Foxe1* results in cleft palate and thyroid malformation (De Felice et al., 1998). Loss-of-function mutations within its forkhead DNA-binding domain cause Bamforth-Lazarus syndrome, which is characterized by thyroid agenesis, choanal atresia, bifid epiglottis, spiky hair and cleft palate (Clifton-Bligh et al., 1998; Castanet et al., 2002). A meta-analysis of 13 genome-wide linkage scans revealed a susceptibility locus on chromosome 9q22, in the vicinity of *FOXE1* and other potential candidate genes (Marazita et al., 2004). In addition, a recent mutation screening detected missense mutations in three unrelated NS CL/P patients (Vieira et al., 2005; Venza et al., 2006). Subsequently, significant associations were reported between *FOXE1* and NS CL/P in multiple populations, although no common coding variants were identified (Venza et al., 2006; Moreno et al., 2009b; Castanet et al., 2010).

Poliovirus receptor-related 1 (PVRL1)

Nectin-1, encoded by *PVRL1*, is an immunoglobulin related transmembrane cell-cell adhesion molecule. Homozygosity for a common nonsense mutation in *PVRL1* (Trp185Stop) causes the autosomal recessive CL/P-ectodermal dysplasia in the indigenous population of Margarita Island (the largest island of the Nueva Esparta State in Venezuela) where there is an unusually high incidence of clefting (5.4/1000) (Suzuki et al., 2000). Heterozygous carriers of the same mutation from a neighboring region in Northern Venezuela were also shown to be at high risk for NS CL/P (Sozen et al., 2001). Rare mutations in sporadic cases and a statistically significant association between a common coding variant (G361V) in *PVRL1* and NS CL/P were found in multiple population (Avila et al., 2006). In addition, mutations in *PVRL1* homologue genes *PVR* and *PVRL2*, and significant associations between a *PVR* variant and NS CL/P were reported in multiple populations from South America (Warrington et al., 2006).

Msh homeobox 1 (MSX1)

Ablation of the murine muscle-segment homeobox 1 (*Msx1*) gene causes a complete cleft of the secondary palate and a variety of other craniofacial defects (Satokata and Maas, 1994). In humans, a nonsense mutation segregating with tooth agenesis and mixed clefting was reported in an extended Dutch family, suggesting an important role for *MSX1* in human clefting (van den Boogaard et al., 2000). Significant associations were reported between SNPs in *MSX1* and *both* NS CL/P and NS CPO in different populations (Lidral et al., 1998; Suzuki et al., 2004; Tongkobpetch et al., 2006). In addition, direct sequencing of the *MSX1* coding regions showed that mutations in *MSX1* could account for approximately 2% of NS CL/P cases (Jezewski et al., 2003). However, no common variants affecting the coding sequence have thus far been identified.

SMT3 suppressor of mif two 3 homolog 1 (SUMO1)

SUMO1 is a 101-amino acid polypeptide involved in posttranslational modification of a variety of proteins. A balanced reciprocal translocation resulting in *SUMO1* haploinsufficiency was identified in a patient with isolated unilateral CLP (Alkuraya et al., 2006). *Sumo1* showed strong expression in the upper lip, primary palate and medial edge epithelium (MEE) of the secondary palate in the mouse at E13.5. Mice with a *Sumo1*-hypomorphic allele had cleft palate (Alkuraya et al., 2006). However, a knockout of *Sumo1* was viable and did not have a cleft phenotype, nor did the heterozygotes (Zhang et al., 2008). A microdeletion encompassing *SUMO1* in a cleft patient supports its role in human clefting (Shi et al., 2008), although other genes near *SUMO1* also warrant continued consideration as genes potentially implicated in clefting. Finally, genetic associations between NS CL/P and *SUMO1* variants have been reported in two ancestrally diverse populations, China (Song et al., 2008) and Ireland (Carter et al., 2010).

T-box 22 (TBX22)

Function-impairing mutations in the T-box DNA-binding domain of the transcription factor gene *TBX22* cause X-linked cleft of the secondary palate (CPX), usually associated with ankyloglossia or tongue-tie (Braybrook et al., 2001). Its expression is localized to the developing palatal shelves and the base of the tongue. Consistent with the human findings, *Tbx22*-null mice also present with submucous cleft palate and ankyloglossia (Braybrook et al., 2002; Pauws et al., 2009). A genome-wide linkage analysis of NS CL/P families identified a susceptibility locus in the vicinity of *TBX22*, suggesting that the linkage signal may emanate from this gene (Prescott et al., 2000). In addition, mutations in *TBX22* were found in individuals with isolated CPO (Marcano et al., 2004; Suphapeetiporn et al., 2007). Using an array of *in vitro* functional assays, Andreou et al. demonstrated that TBX22

functions as a transcriptional repressor and that pathogenic missense mutations in the DNA-binding domain disrupt its DNA-binding affinity and impair its repression ability (Andreou et al., 2007).

Special AT-rich sequence binding protein 2 (SATB2)

SATB2 belongs to a small family of DNA-binding proteins that specifically bind to nuclear matrix-attachment regions (MARs) to regulate gene transcription in a tissue-specific manner through chromatin remodeling (Britanova et al., 2005). An important role for *SATB2* in orofacial clefting was discovered after fine-mapping of translocation breakpoints in the 2q32-q33 region in two unrelated cases with NS CPO (FitzPatrick et al., 2003). The *de novo* translocation breakpoints disrupted the *SATB2* transcriptional unit and separated a putative regulatory element from the gene in these patients. Subsequently, significant associations were reported between genetic variants in *SATB2* and NS CL/P in two Asian populations (Beaty et al., 2006). Mouse *Satb2* is strongly expressed in the developing palate and is 99.6% identical to the human *SATB2* at the protein level (FitzPatrick et al., 2003). The biological significance of this gene was not known until two animal models lacking a functional *Satb2* were reported to have severe craniofacial malformations (Britanova et al., 2006; Dobreva et al., 2006). Notably, mice with a single functional copy of *Satb2* developed various craniofacial defects including cleft palate, closely mimicking the human phenotypes resulting from *SATB2* haploinsufficiency. Losing the second allele severely augmented these phenotypes. In addition, significant changes in the expression patterns of three essential genes involved in craniofacial development and diseases—*Msx1*, *Pax9* and *Alx4*—were observed in $Satb2^{-/-}$ mice (Britanova et al., 2006).

Fibroblast Growth Factor (FGF) signaling pathway

The FGF signaling pathway plays a central role in craniofacial development, essentially through induction and migration of cranial neural crest cells and regulation of epithelial-mesenchymal interactions during fusion of the facial prominences (Nie et al., 2006). To date, 23 mammalian FGF proteins have been recognized along with their seven main receptors (Ornitz and Marie, 2002). The majority of the FGF ligands and the receptors FGFR1 and FGFR2 are broadly expressed in the developing facial primordia (Bachler and Neubuser, 2001). Several members of this family of signaling molecules have been implicated in various birth defects that also afflict craniofacial structures. For example, gain-of-function mutations in FGF receptor genes 1, 2 and 3 are commonly associated with craniosynostosis syndromes (Marie et al., 2005).

Mutations in the FGF receptor 2 gene (*FGFR2*) cause Apert syndrome, a craniosynostosis syndrome characterized by cleft palate in 76% of cases and a Byzantine arch-shaped palate in nearly all cases. Mice deficient for *Fgfr2b* and one of its ligand genes, *Fgf10,* exhibit clefts of the secondary palate (De Moerlooze et al., 2000; Rice et al., 2004). Recently, two potentially etiologic missense mutations in the functional domains of FGFR2 (Riley et al., 2007) and a microdeletion in a region containing *FGFR2* (Osoegawa et al., 2008) were identified in patients with NS CL/P. In addition, statistically significant associations were detected between a common genetic variant in *FGF10* and NS CL/P (Riley et al., 2007), further highlighting the significance of the FGF10-FGFR2 pathway in human orofacial development (Pauws and Stanier, 2007).

Cleft lip and palate are occasionally associated with the autosomal-dominant form of Kallmann syndrome (KAL2), which is characterized by hypogonadotropic hypogonadism with anosmia. KAL2 is caused by loss-of-function mutations in *FGFR1* (Dode et al., 2003). Mice homozygous for a hypomorphic allele of *Fgfr1* exhibit craniofacial defects, including cleft of the secondary palate, and, remarkably, this phenotype can be rescued by restoring

Fgfr1 function specifically in neural crest cells (Trokovic et al., 2003). Mutations in *FGFR1* can also cause nonsyndromic forms of clefting, as evidenced by a Kallmann syndrome patient inheriting a protein truncating mutation from a father with an isolated CL/P, and missense mutations disrupting conserved residues of the FGFR1 protein in two unrelated patients with NS CL/P (Riley et al., 2007).

FGF8 is an important ligand of this pathway and is required for patterning of the first pharyngeal arch under the control of sonic hedgehog (SHH) (Trumpp et al., 1999; Haworth et al., 2007). It is expressed in the mandibular and maxillary ectoderms and induces the expression of LIM homeobox protein 6 (*Lhx6*), distal-less homeobox 1 (*Dlx1*), distal-less homeobox 2 (*Dlx 2*) and BarH-like homeobox 1 (*Barx1*). A *de novo* mutation that changes a highly conserved amino acid residue of FGF8, with a predicted loss-of-function, was found in a case with NS CL/P (Riley et al., 2007). In addition to potentially etiologic rare mutations in the above-mentioned FGF signaling members, the Riley et al. study also reported significant associations between SNPs in *FGF3*, *FGF7*, *FGF18* and *FGFR1* and isolated clefts. Finally, there is a potential connection between alleles in *FGFR2* causing CL/P and alleles contributing to breast cancer. Data from a large Danish cohort study revealed an odds ratio of 1.3 for women born with a cleft to later develop breast cancer (Bille et al., 2005). Several recent studies have also shown a highly significant association of alleles in *FGFR2* with breast cancer (Katoh, 2008).

Transforming growth factor (TGF) signaling pathway

The TGF superfamily of growth factors and their binding receptors play significant roles in craniofacial development. Transforming growth factor α (*TGFA*) was one of the first genes reported to be associated with nonsyndromic clefting (Ardinger et al., 1989; Shiang et al., 1993; Jugessur et al., 2003b). It is strongly expressed in the MEE of fusing palatal shelves and promotes extracellular matrix biosynthesis (Dixon and Ferguson, 1992). In addition, *TGFA* alleles are among the few genetic factors that have shown significant interactions with various environmental factors, including maternal smoking and vitamin use (Shaw et al., 1996; Shaw et al., 1998; Jugessur et al., 2003a; Zeiger et al., 2005; Sull et al., 2009).

Most of our knowledge of the role of TGF signaling in orofacial development stems from animal models and *in vivo* organ culture studies. Two independent mouse knockouts for *Tgfb3* both manifested cleft palate phenotypes (Kaartinen et al., 1995; Proetzel et al., 1995). Targeted overexpression of the Tgf-β3 signal mediator Smad2 in MEE of these mice partially rescued the cleft palate phenotype, indicating a specific requirement for this growth factor in palatal closure (Cui et al., 2005). Interestingly, exogenous application of TGF-β3 also induced palatal fusion in chickens, a species born with a natural cleft palate (Sun et al., 1998). In addition, TGF-β3 signaling is central to MEE disintegration as well as sequential induction of cell cycle arrest in MEE, cell migration and apoptosis at advanced stages of palatal development (Ahmed et al., 2007). Finally, genetic variants in *TGFB3* have been associated with NS CL/P in multiple populations (Lidral et al., 1998; Reutter et al., 2008; Suazo et al., 2010a; Zhu et al., 2010).

Bone morphogenetic protein (BMP) signaling pathway

The BMPs are a collection of secreted cell signaling molecules of the TGF-β superfamily of growth factors. They regulate important developmental processes, including cell proliferation, differentiation and apoptosis (Srichomthong et al., 2005). Members of this signaling pathway are expressed throughout the orofacial primordia in a strictly regulated spatio-temporal pattern, and outgrowth and patterning of the facial primordia are BMP-dosage sensitive (Barlow and Francis-West, 1997; Ashique et al., 2002). Conditional inactivation of the type 1 Bmp receptor gene (*Bmpr1a*) in the orofacial primordia causes

bilateral CL/P with tooth agenesis, whereas conditional deletion of its ligand Bmp4 in the same tissue results in isolated cleft lip only (Liu et al., 2005). The cleft lip accompanied by cleft palate was attributed to increased apoptosis in the ectodermal and mesenchymal tissues of the medial nasal prominence. Interestingly, the cleft lip in the *Bmp4*-mutant mouse was repaired at advanced stages of embryogenesis, perhaps owing to redundancy among BMP factors.

This wound healing property of *BMP4* may also have a parallel in humans. It was recently proposed that *BMP4* may be involved in mild forms of cleft lip, as defects in the *orbicularis oris* (OO) muscle in the mouth may be a manifestation of *in utero* healed cleft lip (Marazita, 2007). Supportive of this hypothesis are the reported potential etiologic mutations in *BMP4* in two individuals with OO muscle defects and one with a microform cleft lip (Suzuki et al., 2009). Additional mutations were also detected in five cases with NS CL/P. Further, pathogenic mutations in *BMP4* have recently been associated with eye, brain and digit abnormalities with no apparent facial dysmorphologies (Bakrania et al., 2008). It is plausible that an ultrasound examination of these patients might reveal OO muscle defects.

Thus far, only two association studies have been published on genetic variants in *BMP4* and risk of NS CL/P. The first investigated three SNPs in *BMP4* (rs762642, rs2855532 and rs1957860) in 150 unrelated NS CL/P trios from Chile (Suazo et al., 2010b). An association was found with haplotypes of rs1957860-rs762642, but not with any individual SNP. The second study reported an association between a single non-synonymous SNP (rs17563) in *BMP4* among 184 patients with NS CL/P and 205 controls from China (Lin et al., 2008).

Sonic Hedgehog (SHH) pathway

The sonic hedgehog (SHH) signaling pathway is involved in various aspects of embryonic development and craniofacial morphogenesis, as illustrated by a wide spectrum of craniofacial defects caused by perturbations in this evolutionarily conserved signaling pathway. Mutations in human *SHH* are responsible for a subset of holoprosencephaly cases, a congenital birth defect characterized by a spectrum of brain and facial abnormalities (Roessler et al., 1996). Mice null for *Shh* die prenatally due to severe midline defects (Chiang et al., 1996). *Shh* is broadly expressed in the ectoderm covering primordial facial structures (Jeong et al., 2004). SHH initiates its signaling by binding to its cell surface receptor Patched to relieve its inhibition of the transmembrane protein Smoothened, which in turn activates the GLI family of zinc-finger transcription factors that regulate the expression of downstream target genes such as *FOXE1* (Brancaccio et al., 2004; Eichberger et al., 2004).

One of the downstream target genes is the Patched receptor gene (*PTCH*) itself, whose induction ensures an autoregulation of the pathway. Mutations in *PTCH* are associated with nevoid basal cell carcinoma (also known as Gorlin syndrome), which includes cleft palate in ~4% of cases. A mutation screen of the *PTCH* coding sequences in 220 multiplex families with NS CL/P revealed missense mutations in its predicted extracellular SHH-binding domain, which may interfere with its binding activity (Mansilla et al., 2006). However, no disease-causing mutations in *SHH* have yet been found in isolated clefts (Orioli et al., 2002).

Loss-of-function mutations in *GLI2*, one of the three canonical transcription factors that convey intracellular SHH messaging, are associated with holoprosencephaly-like features with various combinations of cleft lip/palate (Roessler et al., 2003). Linkage and significant association between SNPs in the *GLI2* region and NS CL/P have been reported in various populations (Beaty et al., 2006). Furthermore, potentially pathogenic missense mutations that disrupt conserved residues in GLI2 were found in sporadic individuals with NS CL/P

(Vieira et al., 2005). Overall, these findings demonstrate that members of the SHH signaling pathway are involved in the pathogenesis of NS CL/P.

Other candidate genes

In addition to these extensively studied genes and pathways, preliminary evidence for a number of other genes exists, supporting their role in NS CL/P. One recently identified gene associated with NS CL/P is cysteine-rich secretory protein LCCL domain containing 2 (*CRISPLD2*), with strong expression in the fusing palatal shelves (Chiquet et al., 2007). Ectopic expression of *Tbx10* (a member of the T-box gene family of transcription factors) in transgenic mice resulted in cleft lip and palate (Bush et al., 2004), and mutations in *TBX10* were also detected in individuals with NS CL/P (Vieira et al., 2005). Similarly, overexpression of the gene for sprouty homolog 2 (*Spry2*) causes stage-dependent craniofacial defects in transgenic mice (Goodnough et al., 2007). Mutations in human *Sprouty2* (*SPRY2*) further delineates its role in clefting (Vieira et al., 2005).

Heterozygous mutations in the tumor protein p63 gene (*TP63*) are responsible for the autosomal dominant ectrodactyly, ectodermal dysplasia, and cleft lip/palate (EEC) syndrome (Celli et al., 1999). A *de novo* missense mutation that disrupts a functional domain of p63 was reported in a NS CL/P patient (Leoyklang et al., 2006). Further, a missense mutation in the gene for RYK receptor-like tyrosine kinase (*RYK*) was also found in a case with isolated clefting (Watanabe et al., 2006). Lastly, significant association between different members of the wingless-type MMTV integration site family (WNT) signaling pathway and isolated clefting have also been reported in various populations (Chiquet et al., 2008), with mouse model studies providing further confirmation (Juriloff et al., 2006; Juriloff and Harris, 2008). A list of NS CL/P candidate gene is provided in Table 2.

Gene-environment (GxE) interactions

It has long been hypothesized that orofacial clefts result from the complex interplay of multiple genes and environmental factors, but only recently have practical approaches become available for a robust investigation of this hypothesis at the genome-wide level (Engelman et al., 2009; Murcray et al., 2009; Gauderman et al., 2010). Analyses of GxE interaction are important, because a failure to incorporate genetic and environmental exposures in a joint analysis of a population composed of both susceptible and non-susceptible individuals will bias observed associations toward the null (Khoury and Wacholder, 2009). Furthermore, they are important in determining the potential for public health intervention on environmental factor(s) which alone could reduce the occurrence/recurrence of clefts, particularly in genetically-susceptible subgroups of the population. This rationale is supported by animal models; the spontaneous CL/P rate among the cleft-susceptible CL/Fr mouse is about 20% compared to <10% in the normal C57BL/6J strain (Juriloff, 2002), but this can be easily increased to almost 100% at dosages of 6-aminonicotinamide (a vitamin B3 inhibitor). Just as some mouse strains are more susceptible to external teratogens (Millicovsky and Johnston, 1981; Juriloff, 2002), human fetuses carrying specific high-risk alleles may be more sensitive to particular teratogenic agents. Thus, identifying teratogens that interact with specific genetic factors will deepen our understanding of the biological mechanisms leading to orofacial clefts.

Although a large number of maternal exposures have been reported to influence the risk of orofacial clefts at critical stages of development, only a handful have survived scrutiny after being tested in large, well-characterized populations. Maternal smoking (Zeiger and Beaty, 2002; Little et al., 2004; Lie et al., 2008); alcohol consumption (especially at binge levels) (Deroo et al., 2008); folic acid and other B-complex vitamin supplementation (Munger, 2002; Hayes, 2002; Wilcox et al., 2007); use of anti-folate medication (Hernandez-Diaz et

al., 2000; Holmes et al., 2001); and specific exposures related to particular parental occupations (Nguyen et al., 2007) are all relevant environmental factors. In addition, the role of maternal illnesses such as hyperthermia (Peterka et al., 1994; Botto et al., 2002; Shahrukh Hashmi et al., 2010), diabetes and obesity (Cedergren and Kallen, 2005; Stothard et al., 2009) have recently been identified as important research gaps for additional public health research (Yazdy et al., 2007).

Maternal risk factors such as cigarette smoking, alcohol consumption, nutritional deficiencies and infectious diseases during pregnancy may adversely affect the intrauterine environment in which the embryo grows. Although both the mother and fetus have an inborn capacity to cope with diverse environmental insults through the action of detoxification enzymes, deleterious variants in these detoxification genes may reduce this ability to biotransform toxic components, rendering the fetus more vulnerable to teratogenic exposures. Below, we briefly review three environmental factors, maternal cigarette smoking, alcohol consumption and folic acid/multivitamin supplement use, for which the accumulated evidence is the most consistent across studies.

Cigarette smoking during the first trimester of pregnancy has been repeatedly associated with an increased risk of clefting (Shi et al., 2008). A meta-analysis strongly supports an odds ratio of ~1.3 for smoking with clefting (Little et al., 2004). Increased risks from exposures can suggest metabolic pathways in which disruptions may play a key role in the pathogenesis of CL/P, with recent evidence of a particularly compelling GxE interaction between fetal glutathione S-transferase theta 1 (*GSTT1*) gene variants and maternal smoking (Shi et al., 2007), and between variants in the gene for alcohol dehydrogenase 1C (class I), gamma polypeptide (*ADH1C*) and alcohol metabolism (Jugessur et al., 2009a; Boyles et al., 2010). In the Boyles et al study, heavy alcohol drinking was associated with risk of clefts *only* if either the mother or the baby carried the slow-metabolizing *ADH1C* variant (Boyles et al., 2010). This finding supports the hypothesis that genetic susceptibility in detoxification genes increases vulnerability of the fetus to alcohol-related orofacial clefts (Shi et al., 2007; Shi et al., 2008).

Through genome-wide expression analyses of B-lymphoblasts derived from NS CL/P patients, Bliek and co-workers identified a large number of folate responsive genes and showed that folate deficiency perturbs normal cell development (Bliek et al., 2008). Several studies have shown that folic acid lowers the risk of orofacial clefts (Badovinac et al., 2007; Wilcox et al., 2007). Whether this is mediated through the action of genes involved in folic acid metabolism has not yet been established. A pathway-wide analysis of 108 SNPs and one insertion polymorphism in 29 genes involved in folate/one-carbon metabolism found no convincing evidence that genetic variants in these folate metabolism genes play a causal role in orofacial clefting (Boyles et al., 2009). A second, more recent analysis of 97 SNPs in 14 genes in or interacting with the folate pathway found suggestive evidence of association with six genes in the folate pathway only when a less conservative approach was used for correcting for multiple testing than the stricter Bonferroni correction (Blanton et al., 2011). These findings suggests that the genetic contribution to orofacial clefts may be independent of pathways by which multivitamin supplementation provides protection from clefts.

In summary, only a few GxE interactions have been conclusive despite the well-recognized role of environmental factors in clefting (Mossey et al., 2009). Known challenges are related to exposure assessment, sample size/statistical power, and study heterogeneity (Clayton and McKeigue, 2001; Akey et al., 2004; Weinberg, 2009; Thomas, 2010). Indeed, most cleft studies are not sufficiently large or phenotypically well-characterized to provide the level of statistical power necessary to tease out GxE interactions. The emergence of collaborative networks such as the Gene, Environment Association Studies (GENEVA) consortium

[(Cornelis et al., 2010); http://www.genevastudy.org] may help resolve some of the sample size issues. Concurrently, novel study designs that increase statistical power and are not prone to confounding from population stratification are needed to advance the study of GxE interactions in clefting (Engelman et al., 2009; Murcray et al., 2009; Shi et al., 2010; Gauderman et al., 2010).

Future directions

Linkage and candidate gene approaches have had their fair share of success in identifying genes and genetic pathways involved in orofacial clefts. Because of the high prevalence and genetic complexity of this disorder, most of these studies have assessed association with common variants, under the assumption that a combination of common susceptibility alleles increases the risk of the disease. As an alternative to the common disease-common variant hypothesis, accumulation of mildly deleterious, low-frequency missense mutations may represent a potential mechanism underlying the pathogenesis of common, complex diseases (Pritchard, 2001; Pritchard and Cox, 2002). A recent quantitative study, supplemented with empirical data from mutation screening of candidate genes involved in obesity, provides a rough estimation of the degree to which such rare variants contribute to complex diseases (Ahituv et al., 2007; Kryukov et al., 2007). These low-frequency variants can only be detected when a sufficiently large number of individuals are sequenced. Indeed, case-control candidate gene resequencing efforts in NS CL/P have revealed rare missense variants with a statistically significant excess in affected individuals when large numbers of cases are sequenced (Jezewski et al., 2003; Riley et al., 2007). Recent advances in massively parallel sequencing technologies will enable us to sequence at least all protein-coding and highly conserved non-coding functional portions of the human genome, if not the entire genome, quickly and more affordably. Several recent studies clearly illustrate the power of targeted exome sequencing in detecting rare, disease-causing mutations (Ng et al., 2009; Ng et al., 2010b). A few studies have already applied whole-genome sequencing approaches to personalized medicine (Ashley et al., 2010; Lupski et al., 2010; Sobreira et al., 2010). In addition, the inclusion of rare variants with minor allele frequency typically below 0.5% in both candidate gene and GWA studies may help account for some of the missing heritability in complex disorders (Gorlov et al., 2008).

With the increasing number of associated genes without obvious amino-acid changing mutations, the significance of genetic variation in non-coding regulatory elements is becoming more recognized. Therefore, mutation screening of highly conserved, potential regulatory elements flanking candidate genes for NS CL/P should be carried out in order to identify previously undetected pathogenic variants. An excellent resource for selecting regulatory element is the genome-wide enhancer screening initiative (Pennacchio et al., 2006), which harnesses the evolutionary conservation of non-coding regions in the human genome. Enhancer activities of randomly selected, highly conserved regions are assessed in transgenic mouse assays and the data are deposited into the VISTA Enhancer database (http://enhancer.lbl.gov), which can then be queried based on their expression patterns in specific tissues or organs (Visel et al., 2007). Enhancer activities of highly conserved regions near candidate genes can facilitate the discovery of regulatory elements of genes expressed in the orofacial region. This can then be followed up by resequencing in NS CL/P populations to search for both rare and common etiologic variants. Again, next generation sequencing technologies will provide an opportunity to perform deep sequencing of potential regulatory elements on a much larger scale than previously possible.

Characterizing the role of regulatory RNAs in craniofacial morphogenesis provides another exciting new avenue for gene discovery, as exemplified by a recent study demonstrating, for the first time, a role for microRNA in palatogenesis (Eberhart et al., 2008). Interestingly,

disruption of a signaling pathway mediated by a member of the platelet-derived growth factor (Pdgf) family had previously been shown to result in palatal clefting in knockout mice (Ding et al., 2004). Further, targeted deletion of the Pdgf receptor alpha gene (*Pdgfra*) leads to neural tube closure defects, including midfacial and palatal clefting (Soriano, 1997). The microRNA in the Eberhart study negatively regulated translation of zebrafish *Pdgfra* mRNA via its 3′UTR region. Since spatio-temporal downregulation of *Pdgfra* is required for the proper migration of a specific subset of neural crest cells into craniofacial structures (Eberhart et al., 2008), these findings provide novel non-protein coding target genes for further evaluation in human orofacial clefting.

Conclusion

Significant knowledge of the risk factors for a range of birth defects has already been gained and is being used to guide public health recommendations for pregnancy planning in order to reduce the overall health burden of these defects. However, further research is needed on environmental and genetic factors to improve preventive measures and risk assessment. To date, smoking, alcohol and folic acid use during the first trimester of pregnancy appear to be the most consistent environmental factors for clefting, although other exposures have also been suggested. Identification of specific genetic and environmental causes of clefting could enable major changes in genetic counseling, improved programs for personalized medicine applications and aid in identifying new biological pathways and gene networks for investigation of the underlying biology. Genetic and epidemiologic studies of NS CL/P currently underway hold the promise of refining our ability for more accurate diagnosis, recurrence risk estimation, and eventually the prevention of cleft lip and palate. Information derived from GxE interaction studies will provide insights into efficient preventive interventions in individuals with susceptible genotypes. Furthermore, the inclusion of relevant subphenotypes may reveal hitherto unrecognized patterns of Mendelian inheritance, raising the exciting possibility of using standard genetic techniques to identify causative genes for isolated clefts. As we are entering the age of personalized medicine, individually tailored medications and vitamin supplementation regimen based on a thorough assessment of the genetic risk profiles of pregnant mothers may become an important measure in reducing the overall health burden of orofacial clefts and other similar birth defects.

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References

Ahituv N, Kavaslar N, Schackwitz W, Ustaszewska A, Martin J, Hebert S, Doelle H, Ersoy B, Kryukov G, Schmidt S, et al. Medical sequencing at the extremes of human body mass. Am J Hum Genet. 2007; 80:779–791. [PubMed: 17357083]

Ahmed S, Liu CC, Nawshad A. Mechanisms of palatal epithelial seam disintegration by transforming growth factor (TGF) beta3. Dev Biol. 2007; 309:193–207. [PubMed: 17698055]

Akey JM, Eberle MA, Rieder MJ, Carlson CS, Shriver MD, Nickerson DA, Kruglyak L. Population history and natural selection shape patterns of genetic variation in 132 genes. PLoS Biol. 2004; 2:e286. [PubMed: 15361935]

Alkuraya FS, Saadi I, Lund JJ, Turbe-Doan A, Morton CC, Maas RL. SUMO1 haploinsufficiency leads to cleft lip and palate. Science. 2006; 313:1751. [PubMed: 16990542]

Andreou AM, Pauws E, Jones MC, Singh MK, Bussen M, Doudney K, Moore GE, Kispert A, Brosens JJ, Stanier P. TBX22 missense mutations found in patients with X-linked cleft palate affect DNA binding, sumoylation, and transcriptional repression. Am J Hum Genet. 2007; 81:700–712. [PubMed: 17846996]

Ardinger HH, Buetow KH, Bell GI, Bardach J, VanDemark DR, Murray JC. Association of genetic variation of the transforming growth factor-alpha gene with cleft lip and palate. Am J Hum Genet. 1989; 45:348–353. [PubMed: 2570526]

Ashique AM, Fu K, Richman JM. Endogenous bone morphogenetic proteins regulate outgrowth and epithelial survival during avian lip fusion. Development. 2002; 129:4647–4660. [PubMed: 12223420]

Ashley EA, Butte AJ, Wheeler MT, Chen R, Klein TE, Dewey FE, Dudley JT, Ormond KE, Pavlovic A, Morgan AA, et al. Clinical assessment incorporating a personal genome. Lancet. 2010; 375:1525–1535. [PubMed: 20435227]

Avila JR, Jezewski PA, Vieira AR, Orioli IM, Castilla EE, Christensen K, Daack-Hirsch S, Romitti PA, Murray JC. PVRL1 variants contribute to non-syndromic cleft lip and palate in multiple populations. Am J Med Genet A. 2006; 140:2562–2570. [PubMed: 17089422]

Bachler M, Neubuser A. Expression of members of the Fgf family and their receptors during midfacial development. Mech Dev. 2001; 100:313–316. [PubMed: 11165488]

Badovinac RL, Werler MM, Williams PL, Kelsey KT, Hayes C. Folic acid-containing supplement consumption during pregnancy and risk for oral clefts: a meta-analysis. Birth Defects Res A Clin Mol Teratol. 2007; 79:8–15. [PubMed: 17133404]

Bakrania P, Efthymiou M, Klein JC, Salt A, Bunyan DJ, Wyatt A, Ponting CP, Martin A, Williams S, Lindley V, et al. Mutations in BMP4 cause eye, brain, and digit developmental anomalies: overlap between the BMP4 and hedgehog signaling pathways. Am J Hum Genet. 2008; 82:304–319. [PubMed: 18252212]

Barlow AJ, Francis-West PH. Ectopic application of recombinant BMP-2 and BMP-4 can change patterning of developing chick facial primordia. Development. 1997; 124:391–398. [PubMed: 9053315]

Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB, Liang KY, Wu T, Murray T, Fallin MD, et al. 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]

Beaty TH, Hetmanski JB, Fallin MD, Park JW, Sull JW, McIntosh I, Liang KY, Vanderkolk CA, Redett RJ, Boyadjiev SA, et al. Analysis of candidate genes on chromosome 2 in oral cleft case-parent trios from three populations. Hum Genet. 2006; 120:501–518. [PubMed: 16953426]

Beiraghi S, Nath SK, Gaines M, Mandhyan DD, Hutchings D, Ratnamala U, McElreavey K, Bartoloni L, Antonarakis GS, Antonarakis SE, et al. Autosomal dominant nonsyndromic cleft lip and palate: significant evidence of linkage at 18q21.1. Am J Hum Genet. 2007; 81:180–188. [PubMed: 17564975]

Bille C, Winther JF, Bautz A, Murray JC, Olsen J, Christensen K. Cancer risk in persons with oral cleft--a population-based study of 8,093 cases. Am J Epidemiol. 2005; 161:1047–1055. [PubMed: 15901625]

Birnbaum S, Ludwig KU, Reutter H, Herms S, Steffens M, Rubini M, Baluardo C, Ferrian M, Almeida de Assis N, Alblas MA, et al. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. Nat Genet. 2009; 41:473–477. [PubMed: 19270707]

Blanton SH, Henry RR, Yuan Q, Mulliken JB, Stal S, Finnell RH, Hecht JT. Folate pathway and nonsyndromic cleft lip and palate. Birth Defects Res A Clin Mol Teratol. 2011; 91:50–60. [PubMed: 21254359]

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; 137:259–262. [PubMed: 16096995]

Bliek BJ, Steegers-Theunissen RP, Blok LJ, Santegoets LA, Lindemans J, Oostra BA, Steegers EA, de Klein A. Genome-wide pathway analysis of folate-responsive genes to unravel the pathogenesis of

orofacial clefting in man. Birth Defects Res A Clin Mol Teratol. 2008; 82:627–635. [PubMed: 18655124]

Botto LD, Erickson JD, Mulinare J, Lynberg MC, Liu Y. Maternal fever, multivitamin use, and selected birth defects: evidence of interaction? Epidemiology. 2002; 13:485–488. [PubMed: 12094106]

Boyles AL, DeRoo LA, Lie RT, Taylor JA, Jugessur A, Murray JC, Wilcox AJ. Maternal alcohol consumption, alcohol metabolism genes, and the risk of oral clefts: a population-based case-control study in Norway, 1996–2001. Am J Epidemiol. 2010; 172:924–931. [PubMed: 20810466]

Boyles AL, Wilcox AJ, Taylor JA, Shi M, Weinberg CR, Meyer K, Fredriksen A, Ueland PM, Johansen AM, Drevon CA, et al. Oral facial clefts and gene polymorphisms in metabolism of folate/one-carbon and vitamin A: a pathway-wide association study. Genet Epidemiol. 2009; 33:247–255. [PubMed: 19048631]

Brancaccio A, Minichiello A, Grachtchouk M, Antonini D, Sheng H, Parlato R, Dathan N, Dlugosz AA, Missero C. Requirement of the forkhead gene Foxe1, a target of sonic hedgehog signaling, in hair follicle morphogenesis. Hum Mol Genet. 2004; 13:2595–2606. [PubMed: 15367491]

Braybrook C, Doudney K, Marcano AC, Arnason A, Bjornsson A, Patton MA, Goodfellow PJ, Moore GE, Stanier P. The T-box transcription factor gene TBX22 is mutated in X-linked cleft palate and ankyloglossia. Nat Genet. 2001; 29:179–183. [PubMed: 11559848]

Braybrook C, Lisgo S, Doudney K, Henderson D, Marcano AC, Strachan T, Patton MA, Villard L, Moore GE, Stanier P, et al. Craniofacial expression of human and murine TBX22 correlates with the cleft palate and ankyloglossia phenotype observed in CPX patients. Hum Mol Genet. 2002; 11:2793–2804. [PubMed: 12374769]

Britanova O, Depew MJ, Schwark M, Thomas BL, Miletich I, Sharpe P, Tarabykin V. Satb2 haploinsufficiency phenocopies 2q32-q33 deletions, whereas loss suggests a fundamental role in the coordination of jaw development. Am J Hum Genet. 2006; 79:668–678. [PubMed: 16960803]

Britanova O, Akopov S, Lukyanov S, Gruss P, Tarabykin V. Novel transcription factor Satb2 interacts with matrix attachment region DNA elements in a tissue-specific manner and demonstrates cell-type-dependent expression in the developing mouse CNS. Eur J Neurosci. 2005; 21:658–668. [PubMed: 15733084]

Bush JO, Lan Y, Jiang R. The cleft lip and palate defects in Dancer mutant mice result from gain of function of the Tbx10 gene. Proc Natl Acad Sci U S A. 2004; 101:7022–7027. [PubMed: 15118109]

Cai J, Ash D, Kotch LE, Jabs EW, Attie-Bitach T, Auge J, Mattei G, Etchevers H, Vekemans M, Korshunova Y, et al. Gene expression in pharyngeal arch 1 during human embryonic development. Hum Mol Genet. 2005; 14:903–912. [PubMed: 15703188]

Carter TC, Molloy AM, Pangilinan F, Troendle JF, Kirke PN, Conley MR, Orr DJ, Earley M, McKiernan E, Lynn EC, et al. Testing reported associations of genetic risk factors for oral clefts in a large Irish study population. Birth Defects Res A Clin Mol Teratol. 2010; 88:84–93. [PubMed: 19937600]

Castanet M, Park SM, Smith A, Bost M, Leger J, Lyonnet S, Pelet A, Czernichow P, Chatterjee K, Polak M. A novel loss-of-function mutation in TTF-2 is associated with congenital hypothyroidism, thyroid agenesis and cleft palate. Hum Mol Genet. 2002; 11:2051–2059. [PubMed: 12165566]

Castanet M, Mallya U, Agostini M, Schoenmakers E, Mitchell C, Demuth S, Raymond FL, Schwabe J, Gurnell M, Chatterjee VK. Maternal Isodisomy for Chromosome 9 Causing Homozygosity for a Novel FOXE1 Mutation in Syndromic Congenital Hypothyroidism. J Clin Endocrinol Metab. 2010; 95:4031–4036. [PubMed: 20484477]

Cedergren M, Kallen B. Maternal obesity and the risk for orofacial clefts in the offspring. Cleft Palate Craniofac J. 2005; 42:367–371. [PubMed: 16001917]

Celli J, Duijf P, Hamel BC, Bamshad M, Kramer B, Smits AP, Newbury-Ecob R, Hennekam RC, Van Buggenhout G, van Haeringen A, et al. Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. Cell. 1999; 99:143–153. [PubMed: 10535733]

Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature. 1996; 383:407–413. [PubMed: 8837770]

Chiquet BT, 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 Genet. 2008; 17:2212–2218. [PubMed: 18413325]

Chiquet BT, Lidral AC, Stal S, Mulliken JB, Moreno LM, Arco-Burgos M, Valencia-Ramirez C, Blanton SH, Hecht JT. CRISPLD2: a novel NSCLP candidate gene. Hum Mol Genet. 2007; 16:2241–2248. [PubMed: 17616516]

Christensen K. The 20th century Danish facial cleft population--epidemiological and genetic-epidemiological studies. Cleft Palate Craniofac J. 1999; 36:96–104. [PubMed: 10213053]

Christensen K, Murray JC. What genome-wide association studies can do for medicine. N Engl J Med. 2007; 356:1094–1097. [PubMed: 17360987]

Christiansen JH, Yang Y, Venkataraman S, Richardson L, Stevenson P, Burton N, Baldock RA, Davidson DR. EMAGE: a spatial database of gene expression patterns during mouse embryo development. Nucleic Acids Res. 2006; 34:D637–641. [PubMed: 16381949]

Clayton D, McKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. Lancet. 2001; 358:1356–1360. [PubMed: 11684236]

Clifton-Bligh RJ, Wentworth JM, Heinz P, Crisp MS, John R, Lazarus JH, Ludgate M, Chatterjee VK. Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. Nat Genet. 1998; 19:399–401. [PubMed: 9697705]

Conrad AL, Dailey S, Richman L, Canady J, Karnell MP, Axelson E, Nopoulos P. Cerebellum Structure Differences and Relationship to Speech in Boys and Girls With Nonsyndromic Cleft of the Lip and/or Palate. Cleft Palate Craniofac J. 2010; 47:469–475. [PubMed: 20180711]

Cornelis MC, Agrawal A, Cole JW, Hansel NN, Barnes KC, Beaty TH, Bennett SN, Bierut LJ, Boerwinkle E, Doheny KF, et al. The Gene, Environment Association Studies consortium (GENEVA): maximizing the knowledge obtained from GWAS by collaboration across studies of multiple conditions. Genet Epidemiol. 2010; 34:364–372. [PubMed: 20091798]

Cuervo R, Covarrubias L. Death is the major fate of medial edge epithelial cells and the cause of basal lamina degradation during palatogenesis. Development. 2004; 131:15–24. [PubMed: 14645125]

Cui XM, Shiomi N, Chen J, Saito T, Yamamoto T, Ito Y, Bringas P, Chai Y, Shuler CF. Overexpression of Smad2 in Tgf-beta3-null mutant mice rescues cleft palate. Dev Biol. 2005; 278:193–202. [PubMed: 15649471]

Dawn Teare M, Barrett JH. Genetic linkage studies. Lancet. 2005; 366:1036–1044. [PubMed: 16168786]

De Felice M, Ovitt C, Biffali E, Rodriguez-Mallon A, Arra C, Anastassiadis K, Macchia PE, Mattei MG, Mariano A, Scholer H, et al. A mouse model for hereditary thyroid dysgenesis and cleft palate. Nat Genet. 1998; 19:395–398. [PubMed: 9697704]

De Moerlooze L, Spencer-Dene B, Revest J, Hajihosseini M, Rosewell I, Dickson C. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. Development. 2000; 127:483–492. [PubMed: 10631169]

Deroo LA, Wilcox AJ, Drevon CA, Lie RT. First-Trimester Maternal Alcohol Consumption and the Risk of Infant Oral Clefts in Norway: A Population-based Case-Control Study. Am J Epidemiol. 2008

Ding H, Wu X, Bostrom H, Kim I, Wong N, Tsoi B, O'Rourke M, Koh GY, Soriano P, Betsholtz C, et al. A specific requirement for PDGF-C in palate formation and PDGFR-alpha signaling. Nat Genet. 2004; 36:1111–1116. [PubMed: 15361870]

Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet. 2011; 12:167–178. [PubMed: 21331089]

Dixon MJ, Ferguson MW. The effects of epidermal growth factor, transforming growth factors alpha and beta and platelet-derived growth factor on murine palatal shelves in organ culture. Arch Oral Biol. 1992; 37:395–410. [PubMed: 1610308]

Dobreva G, Chahrour M, Dautzenberg M, Chirivella L, Kanzler B, Farinas I, Karsenty G, Grosschedl R. SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation. Cell. 2006; 125:971–986. [PubMed: 16751105]

Dode C, Levilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet. 2003; 33:463–465. [PubMed: 12627230]

Eberhart JK, 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; 40:290–298. [PubMed: 18264099]

Eichberger T, Regl G, Ikram MS, Neill GW, Philpott MP, Aberger F, Frischauf AM. FOXE1, a new transcriptional target of GLI2 is expressed in human epidermis and basal cell carcinoma. J Invest Dermatol. 2004; 122:1180–1187. [PubMed: 15140221]

Engelman CD, Baurley JW, Chiu YF, Joubert BR, Lewinger JP, Maenner MJ, Murcray CE, Shi G, Gauderman WJ. Detecting gene-environment interactions in genome-wide association data. Genet Epidemiol. 2009; 33 (Suppl 1):S68–73. [PubMed: 19924704]

FitzPatrick DR, Carr IM, McLaren L, Leek JP, Wightman P, Williamson K, Gautier P, McGill N, Hayward C, Firth H, et al. Identification of SATB2 as the cleft palate gene on 2q32-q33. Hum Mol Genet. 2003; 12:2491–2501. [PubMed: 12915443]

Fogh-Andersen, P. Inheritance of harelip and cleft palate. Copenhagen: Nyt nordisk forlag. Arnold Busck; 1942. Dissertation

Fraser FC. Thoughts on the etiology of clefts of the palate and lip. Acta Genet Stat Med. 1955; 5:358–369. [PubMed: 13339079]

Gauderman WJ, Thomas DC, Murcray CE, Conti D, Li D, Lewinger JP. Efficient genome-wide association testing of gene-environment interaction in case-parent trios. Am J Epidemiol. 2010; 172:116–122. [PubMed: 20543031]

Genisca AE, Frias JL, Broussard CS, Honein MA, Lammer EJ, Moore CA, Shaw GM, Murray JC, Yang W, Rasmussen SA. Orofacial clefts in the National Birth Defects Prevention Study, 1997–2004. Am J Med Genet A. 2009; 149A:1149–1158. [PubMed: 19441124]

Ghassibe 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; 13:1239–1242. [PubMed: 16132054]

Goodnough LH, Brugmann SA, Hu D, Helms JA. Stage-dependent craniofacial defects resulting from Sprouty2 overexpression. Dev Dyn. 2007; 236:1918–1928. [PubMed: 17576140]

Gorlov IP, Gorlova OY, Sunyaev SR, Spitz MR, Amos CI. Shifting paradigm of association studies: value of rare single-nucleotide polymorphisms. Am J Hum Genet. 2008; 82:100–112. [PubMed: 18179889]

Grant SF, Wang K, Zhang H, Glaberson W, Annaiah K, Kim CE, Bradfield JP, Glessner JT, Thomas KA, Garris M, et al. 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]

Gritli-Linde A. Molecular control of secondary palate development. Dev Biol. 2007; 301:309–326. [PubMed: 16942766]

Grosen D, Chevrier C, 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]

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]

Haworth KE, Wilson JM, Grevellec A, Cobourne MT, Healy C, Helms JA, Sharpe PT, Tucker AS. Sonic hedgehog in the pharyngeal endoderm controls arch pattern via regulation of Fgf8 in head ectoderm. Dev Biol. 2007; 303:244–258. [PubMed: 17187772]

Hayes, C. Environmental risk factors and oral clefts. In: Wyszynski, DFE., editor. Cleft lip and palate: from origin to treatment. New York: Oxford University press; 2002.

Helms JA, Cordero D, Tapadia MD. New insights into craniofacial morphogenesis. Development. 2005; 132:851–861. [PubMed: 15705856]

Hernandez-Diaz S, Werler MM, Walker AM, Mitchell AA. Folic acid antagonists during pregnancy and the risk of birth defects. N Engl J Med. 2000; 343:1608–1614. [PubMed: 11096168]

Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A. 2009; 106:9362–9367. [PubMed: 19474294]

Hirschhorn JN. Genomewide association studies--illuminating biologic pathways. N Engl J Med. 2009; 360:1699–1701. [PubMed: 19369661]

Holmes LB, Harvey EA, Coull BA, Huntington KB, Khoshbin S, Hayes AM, Ryan LM. The teratogenicity of anticonvulsant drugs. N Engl J Med. 2001; 344:1132–1138. [PubMed: 11297704]

Huang Y, Wu J, Ma J, Beaty TH, Sull JW, Zhu L, Lu D, Wang Y, Meng T, Shi B. Association between IRF6 SNPs and oral clefts in West China. J Dent Res. 2009; 88:715–718. [PubMed: 19734457]

Ingraham CR, Kinoshita A, Kondo S, Yang B, Sajan S, Trout KJ, Malik MI, Dunnwald M, Goudy SL, Lovett M, et al. Abnormal skin, limb and craniofacial morphogenesis in mice deficient for interferon regulatory factor 6 (Irf6). Nat Genet. 2006; 38:1335–1340. [PubMed: 17041601]

Jeong J, Mao J, Tenzen T, Kottmann AH, McMahon AP. Hedgehog signaling in the neural crest cells regulates the patterning and growth of facial primordia. Genes Dev. 2004; 18:937–951. [PubMed: 15107405]

Jezewski PA, Vieira AR, Nishimura C, Ludwig B, Johnson M, O'Brien SE, Daack-Hirsch S, Schultz RE, Weber A, Nepomucena B, et al. Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. J Med Genet. 2003; 40:399–407. [PubMed: 12807959]

Jiang R, Bush JO, Lidral AC. Development of the upper lip: morphogenetic and molecular mechanisms. Dev Dyn. 2006; 235:1152–1166. [PubMed: 16292776]

Jugessur A, Shi M, Gjessing HK, Lie RT, Wilcox AJ, Weinberg CR, Christensen K, Boyles AL, Daack-Hirsch S, Trung TN, et al. Genetic determinants of facial clefting: analysis of 357 candidate genes using two national cleft studies from Scandinavia. PLoS ONE. 2009a; 4:e5385. [PubMed: 19401770]

Jugessur A, Shi M, Gjessing HK, Lie RT, Wilcox AJ, Weinberg CR, Christensen K, Boyles AL, Daack-Hirsch S, Nguyen TT, et al. Fetal genetic risk of isolated cleft lip only versus isolated cleft lip and palate: A subphenotype analysis using two population-based studies of orofacial clefts in scandinavia. Birth Defects Res A Clin Mol Teratol. 2011; 91:85–92. [PubMed: 21319277]

Jugessur A, Murray JC. Orofacial clefting: recent insights into a complex trait. Curr Opin Genet Dev. 2005; 15:270–278. [PubMed: 15917202]

Jugessur A, Lie RT, Wilcox AJ, Murray JC, Taylor JA, Saugstad OD, Vindenes HA, Abyholm FE. Cleft palate, transforming growth factor alpha gene variants, and maternal exposures: assessing gene-environment interactions in case-parent triads. Genet Epidemiol. 2003a; 25:367–374. [PubMed: 14639706]

Jugessur A, Lie RT, Wilcox AJ, Murray JC, Taylor JA, Saugstad OD, Vindenes HA, Abyholm F. Variants of developmental genes (TGFA, TGFB3, and MSX1) and their associations with orofacial clefts: a case-parent triad analysis. Genet Epidemiol. 2003b; 24:230–239. [PubMed: 12652527]

Jugessur A, Farlie PG, Kilpatrick N. The genetics of isolated orofacial clefts: from genotypes to subphenotypes. Oral Dis. 2009b; 15:437–453. [PubMed: 19583827]

Jugessur A, Rahimov F, Lie RT, Wilcox AJ, Gjessing HK, Nilsen RM, Nguyen TT, Murray JC. Genetic variants in IRF6 and the risk of facial clefts: single-marker and haplotype-based analyses in a population-based case-control study of facial clefts in Norway. Genet Epidemiol. 2008; 32:413–424. [PubMed: 18278815]

Juriloff, DM. Mapping studies in animal models. In: Wyszynski, DF., editor. Cleft Lip and Palate: From Origin to Treatment. New York: Oxford University Press; 2002. p. 265-282.

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; 76:574–579. [PubMed: 16998816]

Juriloff DM, Harris MJ. Mouse genetic models of cleft lip with or without cleft palate. Birth Defects Res A Clin Mol Teratol. 2008; 82:63–77. [PubMed: 18181213]

Kaartinen V, Voncken JW, Shuler C, Warburton D, Bu D, Heisterkamp N, Groffen J. Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. Nat Genet. 1995; 11:415–421. [PubMed: 7493022]

Katoh M. Cancer genomics and genetics of FGFR2 (Review). Int J Oncol. 2008; 33:233–237. [PubMed: 18636142]

Khoury MJ, Wacholder S. Invited commentary: from genome-wide association studies to gene-environment-wide interaction studies--challenges and opportunities. Am J Epidemiol. 2009; 169:227–230. discussion 234–225. [PubMed: 19022826]

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:2697–2702. [PubMed: 20949506]

Knight AS, Schutte BC, Jiang R, Dixon MJ. Developmental expression analysis of the mouse and chick orthologues of IRF6: the gene mutated in Van der Woude syndrome. Dev Dyn. 2006; 235:1441–1447. [PubMed: 16245336]

Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, Howard E, de Lima RL, Daack-Hirsch S, Sander A, et al. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nat Genet. 2002; 32:285–289. [PubMed: 12219090]

Kryukov GV, Pennacchio LA, Sunyaev SR. Most rare missense alleles are deleterious in humans: implications for complex disease and association studies. Am J Hum Genet. 2007; 80:727–739. [PubMed: 17357078]

Lander ES, Schork NJ. Genetic dissection of complex traits. Science. 1994; 265:2037–2048. [PubMed: 8091226]

Leoyklang P, Siriwan P, Shotelersuk V. A mutation of the p63 gene in non-syndromic cleft lip. J Med Genet. 2006; 43:e28. [PubMed: 16740912]

Letra A, Menezes R, Granjeiro JM, Vieira AR. Defining subphenotypes for oral clefts based on dental development. J Dent Res. 2007; 86:986–991. [PubMed: 17890676]

Lidral AC, Moreno LM. Progress toward discerning the genetics of cleft lip. Curr Opin Pediatr. 2005; 17:731–739. [PubMed: 16282779]

Lidral AC, Romitti PA, Basart AM, Doetschman T, Leysens NJ, Daack-Hirsch S, Semina EV, Johnson LR, Machida J, Burds A, et al. Association of MSX1 and TGFB3 with nonsyndromic clefting in humans. Am J Hum Genet. 1998; 63:557–568. [PubMed: 9683588]

Lie RT, Wilcox AJ, Taylor J, Gjessing HK, Saugstad OD, Aabyholm F, Vindenes H. Maternal smoking and oral clefts: the role of detoxification pathway genes. Epidemiology. 2008; 19:606–615. [PubMed: 18449058]

Lin JY, Chen YJ, Huang YL, Tang GP, Zhang L, Deng B, Li M, Ma H, Luan RS. Association of bone morphogenetic protein 4 gene polymorphisms with nonsyndromic cleft lip with or without cleft palate in Chinese children. DNA Cell Biol. 2008; 27:601–605. [PubMed: 18771417]

Little HJ, Rorick NK, Su LI, Baldock C, Malhotra S, Jowitt T, Gakhar L, Subramanian R, Schutte BC, Dixon MJ, et al. Mis-sense mutations that cause Van der Woude syndrome and popliteal pterygium syndrome affect the DNA-binding and transcriptional activation functions of IRF6. Hum Mol Genet. 2009; 18:535–545. [PubMed: 19036739]

Little J, Cardy A, Munger RG. Tobacco smoking and oral clefts: a meta-analysis. Bull World Health Organ. 2004; 82:213–218. [PubMed: 15112010]

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; 132:1453–1461. [PubMed: 15716346]

Lupski JR, Reid JG, Gonzaga-Jauregui C, Rio Deiros D, Chen DC, Nazareth L, Bainbridge M, Dinh H, Jing C, Wheeler DA, et al. Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. N Engl J Med. 2010; 362:1181–1191. [PubMed: 20220177]

Mangold E, Ludwig KU, Birnbaum S, Baluardo C, Ferrian M, Herms S, Reutter H, de Assis NA, Chawa TA, Mattheisen M, et al. 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]

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, et al. Finding the missing heritability of complex diseases. Nature. 2009; 461:747–753. [PubMed: 19812666]

Manolio TA, Rodriguez LL, Brooks L, Abecasis G, Ballinger D, Daly M, Donnelly P, Faraone SV, Frazer K, Gabriel S, et al. New models of collaboration in genome-wide association studies: the Genetic Association Information Network. Nat Genet. 2007; 39:1045–1051. [PubMed: 17728769]

Mansilla MA, Cooper ME, Goldstein T, Castilla EE, Lopez Camelo JS, Marazita ML, Murray JC. Contributions of PTCH gene variants to isolated cleft lip and palate. Cleft Palate Craniofac J. 2006; 43:21–29. [PubMed: 16405370]

Marazita ML. 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. Orthod Craniofac Res. 2007; 10:82–87. [PubMed: 17552944]

Marazita ML, Murray JC, Lidral AC, Arcos-Burgos M, Cooper ME, Goldstein T, Maher BS, Daack-Hirsch S, Schultz R, Mansilla MA, et al. Meta-analysis of 13 genome scans reveals multiple cleft lip/palate genes with novel loci on 9q21 and 2q32-35. Am J Hum Genet. 2004; 75:161–173. [PubMed: 15185170]

Marazita ML, Field LL, Cooper ME, Tobias R, Maher BS, Peanchitlertkajorn S, Liu YE. Genome scan for loci involved in cleft lip with or without cleft palate, in Chinese multiplex families. Am J Hum Genet. 2002; 71:349–364. [PubMed: 12087515]

Marazita ML, Lidral AC, Murray JC, Field LL, Maher BS, Goldstein McHenry T, Cooper ME, Govil M, Daack-Hirsch S, Riley B, et al. 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]

Marcano AC, Doudney K, Braybrook C, Squires R, Patton MA, Lees MM, Richieri-Costa A, Lidral AC, Murray JC, Moore GE, et al. TBX22 mutations are a frequent cause of cleft palate. J Med Genet. 2004; 41:68–74. [PubMed: 14729838]

Marie PJ, Coffin JD, Hurley MM. FGF and FGFR signaling in chondrodysplasias and craniosynostosis. J Cell Biochem. 2005; 96:888–896. [PubMed: 16149058]

Martin, E. Linkage Disequilibrium and Association Analysis. In: Haines, J.; Pericak-Vance, M., editors. Genetic Analysis of Complex Diseases. Hoboken, NJ: John Wiley & Sons, Inc; 2006. p. 329-349.

Martin RA, Hunter V, Neufeld-Kaiser W, Flodman P, Spence MA, Furnas D, Martin KA. Ultrasonographic detection of orbicularis oris defects in first degree relatives of isolated cleft lip patients. Am J Med Genet. 2000; 90:155–161. [PubMed: 10607956]

Mefford HC, Clauin S, Sharp AJ, Moller RS, Ullmann R, Kapur R, Pinkel D, Cooper GM, Ventura M, Ropers HH, et al. Recurrent reciprocal genomic rearrangements of 17q12 are associated with renal disease, diabetes, and epilepsy. Am J Hum Genet. 2007; 81:1057–1069. [PubMed: 17924346]

Meng L, Bian Z, Torensma R, Von den Hoff JW. Biological mechanisms in palatogenesis and cleft palate. J Dent Res. 2009; 88:22–33. [PubMed: 19131313]

Millicovsky G, Johnston MC. Hyperoxia and hypoxia in pregnancy: simple experimental manipulation alters the incidence of cleft lip and palate in CL/Fr mice. Proc Natl Acad Sci U S A. 1981; 78:5722–5723. [PubMed: 6946511]

Milunsky JM, Maher TA, Zhao G, Roberts AE, Stalker HJ, Zori RT, Burch MN, Clemens M, Mulliken JB, Smith R, et al. TFAP2A mutations result in branchio-oculo-facial syndrome. Am J Hum Genet. 2008; 82:1171–1177. [PubMed: 18423521]

Mitchell LE, Healey SC, Chenevix-Trench G. Evidence for an association between nonsyndromic cleft lip with or without cleft palate and a gene located on the long arm of chromosome 4. Am J Hum Genet. 1995; 57:1130–1136. [PubMed: 7485164]

Moreno LM, Mansilla MA, Bullard SA, Cooper ME, Busch TD, Machida J, Johnson MK, Brauer D, Krahn K, Daack-Hirsch S, et al. FOXE1 association with both isolated cleft lip with or without cleft palate, and isolated cleft palate. Hum Mol Genet. 2009a; 18:4879–4896. [PubMed: 19779022]

Moreno LM, Mansilla MA, Bullard SA, Cooper ME, Busch TD, Machida J, Johnson MK, Brauer D, Krahn K, Daack-Hirsch S, et al. FOXE1 Association with both Isolated Cleft Lip with or without Cleft Palate; and Isolated Cleft Palate. Hum Mol Genet. 2009b

Mossey, PA.; Little, J. Epidemiology of oral clefts: An international perspective. In: Wyszynski, D., editor. Cleft lip and palate: From origin to treatment. Oxford: Oxford University Press; 2002. p. 127-144.

Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. Lancet. 2009; 374:1773–1785. [PubMed: 19747722]

Munger, RG. Maternal nutrition and oral clefts. In: Wyszynski, DFE., editor. Cleft lip and palate: from origin to treatment. New York: Oxford University press; 2002.

Murcray CE, Lewinger JP, Gauderman WJ. Gene-environment interaction in genome-wide association studies. Am J Epidemiol. 2009; 169:219–226. [PubMed: 19022827]

Murray JC, Daack-Hirsch S, Buetow KH, Munger R, Espina L, Paglinawan N, Villanueva E, Rary J, Magee K, Magee W. Clinical and epidemiologic studies of cleft lip and palate in the Philippines. Cleft Palate Craniofac J. 1997; 34:7–10. [PubMed: 9003905]

Nawshad A, Hay ED. TGFbeta3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. J Cell Biol. 2003; 163:1291–1301. [PubMed: 14691138]

Neiswanger K, Weinberg SM, Rogers CR, Brandon CA, Cooper ME, Bardi KM, Deleyiannis FW, Resick JM, Bowen A, Mooney MP, et al. Orbicularis oris muscle defects as an expanded phenotypic feature in nonsyndromic cleft lip with or without cleft palate. Am J Med Genet A. 2007; 143:1143–1149. [PubMed: 17497721]

Neiswanger K, Chirigos KW, Klotz CM, Cooper ME, Bardi KM, Brandon CA, Weinberg SM, Vieira AR, Martin RA, Czeizel AE, et al. Whorl patterns on the lower lip are associated with nonsyndromic cleft lip with or without cleft palate. Am J Med Genet A. 2009; 149A:2673–2679. [PubMed: 19921634]

Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003; 31:3812–3814. [PubMed: 12824425]

Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, et al. Targeted capture and massively parallel sequencing of 12 human exomes. Nature. 2009; 461:272–276. [PubMed: 19684571]

Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, et al. Exome sequencing identifies the cause of a mendelian disorder. Nat Genet. 2010a; 42:30–35. [PubMed: 19915526]

Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet. 2010b

Nguyen RH, Wilcox AJ, Moen BE, McConnaughey DR, Lie RT. Parent's occupation and isolated orofacial clefts in Norway: a population-based case-control study. Ann Epidemiol. 2007; 17:763–771. [PubMed: 17664071]

Nie X, Luukko K, Kettunen P. FGF signalling in craniofacial development and developmental disorders. Oral Dis. 2006; 12:102–111. [PubMed: 16476029]

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; 4:1–9. [PubMed: 11839951]

Orioli IM, Vieira AR, Castilla EE, Ming JE, Muenke M. Mutational analysis of the Sonic Hedgehog gene in 220 newborns with oral clefts in a South American (ECLAMC) population. Am J Med Genet. 2002; 108:12–15. [PubMed: 11857543]

Ornitz DM, Marie PJ. FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. Genes Dev. 2002; 16:1446–1465. [PubMed: 12080084]

Osoegawa K, Vessere GM, Utami KH, Mansilla MA, Johnson MK, Riley BM, L'Heureux J, Pfundt R, Staaf J, van der Vliet WA, et al. Identification of novel candidate genes associated with cleft lip and palate using array comparative genomic hybridisation. J Med Genet. 2008; 45:81–86. [PubMed: 17873121]

Park JW, McIntosh I, Hetmanski JB, Jabs EW, Vander Kolk CA, Wu-Chou YH, Chen PK, Chong SS, Yeow V, Jee SH, et al. Association between IRF6 and nonsyndromic cleft lip with or without cleft palate in four populations. Genet Med. 2007; 9:219–227. [PubMed: 17438386]

Pauws E, Hoshino A, Bentley L, Prajapati S, Keller C, Hammond P, Martinez-Barbera JP, Moore GE, Stanier P. Tbx22null mice have a submucous cleft palate due to reduced palatal bone formation and also display ankyloglossia and choanal atresia phenotypes. Hum Mol Genet. 2009; 18:4171–4179. [PubMed: 19648291]

Pauws E, Stanier P. FGF signalling and SUMO modification: new players in the aetiology of cleft lip and/or palate. Trends Genet. 2007; 23:631–640. [PubMed: 17981355]

Pearson TA, Manolio TA. How to interpret a genome-wide association study. JAMA. 2008; 299:1335–1344. [PubMed: 18349094]

Pennacchio LA, Ahituv N, Moses AM, Prabhakar S, Nobrega MA, Shoukry M, Minovitsky S, Dubchak I, Holt A, Lewis KD, et al. In vivo enhancer analysis of human conserved non-coding sequences. Nature. 2006; 444:499–502. [PubMed: 17086198]

Peterka M, Tvrdek M, Likovsky Z, Peterkova R, Fara M. Maternal hyperthermia and infection as one of possible causes of orofacial clefts. Acta Chir Plast. 1994; 36:114–118. [PubMed: 7610756]

Prescott NJ, Lees MM, Winter RM, Malcolm S. Identification of susceptibility loci for nonsyndromic cleft lip with or without cleft palate in a two stage genome scan of affected sib-pairs. Hum Genet. 2000; 106:345–350. [PubMed: 10798365]

Pritchard JK, Cox NJ. The allelic architecture of human disease genes: common disease-common variant…or not? Hum Mol Genet. 2002; 11:2417–2423. [PubMed: 12351577]

Pritchard JK. Are rare variants responsible for susceptibility to complex diseases? Am J Hum Genet. 2001; 69:124–137. [PubMed: 11404818]

Proetzel G, Pawlowski SA, Wiles MV, Yin M, Boivin GP, Howles PN, Ding J, Ferguson MW, Doetschman T. Transforming growth factor-beta 3 is required for secondary palate fusion. Nat Genet. 1995; 11:409–414. [PubMed: 7493021]

Radhakrishna U, Ratnamala U, Gaines M, Beiraghi S, Hutchings D, Golla J, Husain SA, Gambhir PS, Sheth JJ, Sheth FJ, et al. Genomewide scan for nonsyndromic cleft lip and palate in multigenerational Indian families reveals significant evidence of linkage at 13q33.1–34. Am J Hum Genet. 2006; 79:580–585. [PubMed: 16909398]

Rahimov F, Marazita ML, Visel A, Cooper ME, Hitchler MJ, Rubini M, Domann FE, Govil M, Christensen K, Bille C, et al. Disruption of an AP-2alpha binding site in an IRF6 enhancer is associated with cleft lip. Nat Genet. 2008; 40:1341–1347. [PubMed: 18836445]

Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res. 2002; 30:3894–3900. [PubMed: 12202775]

Reutter H, Birnbaum S, Mende M, Lauster C, Schmidt G, Henschke H, Saffar M, Martini M, Lauster R, Schiefke F, et al. TGFB3 displays parent-of-origin effects among central Europeans with nonsyndromic cleft lip and palate. J Hum Genet. 2008; 53:656–661. [PubMed: 18480962]

Rice R, Spencer-Dene B, Connor EC, Gritli-Linde A, McMahon AP, Dickson C, Thesleff I, Rice DP. Disruption of Fgf10/Fgfr2b-coordinated epithelial-mesenchymal interactions causes cleft palate. J Clin Invest. 2004; 113:1692–1700. [PubMed: 15199404]

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; 38:1329–1334. [PubMed: 17041603]

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. 2007; 143A:3228–3234. [PubMed: 17963255]

Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, Russo ET, Vieira AR, Dode C, Mohammadi M, et al. Impaired FGF signaling contributes to cleft lip and palate. Proc Natl Acad Sci USA. 2007; 104:4512–4517. [PubMed: 17360555]

Roessler E, Belloni E, Gaudenz K, Jay P, Berta P, Scherer SW, Tsui LC, Muenke M. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. Nat Genet. 1996; 14:357–360. [PubMed: 8896572]

Roessler E, Du YZ, Mullor JL, Casas E, Allen WP, Gillessen-Kaesbach G, Roeder ER, Ming JE, Ruiz i Altaba A, Muenke M. Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. Proc Natl Acad Sci U S A. 2003; 100:13424–13429. [PubMed: 14581620]

Sadler, TW. Langman's medical embryology. Philadelphia: Lippincott Williams & Wilkins; 2006. Head and Neck; p. 257-284.

Sander A, Schmelzle R, Murray J. Evidence for a microdeletion in 1q32-41 involving the gene responsible for Van der Woude syndrome. Hum Mol Genet. 1994; 3:575–578. [PubMed: 8069301]

Satokata I, Maas R. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. Nat Genet. 1994; 6:348–356. [PubMed: 7914451]

Scapoli L, Palmieri A, 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; 76:180–183. [PubMed: 15558496]

Schorle H, Meier P, Buchert M, Jaenisch R, Mitchell PJ. Transcription factor AP-2 essential for cranial closure and craniofacial development. Nature. 1996; 381:235–238. [PubMed: 8622765]

Shahrukh Hashmi S, Gallaway MS, Waller DK, Langlois PH, Hecht JT. Maternal fever during early pregnancy and the risk of oral clefts. Birth Defects Res A Clin Mol Teratol. 2010; 88:186–194. [PubMed: 20099315]

Shaw GM, Wasserman CR, Murray JC, Lammer EJ. Infant TGF-alpha genotype, orofacial clefts, and maternal periconceptional multivitamin use. Cleft Palate Craniofac J. 1998; 35:366–370. [PubMed: 9684776]

Shaw GM, Wasserman CR, Lammer EJ, O'Malley CD, Murray JC, Basart AM, Tolarova MM. Orofacial clefts, parental cigarette smoking, and transforming growth factor-alpha gene variants. Am J Hum Genet. 1996; 58:551–561. [PubMed: 8644715]

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; 84:16–29. [PubMed: 18383123]

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; 80:76–90. [PubMed: 17160896]

Shi M, Umbach DM, Weinberg CR. Testing haplotype-environment interactions using case-parent triads. Hum Hered. 2010; 70:23–33. [PubMed: 20413979]

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; 85:42–51. [PubMed: 19137569]

Shiang R, Lidral AC, Ardinger HH, Buetow KH, Romitti PA, Munger RG, Murray JC. Association of transforming growth-factor alpha gene polymorphisms with nonsyndromic cleft palate only (CPO). Am J Hum Genet. 1993; 53:836–843. [PubMed: 8105683]

Sivertsen A, Wilcox AJ, Skjaerven R, Vindenes HA, Abyholm F, Harville E, Lie RT. Familial risk of oral clefts by morphological type and severity: population based cohort study of first degree relatives. Bmj. 2008a; 336:432–434. [PubMed: 18250102]

Sivertsen A, Wilcox AJ, Skjaerven R, Vindenes HA, Abyholm F, Harville E, Lie RT. Familial risk of oral clefts by morphological type and severity: population based cohort study of first degree relatives. Bmj. 2008b

Sobreira NL, Cirulli ET, Avramopoulos D, Wohler E, Oswald GL, Stevens EL, Ge D, Shianna KV, Smith JP, Maia JM, et al. Whole-genome sequencing of a single proband together with linkage analysis identifies a Mendelian disease gene. PLoS Genet. 2010; 6:e1000991. [PubMed: 20577567]

Song T, Li G, Jing G, Jiao X, Shi J, Zhang B, Wang L, Ye X, Cao F. SUMO1 polymorphisms are associated with non-syndromic cleft lip with or without cleft palate. Biochem Biophys Res Commun. 2008; 377:1265–1268. [PubMed: 18983974]

Soriano P. The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. Development. 1997; 124:2691–2700. [PubMed: 9226440]

Sozen MA, Suzuki K, Tolarova MM, Bustos T, Fernandez Iglesias JE, Spritz RA. Mutation of PVRL1 is associated with sporadic, non-syndromic cleft lip/palate in northern Venezuela. Nat Genet. 2001; 29:141–142. [PubMed: 11559849]

Srichomthong C, Siriwan P, Shotelersuk V. Significant association between IRF6 820G->A and non-syndromic cleft lip with or without cleft palate in the Thai population. J Med Genet. 2005; 42:e46. [PubMed: 15994871]

Stanier P, Moore GE. Genetics of cleft lip and palate: syndromic genes contribute to the incidence of non-syndromic clefts. Hum Mol Genet. 2004; 13(Spec No 1):R73–81. [PubMed: 14722155]

Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. JAMA. 2009; 301:636–650. [PubMed: 19211471]

Suazo J, Santos JL, Scapoli L, Jara L, Blanco R. Association Between TGFB3 and Nonsyndromic Cleft Lip With or Without Cleft Palate in a Chilean Population. Cleft Palate Craniofac J. 2010a; 47:513–517. [PubMed: 20170386]

Suazo J, Santos JL, Jara L, Blanco R. Association between bone morphogenetic protein 4 gene polymorphisms with nonsyndromic cleft lip with or without cleft palate in a chilean population. DNA Cell Biol. 2010b; 29:59–64. [PubMed: 19839778]

Sull JW, Liang KY, Hetmanski JB, Wu T, Fallin MD, Ingersoll RG, Park JW, Wu-Chou YH, Chen PK, Chong SS, et al. Evidence that TGFA influences risk to cleft lip with/without cleft palate through unconventional genetic mechanisms. Hum Genet. 2009; 126:385–394. [PubMed: 19444471]

Sun D, Vanderburg CR, Odierna GS, Hay ED. TGFbeta3 promotes transformation of chicken palate medial edge epithelium to mesenchyme in vitro. Development. 1998; 125:95–105. [PubMed: 9389667]

Sun D, Baur S, Hay ED. Epithelial-mesenchymal transformation is the mechanism for fusion of the craniofacial primordia involved in morphogenesis of the chicken lip. Dev Biol. 2000; 228:337–349. [PubMed: 11112334]

Suphapeetiporn K, Tongkobpetch S, Siriwan P, Shotelersuk V. TBX22 mutations are a frequent cause of non-syndromic cleft palate in the Thai population. Clin Genet. 2007; 72:478–483. [PubMed: 17868388]

Suzuki K, Hu D, Bustos T, Zlotogora J, Richieri-Costa A, Helms JA, Spritz RA. Mutations of PVRL1, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. Nat Genet. 2000; 25:427–430. [PubMed: 10932188]

Suzuki S, Marazita ML, Cooper ME, Miwa N, Hing A, Jugessur A, Natsume N, Shimozato K, Ohbayashi N, Suzuki Y, et al. Mutations in BMP4 are associated with subepithelial, microform, and overt cleft lip. Am J Hum Genet. 2009; 84:406–411. [PubMed: 19249007]

Suzuki Y, Jezewski PA, Machida J, Watanabe Y, Shi M, Cooper ME, Viet le T, Nguyen TD, Hai H, Natsume N, et al. In a Vietnamese population, MSX1 variants contribute to cleft lip and palate. Genet Med. 2004; 6:117–125. [PubMed: 15354328]

The Treacher Collins Syndrome Collaborative Group. Positional cloning of a gene involved in the pathogenesis of Treacher Collins syndrome. Nat Genet. 1996; 12:130–136. [PubMed: 8563749]

Thomas D. Gene-environment-wide association studies: emerging approaches. Nat Rev Genet. 2010; 11:259–272. [PubMed: 20212493]

Tolarova MM, Cervenka J. Classification and birth prevalence of orofacial clefts. Am J Med Genet. 1998; 75:126–137. [PubMed: 9450872]

Tongkobpetch S, Siriwan P, Shotelersuk V. MSX1 mutations contribute to nonsyndromic cleft lip in a Thai population. J Hum Genet. 2006; 51:671–676. [PubMed: 16868654]

Trokovic N, Trokovic R, Mai P, Partanen J. Fgfr1 regulates patterning of the pharyngeal region. Genes Dev. 2003; 17:141–153. [PubMed: 12514106]

Trumpp A, Depew MJ, Rubenstein JL, Bishop JM, Martin GR. Cre-mediated gene inactivation demonstrates that FGF8 is required for cell survival and patterning of the first branchial arch. Genes Dev. 1999; 13:3136–3148. [PubMed: 10601039]

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]

Vanderas AP. Incidence of cleft lip, cleft palate, and cleft lip and palate among races: a review. Cleft Palate J. 1987; 24:216–225. [PubMed: 3308178]

Vaziri Sani F, Hallberg K, Harfe BD, McMahon AP, Linde A, Gritli-Linde A. Fate-mapping of the epithelial seam during palatal fusion rules out epithelial-mesenchymal transformation. Dev Biol. 2005; 285:490–495. [PubMed: 16109396]

Venza M, Visalli M, Venza I, Torino C, Saladino R, Teti D. FOXE1 gene mutation screening by multiplex PCR/DHPLC in CHARGE syndrome and syndromic and non-syndromic cleft palate. J Chromatogr B Analyt Technol Biomed Life Sci. 2006; 836:39–46.

Vieira AR, McHenry TG, Daack-Hirsch S, Murray JC, Marazita ML. A genome wide linkage scan for cleft lip and palate and dental anomalies. Am J Med Genet A. 2008; 146A:1406–1413. [PubMed: 18442096]

Vieira AR, Avila JR, Daack-Hirsch S, Dragan E, Felix TM, Rahimov F, Harrington J, Schultz RR, Watanabe Y, Johnson M, et al. Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. PLoS Genet. 2005; 1:e64. [PubMed: 16327884]

Visel A, Minovitsky S, Dubchak I, Pennacchio LA. VISTA Enhancer Browser--a database of tissue-specific human enhancers. Nucleic Acids Res. 2007; 35:D88–92. [PubMed: 17130149]

Vissers LE, Veltman JA, van Kessel AG, Brunner HG. Identification of disease genes by whole genome CGH arrays. Hum Mol Genet. 2005; 14(Spec No 2):R215–223. [PubMed: 16244320]

Warrington A, Vieira AR, Christensen K, Orioli IM, Castilla EE, Romitti PA, Murray JC. Genetic evidence for the role of loci at 19q13 in cleft lip and palate. J Med Genet. 2006; 43:e26. [PubMed: 16740910]

Watanabe A, Akita S, Tin NT, Natsume N, Nakano Y, Niikawa N, Uchiyama T, Yoshiura K. A mutation in RYK is a genetic factor for nonsyndromic cleft lip and palate. Cleft Palate Craniofac J. 2006; 43:310–316. [PubMed: 16681403]

Wehby GL, Cassell CH. The impact of orofacial clefts on quality of life and healthcare use and costs. Oral Dis. 2010; 16:3–10. [PubMed: 19656316]

Weinberg CR. Less is more, except when less is less: Studying joint effects. Genomics. 2009; 93:10–12. [PubMed: 18598750]

Weinberg SM, Neiswanger K, Martin RA, Mooney MP, Kane AA, Wenger SL, Losee J, Deleyiannis F, Ma L, De Salamanca JE, et al. The Pittsburgh Oral-Facial Cleft study: expanding the cleft phenotype. Background and justification. Cleft Palate Craniofac J. 2006; 43:7–20. [PubMed: 16405378]

Weinberg SM, 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; 12:271–281. [PubMed: 19840279]

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 A. 2008; 146A:409–420. [PubMed: 18203157]

Wilcox AJ, 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; 334:464. [PubMed: 17259187]

Yazdy MM, Honein MA, Rasmussen SA, Frias JL. Priorities for future public health research in orofacial clefts. Cleft Palate Craniofac J. 2007; 44:351–357. [PubMed: 17608558]

Zeiger JS, Beaty TH, Liang KY. Oral clefts, maternal smoking, and TGFA: a meta-analysis of gene-environment interaction. Cleft Palate Craniofac J. 2005; 42:58–63. [PubMed: 15643916]

Zeiger, JS.; Beaty, TH. Gene-environment interaction and risk to oral clefts. In: Wyszynski, DFE., editor. Cleft lip and palate: from origin to treatment. New York: Oxford University press; 2002.

Zhang FP, Mikkonen L, Toppari J, Palvimo JJ, Thesleff I, Janne OA. Sumo-1 function is dispensable in normal mouse development. Mol Cell Biol. 2008; 28:5381–5390. [PubMed: 18573887]

Zhu J, Hao L, Li S, Bailey LB, Tian Y, Li Z. MTHFR, TGFB3, and TGFA polymorphisms and their association with the risk of non-syndromic cleft lip and cleft palate in China. Am J Med Genet A. 2010; 152A:291–298. [PubMed: 20082468]

Zucchero TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, Caprau D, Christensen K, Suzuki Y, Machida J, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. N Engl J Med. 2004; 351:769–780. [PubMed: 15317890]

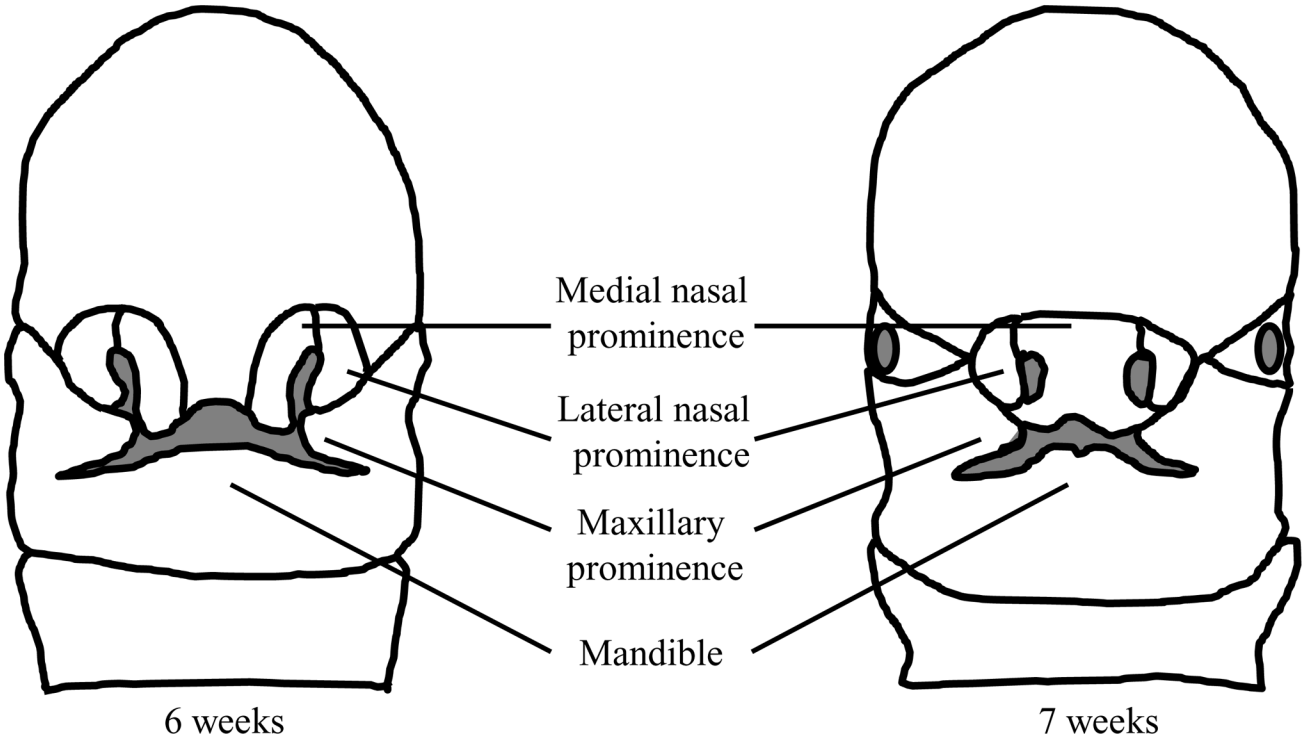

Figure 1. Development of the orofacial structures

The medial nasal prominences enlarge and merge with each other during the 6^{th}–7^{th} weeks of gestation to form the intermaxillary segment providing the basis for both the philtrum and primary palate. The intermaxillary segment fuses with the flanking maxillary prominences giving rise to the upper lip. The lateral nasal prominences form the sides of the nose. The paired bilateral maxillary prominences form the upper jaw, and the paired mandibular prominences develop into the lower jaw. Adapted from (Helms et al., 2005).

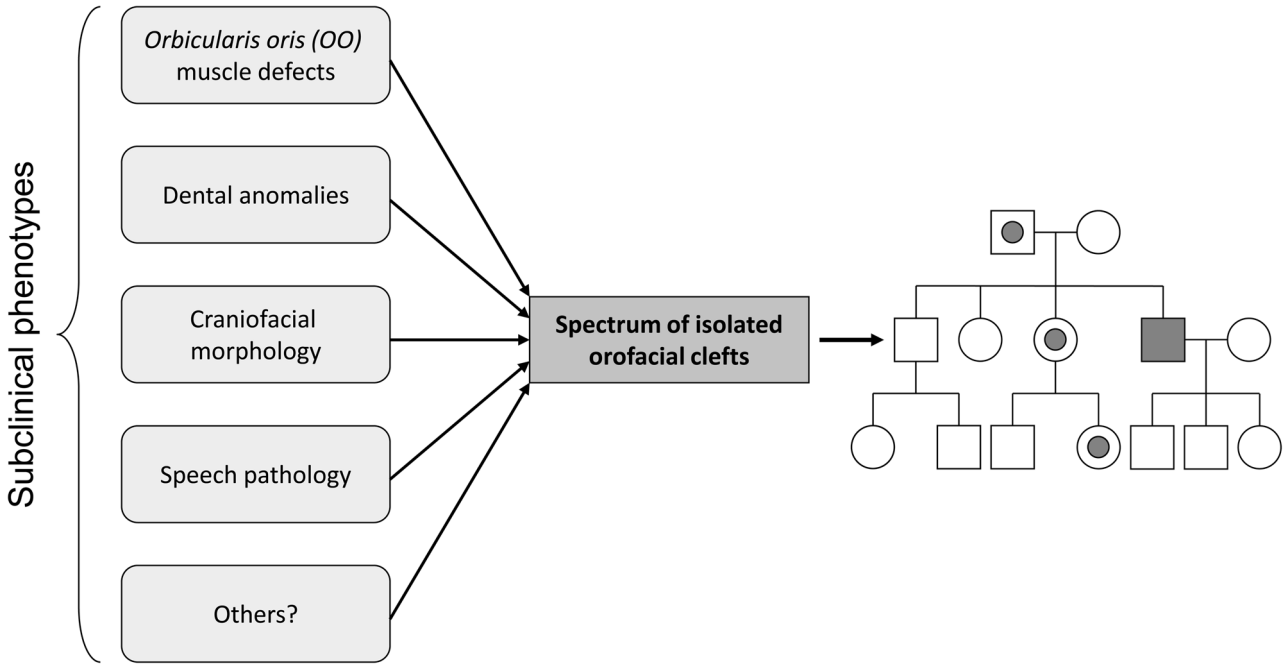

Figure 2. Refining the isolated orofacial cleft phenotype for enhanced linkage analysis

Subclinical features (or 'subphenotypes') such as defects in the muscle that surrounds the mouth (*Orbicularis Oris*), dental anomalies, craniofacial morphology and speech-related disorders may be part of the overall spectrum of isolated clefting. Therefore, their inclusion in classic linkage approaches may boost the search for causal genetic variants. "Others?" in the figure refers to additional subphenotypes, such as variations in brain morphology; lip pits/prints, dermatoglyphic patterns, etc. A circle inside a pedigree symbol refers to an apparently 'unaffected' individual who harbors at least one of the subphenotypes and is therefore categorized as a potential gene-carrier; filled symbols refer to individuals with an overt cleft.

Table 1

Review of GWA studies of nonsyndromic orofacial clefts.

Study	Study population and origin	Study design	Genotyping platform	Locus/gene with hit	Candidate gene in region*	Most significant SNP	P-value associated with SNP	OR/RR (95% CI)	Attributable risk (AR) for SNP	Results*
Birnbaum *et al.* 2009	Discovery sample: 224 NS CL/P cases and 383 controls of Central European Origin; Confirmation sample: 462 NS CL/P cases and 954 controls. SNP association study: 295 NS CPO cases.	Case-control; case-parent trio	Illumina BeadChip HumanHap550k	8q24.21	*PVT1; GSDMC; CCDC26*	rs987525	3.34×10^{-24}	OR=2.57 (2.02–3.26) for heterozygous genotype; OR=6.05 (3.88–9.43) for homozygous genotype.	41%	No association detected between rs987525 and NS CPO ($P = 0.788$).
Grant *et al.* 2009	111 NS CL/P cases and 5951 controls from the US, of European descent.	Case-control	Illumina Infinium II HumanHap550k BeadChip	8q24 18q22	None suggested	rs987525 on 8q24; rs17085106 on 18q22	9.18×10^{-8}; 3.78×10^{-8}	OR=2.09 (1.59–2.76) for rs987525; OR=4.07 (2.37–7.00) for rs17085106.	NA	Like the 8q24 signal, the 18q22 signal also resides in a gene-poor region. No association found with *IRF6*.
Mangold *et al.* 2010	401 NS CL/P cases and 1323 controls of Central European origin.	Case-control; Case-parent trio	Illumina BeadChips (Human610-Quad and HumanHap550k)	17q22 10q25.3	*NOG* (17q22); *KIAA1598* and *VAX1* (10q25.3)	rs227731 on 17q22; rs7078160 on 10q25.3	1.07×10^{-8}; 1.92×10^{-8}	RR=1.84 (1.34–2.53) for rs227731 in homozygotes; RR=2.17 (1.32–3.56) for rs7078160 in homozygotes.	23.9% for rs227731; 12.3% for rs7078160; joint population AR=54.6%	The study population is an extension of the Birnbaum et al. 2009 study. 177 additional NS CL/P cases and 940 controls were genotyped. Three was suggestive evidence of association with three other loci at 13q31.1, 15q13.3, and 2p21, respectively.
Beaty *et al.* 2010	NS CLO and NS CLP trios from Europe, US, China, Taiwan, Singapore, Korea and the Philippines. For specific numbers, see Supplementary Table 1 of the article.	Case-parent trio	Illumina Human660W-Quad v1 DNA Analysis BeadChip Kit	8q24 1q32 20q12 1p22.1	*IRF6* (1q32); *MAFB* (20q12); *ABCA4* (1p22.1)	rs13041247 in *MAFB*; rs560426 in *ABCA4*	1.44×10–11 for rs13041247; 5.01×10–12 for rs560426	OR per minor allele=0.70 (0.64–0.78) for rs13041247 and 1.43 (1.29–1.59) for rs560426.		Three genes (*PAX7* on 1p36, *VAX1* on 10q25.3, and *NTN1* on 17p13) had one or more SNPs near genome-wide significance.

*Gene acronyms from Entrez Gene (http://www.ncbi.nlm.nih.gov/sites/gene): *PVT1*, Pvt1 oncogene (non-protein coding); *GSDMC*, gasdermin C; *CCDC26*, coiled-coil domain containing 26; *NOG*, noggin; *KIAA1598*, not available; *IRF6*, interferon regulatory factor 6; *MAFB*, v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian); *ABCA4*, ATP-binding cassette, subfamily A (ABC1), member 4; *PAX7*, paired box 7; *VAX1*, ventral anterior homeobox 1; *NTN1*, netrin 1

Table 2

Genes implicated in nonsyndromic cleft lip with or without cleft palate (NS CL/P)

Gene	Chromosomal location	Linkage	Association	Mutations	Deletions	Animal models
IRF6	1q32.2	+	+	−	−	+
FOXE1	9q22.3	+	+	+	−	+
MSX1	4p16.2	−	+	+	−	+
FGFR1	8p12	−	−	+	+	+
BMP4	14q22.2	−	−	+	−	+
SUMO1	2q33.1	−	−	−	+	+
TBX22	Xq21.1	−	+	+	−	−
TP63	3q28	−	−	+	−	+
PVRL1	11q23.3	+	+	+	−	−
TGFB3	14q24.3	−	+	−	−	+
TGFA	2p13.3	−	+	−	−	−
TFAP2A	6p24.3	−	−	−	+	+
CRISPLD2	16q24.1	−	+	−	−	−
RYK	3q22.1	−	−	+	−	+
TBX10	11q13.2	−	−	+	−	+
SPRY2	13q31.1	−	−	+	−	+
GABRB3	15q12	−	+	−	−	+

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