B-ENT, 2015, **11**, 173-182

Review: Facial endophenotypes in non-syndromic orofacial clefting

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Key-words. Endophenotype; cleft; lip; palate; genetics; orofacial

Abstract. *Review: Facial endophenotypes in non-syndromic orofacial clefting*. Cleft lip and/or palate (CL/P) is one of the most frequent congenital malformations, with a frequency of 1 in 700 live births. Non-syndromic orofacial clefting is a multifactorial condition, with both a genetic and an environmental component. Although numerous studies have been published addressing the genetic etiology of CL/P, this factor remains incompletely understood. A promising approach to find candidate gene regions for CL/P is the investigation of endophenotypes, which are characteristics associated with a certain condition and that can be an expression of underlying susceptibility genes. This review focuses on the known facial endophenotypes in CL/P (such as distortion of the orbicularis oris muscle and facial features in non-affected relatives of patients with CL/P) and genes that could be associated with these characteristics. Possibilities for further endophenotype-related studies in the field of non-syndromic CL/P are discussed.

Introduction

Orofacial clefts are among the most common birth defects, with a prevalence of approximately 1 in 700 live births.[1] Orofacial clefts can be responsible for major social and psychological burden in the lives of the patients and their families and require a long-term and multidisciplinary follow-up, including several surgical procedures, orthodontics, and speech therapy.[2,3] Non-syndromic orofacial clefting is considered to be multifactorial, with both genetic and environmental factors contributing to its etiology. This dual contribution is often described using a threshold model: The condition arises when the combined genetic and environmental predisposing factors exceed a certain threshold.[4] Orofacial clefts can be subdivided into two classes with different prevalences and etiologies: cleft lip with or without cleft palate (CL/P) and isolated cleft palate (CP) (Figure 1). The prevalence of CP is slightly lower (5 per 10,000 births) compared that of CL/P (6.64 per 10,000 births), and CP is more often part of a syndromic diagnosis (50% versus 30% of CL/P).[2,5] The prevalence of orofacial clefts is widely variable across geographic regions and ethnic groups.[5] Rates of CL/P are especially high in Latin America and Asia and lower in Israel, South Africa, and Southern Europe. CP, on the other hand, is more prevalent in Canada and parts of Northern Europe and less so in parts of Latin America and South Africa. CL/P and CP presumably have different etiologies because lip closure and palatal closure occur during different embryonic developmental stages.[5] At the end of the sixth week, the medial nasal processes merge with each other and with the maxillary processes. This merging leads to the formation of the upper lip and primary palate. The palatal shelves do not fuse until the seventh week of embryonic development, giving rise to the hard and soft palates.[2] Furthermore, CL/P is more frequent among males while CP occurs more often in females. However, sometimes CL/P and CP arise within the same pedigree, indicating some possible overlap in etiology.[6] In addition, Ludwig et al. and Moreno et al. found an association between variations in *FOXE1* and the occurrence of both CL/P and CP.[7,8] Finally, variations in *IRF6* and *MSX* genes are associated with both cleft types.[6,9]

Figure 1
Orofacial clefting is a clinically heterogeneous condition. (A) Left to right, unilateral to bilateral cleft lip. (B) (left to right) Unilateral cleft lip with cleft palate, bilateral cleft lip with cleft palate, and isolated cleft palate.

Several environmental influences (such as maternal smoking, alcohol consumption, obesity, fever) play a role in the etiology of orofacial clefting. This review, however, will focus mainly on the genetic contributions. Improved knowledge about the etiology of orofacial clefting may lead to more effective and personalized genetic counseling and give new insights into preventive and therapeutic measures,[10] including bringing extra attention to environmental influences in individuals who are considered to be genetically at risk.[11]

Orofacial clefts are a consequence of the failure of complex craniofacial developmental processes. Therefore, variations in genes regulating these processes are likely (partly) responsible for orofacial clefting. These developmental events occur in different stages and levels of the embryonic fusion of the facial prominences, which explains the broad variety of characteristics in orofacial clefting.[6] An underlying genetic etiology can be assumed because non-syndromic CL/P and CP have a high rate of familial recurrence (3-5%, depending on the number of affected family members and the severity of the defect).[12] Furthermore, the concordance rate is higher in monozygotic twins (60%) compared to dizygotic twins (10%).[13]

The term 'phenotype' describes the composite of a person's observable characteristics. Sometimes phenotypic features are associated with a condition without being a deviation from the standard. Endophenotypes are characteristics (behavioral or anatomical) associated with a condition that are also present in non-affected family members. They are considered an expression of underlying susceptibility genes for the condition. Leboyer *et al*. first mentioned the concept of endophenotypes in a publication on the genetics of psychiatric disorders.[14] Traits that show the following characteristics can be classified as an endophenotype:[15]

1. The endophenotype is associated with a condition in the population.
2. The endophenotype is inheritable.
3. The endophenotype is primarily state independent (manifests in an individual independent of the condition).
4. The endophenotype and the condition co-segregate within a family.
5. The endophenotype has a higher prevalence in non-affected family members compared to the general population.

A typical example of an endophenotype in a psychiatric setting is pursuit eye tracking in patients

with schizophrenia. These individuals have trouble pursuing very fast targets, which is also noted in their non-affected relatives.[16]

Because the endophenotype lies on the path from genotype to phenotype, it can be the key to a more straightforward genetic analysis and an indication to include certain genes in linkage and association studies through a candidate symptom approach. The gene that is responsible for the endophenotype can be causal for the CL/P phenotype or can be in linkage disequilibrium with (one of) the genes responsible for the condition. The endophenotype can thus help to identify candidate locations for disease-specific genes.[14] It is important, however, to keep in mind that endophenotypes presumably also have a polygenic basis, which makes genetic analysis complicated.[15] Several endophenotypes have been described in patients with non-syndromic CL/P and CP and their non-affected first-degree relatives.

This review focuses on progress in genetic studies on CL/P and CP through research on facial endophenotypes. We performed a PubMed literature search using the keywords 'facial', 'endophenotype', 'cleft lip', 'palate', and 'genetics'. These keywords were used in different combinations to obtain a complete overview of the literature.

Facial endophenotypes

Most studies on cleft-related endophenotypes have focused on subtle variations in facial morphology in non-affected relatives. These studies have indeed shown the presence of specific facial characteristics in non-affected relatives of patients with CL/P (see below). Furthermore, differences between relatives of CL/P patients and controls have been observed in the anatomy of the orbicularis oris muscle, nasolabial fold continuity, and occurrence of lip whorls. A detailed description of these facial endophenotypes follows.

Anatomy of the orbicularis oris muscle

The orbicularis oris muscle anatomy is obviously deviated in CL/P patients as a result of the cleft lip. In minor incomplete clefts (microform clefts), the orbicularis oris muscle is interrupted or narrowed without distortion, with intact overlying lip skin and mucosa, as De Mey *et al*. showed using histological sections.[17] The degree of alar deformity is associated with the severity of the orbicularis oris muscle distortion. In complete clefts, there is a complete interruption of the orbicularis oris muscle. This finding is not surprising because orbicularis oris muscle interruption arises from defects in the development of mesodermally derived maxillary processes and thus fits completely in the CL/P developmental spectrum. More striking, Martin *et al*. showed that subclinical orbicularis oris muscle defects, as demonstrated by ultrasound, are also present in non-affected first-degree relatives of patients with a cleft lip (Figure 2).[18] This feature was confirmed by Neiswanger *et al*.,[19] who detected anatomical orbicularis oris muscle discontinuities in 10.3% of non-affected first-degree relatives of non-syndromic CL/P patients, compared to 5.8% of controls.[19] The relatives showed no overt signs of CL/P. This difference was especially notable in male relatives compared to male controls. The possibility of a factor present in females that enhances lip fusion or healing of the cleft lip in utero could explain sex differences. Marazita *et al*. described an increase in potentially damaging mutations in *BMP4* (which plays a possible role in rescuing the cleft phenotype in utero) in relatives and patients with orbicularis oris muscle defects.[20] The subepithelial orbicularis oris muscle defect fulfills all criteria of a subclinical endophenotype. When defects in the orbicularis oris muscle can be routinely determined, high- and low-risk families or individuals in the family can be identified, leading to more personalized genetic counseling.

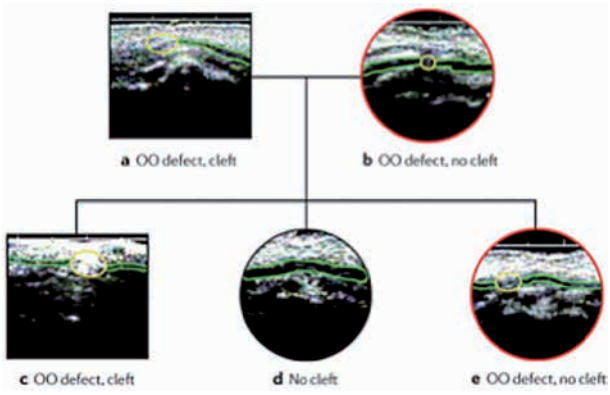

Figure 2
Ultrasound images from the upper lip of patients with non-syndromic CL/P and their non-affected first-degree relatives, investigated by Dixon *et al*.[4] Disruption of the orbicularis oris muscle (indicated in green) is seen in two non-affected first-degree relatives (indicated in red).

Nasolabial fold discontinuity during speech

Schmidt *et al*. studied the movement of soft tissue in the midfacial region in patients with CL/P and non-affected first-degree relatives compared to controls.[21] They hypothesized that subclinical defects in the orbicularis oris muscle would affect not only the muscle itself but also the interaction with other facial muscles and the skin. This influence could cause appearance-related changes such as furrowing, dimpling, and nasal flaring. More nasolabial fold discontinuity was indeed found in CL/P patients and non-affected first-degree relatives with a defect in the orbicularis oris muscle, compared to controls. In patients with unilateral CL/P, nasolabial fold discontinuity was detected on both the cleft side and the opposite side. The authors suggested that orbicularis oris muscle defects and nasolabial fold discontinuity arise from the same genetic origin but have different effects on the soft tissue of the midface.

Whorl patterns on the lower lip

Although whorl lip print patterns (circular grooves on the lip) are a typical symptom of the autosomal-dominant Van der Woude syndrome (OMIM: 119300), these patterns are also significantly more often noted in patients with non-syndromic CL/P and their relatives compared to unrelated controls (Figure 3).[22] Lip prints are, just like dermatoglyphic patterns, unique to an individual and constant over time. Furthermore, *IRF6* is a good candidate gene for causing these lower lip whorls because variations in *IRF6* are responsible for Van der Woude syndrome.[22] Variations in *IRF6* are not sufficient to cause Van der Woude syndrome, but it is likely that they give rise to the existence of lower lip whorls and therefore play a role in the underlying genetics of non-syndromic CL/P.

Facial morphology

The craniofacial phenotype in CL/P and CP is probably more complex than the phenotype directly related to the cleft and the result of secondary changes arising from early facial surgical procedures and orthodontics. The notion that certain facial features are predictive for an offspring with CL/P is not new; this hypothesis was first investigated by Fraser *et al*. in 1970.[23] Many of the genes responsible for the formation of the face are also candidate genes for orofacial clefting. However, cephalometric and direct anthropometric studies have yielded variable and contradictory results.[24] Advances in non-invasive three-dimensional (3D) surface imaging have moved the study of facial morphology to a new level (Figure 4). Using this technology, Weinberg *et al*. showed facial differences in non-affected parents of multiplex cleft families, differences that included a flattening of the facial profile (midface retrusion), excess interorbital width, excess upper face and cranial base width, increased lower face height, and reduced upper facial height.[25] Increased nasal cavity width is the strongest trait in non-affected relatives of patients with CL/P.[26] It has been hypothesized

Figure 3
Whorl patterns on the lower lip of a patient with non-syndromic CL/P, as described by Neiswanger *et al*.[22] Lip-prints obtained using the Faurot inkless fingerprinting system (Faurot Forensic Products, Inc., Raleigh, NC).

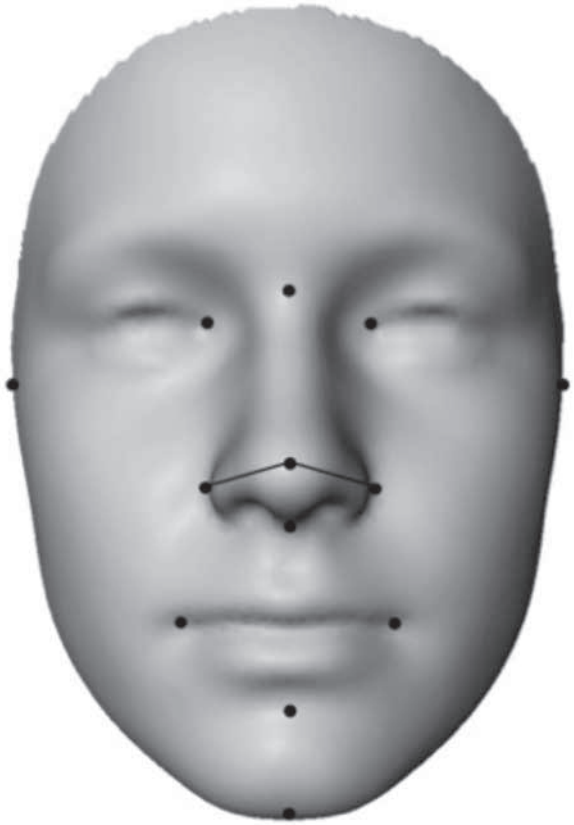

Figure 4
Average face with nine landmarks indicated. Distances between landmarks are used to describe facial differences between study groups. In this figure, the lines are used to measure dimensions of the nose.

that excess width of the embryonic face can lead to clefting because the greater width hampers the merging of facial prominences. However, these 3D studies of cleft-related facial characteristics are based on an analysis of only a limited number of predetermined points on the face, rendering the technique relatively insensitive for measuring the full scope of subtle endophenotypic facial traits. Facial growth and normal facial variation are also determined by several candidate CL/P genes, such as *BMP4*, *MSX1*, *SHH*, *CCDC26*, *GREM1*, and *FGF8*.[27] Of importance, Miller *et al*. characterized the facial shape in non-affected first-degree relatives and found an association between these facial features and single nucleotide polymorphisms (SNPs) in *IRF6* and *ABCA4-ARHGAP29*.[28] Thus, craniofacial shape in parents can be an endophenotype for orofacial clefting, but the relationship between facial shape and the underlying genotype is very complex.[29]

Genetic basis of facial endophenotypes

IRF6

IRF6, a transcription factor, is the only gene with a proven function in the etiology of non-syndromic CL/P. *IRF6* is strongly expressed in the ectoderm of the palatal shelves prior to the formation of the secondary palate.[30] Loss-of-function variations in *IRF6* cause the autosomal-dominant Van der Woude and popliteal pterygium (OMIM: 119500) syndromes. The clefting phenotype of Van der Woude syndrome is comparable to non-syndromic CL/P and CP, although these syndromic patients can often be identified by the presence of lip whorls. Because whorls in the lower lip are also noted in some patients with non-syndromic CL/P, variations in *IRF6* likely are responsible for this endophenotype.[22] The *IRF6* SNP rs2013162 shows a significant correlation with non-syndromic CL/P,[31] and rs2235543 plays a role in craniofacial development, especially in the retrusion of the forehead and profile convexity.[28]

ABCA4/ARHGAP29

ABCA4 and the more downstream *ARHGAP29* are involved in the development of the retina and in normal craniofacial development,[28] and the *ARHGAP29/ABCA4* pathway is likely to interact with the IRF6 pathway. Therefore, these genes possibly play a role in the etiology of CL/P or CP and could be among the causal genes for the specific facial shape in parents of patients with CL/P or CP. Furthermore, Leslie *et al*. found more orbicularis oris muscle defects in non-affected relatives of patients with CL/P carrying SNPs in these genes. Especially, the SNP rs560426 in *ABCA4* is associated with non-syndromic CL/P, both in genome-wide association studies and case–parent trios.[9] Miller *et al*. found a relationship between variations in these genes and wider mouths and upturned noses in non-affected first-degree relatives of patients with CL/P.[28]

MSX1 and MSX2

MSX1 functions as a transcriptional repressor during embryogenesis, with a known role in craniofacial development.[32] *MSX1* and *MSX2* work in an overlapping manner, indicating that several *MSX* genes are necessary for normal craniofacial

Table 1
Genes with a known role in facial endophenotypes of non-syndromic CL/P

Gene	OMIM #	Mutation	Endophenotype
IRF6	607199	rs2013126	lower lip whorls
		rs2235543	facial morphology
ABCA4	601691	rs560426	facial morphology orbicularis oris defects
ARHGAP29	610496	unknown	orbicularis oris defects
MSX1	142983	rs6446693 rs1106514 rs12532	facial morphology
MSX2	123101	unknown	facial morphology
BMP4	112262	unknown	orbicularis oris defects
FGF8	600483	unknown	facial morphology
FGFR2	176943	unknown	facial morphology
FOXE1	602617	rs4460698	facial morphology
SOX9	608160	unknown	facial morphology
PAX3	606597	unknown	facial morphology
PAX7	167410	unknown	facial morphology
PTCH1	601309	rs357564	facial morphology

development. *Msx1* is also expressed in the murine palatal mesenchyme.[33] In humans, *MSX2* is expressed in the orofacial skeleton, such as the mandibular and maxillary bones. In humans and mice, loss-of-function variants in the *MSX* genes lead to non-syndromic cleft palate and tooth agenesis.[32] Mutations in *MSX1* are identified in 2% of patients with non-syndromic orofacial clefting.[11] Furthermore, *MSX* genes play a role in normal craniofacial development by enhancing the expression of *BMP4*, supporting the hypothesis that variations in *MSX* genes can cause differences in craniofacial morphology in patients with CL/P or CP and their relatives.[6] The SNP with the most powerful association with CL/P is rs6446693, based on a candidate gene association study.[34] The SNP rs1106514 is associated with CP, and the SNP rs12532 is associated with convex facial profiles.[28]

BMP4

BMP4 encodes for a signaling molecule with a role in skeletal development and lip and palate fusion.[6] Often, microforms of CL/P are observed, such as scar-like ridges above the lip. The hypothesis is that *BMP4* encodes for factors that regulate rescue of the cleft lip phenotype in utero. Therefore, an association among orbicularis oris muscle defects, microform clefts, and variations in *BMP4* can be expected. Variations in this gene could also possibly play a role in nasolabial fold discontinuity during speech in relatives of patients with CL/P.

FGF signaling pathway

The fibroblast growth factor (FGF) signaling pathway is involved in three stages of craniofacial development (neural crest induction, skeletogenesis, and epithelial–mesenchymal interactions) and is expected to be involved in the genetic etiology of non-syndromic CL/P and CP because it regulates palatal shelf growth.[35,36] Variations in *FGFR2*, *FGF3*, *FGF7*, *FGF10*, *FGF18*, and *FGF19* are thought to play a role in the etiology of non-syndromic CL/P.[37] *FGFR2* also interacts with maternal smoking behavior and vitamin supplementation during pregnancy, increasing the risk of clefting.[36] This link is a perfect example of gene-environment interaction in the etiology of non-syndromic CL/P and CP. Furthermore, the FGF pathway is involved in normal facial morphogenesis.[37]

FOXE1

FOXE1 is a transcription factor involved in the development of the palatal shelves.[6] A strong association between both CL/P and CP and variations in *FOXE1* (in the 9q22–33 region) has been described.[7] The SNP rs4460698 is associated with CL/P and CP,[7] and variations in *FOXE1* cause Bamforth–Lazarus syndrome (OMIM: 241850), characterized by orofacial clefts and thyroid dysgenesis, supporting the hypothesis that *FOXE1* is a candidate gene in the etiology of both CL/P and CP. This possibility is remarkable because it is generally assumed that CL/P and CP have different etiologies. Recent studies have, however, provided evidence of an overlapping etiology for the two cleft types.[7] Furthermore, variations in *FOXE1* are associated with ocular hypertelorism,[38] which is part of the facial endophenotype of CL/P as described by Weinberg *et al*.[25]

SOX9

SOX9 has a role in facial morphogenesis because it is critical for the development of neural crest progenitors. Mutations in *SOX9* cause micrognathia, macroglossia, and cleft palate, sometimes within the context of campomelic dysplasia (OMIM: 114290).[39] Mutations within the enhancer elements of *SOX9* are known to be involved in Pierre Robin sequence (OMIM: 261800).[40]

PAX3 and PAX7

PAX3 has been identified as a key factor in normal facial development. Variations in this gene affect the nasion position, and nasal dysmorphology is one of the most stigmatizing facial characteristics for patients with CL/P. Therefore, it could be hypothesized that *PAX3* also has an influence in the etiology of CL/P or CP.

PAX7 is a transcription factor that has a role in neural crest development.[41] In mice, it is expressed in nasal structures and can be involved in malformations of the maxilla and the nose, which indicates its role in craniofacial development.[42] It is important to keep in mind that although variations in this gene were shown in different trio studies in different populations to have a role in orofacial clefting, there is also a significant imprinting effect.[10]

PTCH1

PTCH1 has a complex role in craniofacial development. Mutations in this gene cause Gorlin syndrome (OMIM: 109400), characterized by macrocephaly, mid-facial hypertelorism, a broad nasal bridge, and a high-arch secondary palate. The loss of *Ptch1* in mice causes craniofacial abnormalities, including clefting phenotypes, because of its influence on the *Shh*, *Wnt*, and *Fgf* pathways.[43] The SNP rs357564, described within *PTCH1*, is expected to have an influence in the etiology of CL/P.[44]

Discussion and future prospects

Cleft lip and palate are common birth defects with a variable phenotypic expression. The etiology of non-syndromic CL/P and CP is complex, multifactorial, and incompletely understood. Not only can this condition be considered multigenic but also genomic imprinting, reduced penetrance, interaction with the environment, and epigenetic regulation of the gene should be taken into consideration when investigating the genetic etiology of non-syndromic CL/P and CP.[45,46]

Several approaches, such as linkage analysis, genome-wide association studies, and animal studies, are used in an effort to clarify the genetic etiology of CP and CL/P, but many factors remain unidentified. Although these large genetic studies have indeed provided promising results, more accurate phenotyping of individuals with CL/P and CP and their relatives may increase the power to detect candidate genes for clefting. Testing candidate genes is a promising approach that has yielded some success. In this approach, genes known to play a role in craniofacial development and that are important during the embryonic stages of lip and palate closure are presumed to be involved in the genetic etiology of CL/P or CP. To fine-tune this approach, non-affected first-degree relatives of patients with CL/P or CP have to be involved in the research. They often show characteristics that are related to the CL/P or CP, without having the cleft phenotype. These characteristics, or endophenotypes, could be expressions of underlying susceptibility genes, and the genes that are causal for the endophenotypic characteristics can also be causal for CL/P or CP. If not, there is a likelihood that they are in linkage disequilibrium with the

causal genes of CL/P or CP. The genetic origin of endophenotypes could thus be a tool to identify candidate locations for disease-specific genes. To identify the genetic origin of endophenotypes, a thorough screening of the literature is necessary. These genes can be included in association, linkage, and expression studies focused on identifying causal factors in CL/P and CP.

Furthermore, the power of genetic research can be increased by identifying low- and high-risk families based on the presence of one or more endophenotypes within these families. Not only is identifying new causal genes for CL/P or CP through the candidate-gene approach possible but also this approach will give more insight into the mechanism of disease and the developmental pathways involved in facial morphology.

Therefore, it is important that these characteristics are well described in a routine manner, using the most sensitive and up-to-date techniques. For instance, a lot of research has been performed to describe the craniofacial differences between non-affected relatives of patients with non-syndromic CL/P and CP and control populations. Initially, these analyses were done using classical anthropometric measurements such as point-to-point measurements of landmarks, and later, laser-scanning techniques were used. Now, 3D imaging is considered to be the most sensitive imaging technique; however, the limited number of landmarks used to analyze these images often dampens the sensitivity of the technique. The use of a spatially dense system can give more and more-detailed information about facial characteristics in the relatives group. Current approaches involve thousands of data points from individual faces, and these points are then compared to a reference dataset. Claes *et al*. already have successfully linked facial characteristics with SNPs using the bootstrapped response-based imputation modeling technique.[47]

A detailed description of phenotypic features including endophenotypic characteristics in both patients and relatives may provide clues for the genetic etiology of this complex disorder in a specific patient. The gradual unraveling of the link between genotype and phenotype may lead to the identification of relevant susceptibility genes in an increased number of patients and ultimately to a more accurate and personalized estimate of the recurrence risk.

References

1. Stanier P. Genetics of cleft lip and palate: syndromic genes contribute to the incidence of non-syndromic clefts. *Hum Mol Genet*. 2004;13(suppl 1):73R-81.
2. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. *Lancet*. 2009;374(9703):1773-1785.
3. Hellings PW, Hens G, Schoenaers J, Jorissen M, Vander Poorten V. Unique approach to secondary cleft-lip rhinoplasty in facial plastic surgery. *B-ENT*. 2010;6 (Suppl 15):97-101.
4. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev Genet*. 2011;12(3):167-178.
5. IPDTOC Working Group. Prevalence at birth of cleft lip with or without cleft palate: data from the International Perinatal Database of Typical Oral Clefts (IPDTOC). *Cleft Palate-Craniofacial J Off Publ Am Cleft Palate-Craniofacial Assoc*. 2011;48(1):66-81.
6. Jugessur A, Farlie P, Kilpatrick N. The genetics of isolated orofacial clefts: from genotypes to subphenotypes. *Oral Dis*. 2009;15(7):437-453.
7. Ludwig, KU, Böhmer AC, Rubini M, Mossey PA, Herms S, Nowak S, Reutter H, Alblas MA, Lippke B, Barth S, Paredes-Zenteno M, Munoz-Jimenez SG, Ortiz-Lopez R, Kreusch T, Hemprich A, Martini M, Braumann B, Jäger A, Pötzsch B, Molloy A, Peterlin B, Hoffmann P, Nöthen MM, Rojas-Martinez A, Knapp M, Steegers-Theunissen RP, Mangold E. Strong Association of Variants around FOXE1 and Orofacial Clefting. *J Dent Res*. 2014;93(4):376-381.
8. Moreno LM, Mansilla MA, Bullard SA, Cooper ME, Busch TD, Machida J, Johnson MK, Brauer D, Krahn K, Daack-Hirsch S, L'heureux J, Valencia-Ramirez C, Rivera D, Lopez AM, Moreno MA, Hing A, Lammer EJ, Jones M, Christensen K, Lie RT, Jugessur A, Wilcox AJ, Chines P, Pugh E, Doheny K, Arcos-Burgos M, Marazita ML, Murray JC, Lidral AC. FOXE1 association with both isolated cleft lip with or without cleft palate, and isolated cleft palate. *Hum Mol Genet*. 2009;18(24):4879-4896.
9. Beaty TH, Taub MA, Scott AF, Murray JC, Marazita ML, Schwender H, Parker MM, Hetmanski JB, Balakrishnan P, Mansilla MA, Mangold E, Ludwig KU, Noethen MM, Rubini M, Elcioglu N, Ruczinski I. Confirming genes influencing risk to cleft lip with/without cleft palate in a case – parent trio study. *Hum Genet*. 2013;132(7):771-781.
10. Mangold E, Ludwig KU, Nöthen MM. Breakthroughs in the genetics of orofacial clefting. *Trends Mol Med*. 2011; 17(12):725-733.
11. Lidral AC, Moreno LM. Progress toward discerning the genetics of cleft lip. *Curr Opin Pediatr*. 2005;17(6):731-739.
12. Van Esch H, Verdonck A, Carels CE. Heredity of non-syndromal orofacial clefts [article in Dutch]. *Ned Tijdschr Tandheelkd*. 2008;115(2):101-104.
13. Christensen K, Fogh-Andersen P. Cleft lip (+/- cleft palate) in Danish twins, 1970-1990. *Am J Med Genet*. 1993;47(6): 910-916.

14. Leboyer M, Bellivier F, Nosten-Bertrand M, Jouvent R, Pauls D, Mallet J. Psychiatric genetics: search for phenotypes. *Trends Neurosci*. 1998;21(3):102-105.
15. Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am . Psychiatry*. 2003;160(4):636-645.
16. Hong LE, Mitchell BD, Avila MT, Adami H, McMahon RP, Thaker GK. Familial aggregation of eye-tracking endophenotypes in families of schizophrenic patients. *Arch Gen Psychiatry*. 2006;63(3):259-264.
17. De Mey A, Van Hoof I, De Roy G, Lejour M. Anatomy of the orbicularis oris muscle in cleft lip. *Br J Plast Surg*. 1989;42(6):710-714.
18. 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(2):155-161.
19. Neiswanger K, Weinberg SM, Rogers CR, Brandon CA, Cooper ME, Bardi KM, Deleyiannis FW, Resick JM, Bowen A, Mooney MP, de Salamanca JE, Gonzalez B, Maher BS, Martin RA, Marazita ML. Orbicularis oris muscle defects as an expanded phenotypic feature in nonsyndromic cleft lip with or without cleft palate. *Am J Med Genet A*. 2007;143A(11):1143-1149.
20. Marazita ML, Lidral AC, Murray JC, Field LL, Maher BS, Goldstein McHenry T, Cooper ME, Govil M, Daack-Hirsch S, Riley B, Jugessur A, Felix T, Morene L, Mansilla MA, Vieira AR, Doheny K, Pugh E, Vanencia-Ramirez C, Arcos-Burgos M. Genome Scan, Fine-Mapping, and Candidate Gene Analysis of Non-Syndromic Cleft Lip with or without Cleft Palate Reveals Phenotype-Specific Differences in Linkage and Association Results. *Hum Hered*. 2009;68(3):151-170.
21. Schmidt KL, Neiswanger K, Cohn E, Desensi R, Brandon C, Bardi K, Marazita ML. Nasolabial Fold Discontinuity During Speech as a Possible Extended Cleft Phenotype. *Cleft Palate-Craniofacial J Off Publ Am Cleft Palate-Craniofacial Assoc*. 2013;50(2):201-206.
22. Neiswanger K, Walker K, Klotz CM, Cooper ME, Bardi KM, Brandon CA, Weinberg SM, Vieira AR, Martin RA, Czeizel AE, Castilla EE, Poletta FA, Marazita ML. Whorl patterns on the lower lip are associated with nonsyndromic cleft lip with or without cleft palate. *Am J Med Genet A*. 2009;149A(12):2673-267.
23. Fraser FC, Pashayan H. Relation of face shape to susceptibility to congenital cleft lip. A preliminary report. *J Med Genet*. 1970;7(2):112-117.
24. Claes P, Walters M, Clement J. Improved facial outcome assessment using a 3D anthropometric mask. *Int J Oral Maxillofac Surg*. 2012;41(3):324-330.
25. 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(4):409-420.
26. Weinberg SM, Neiswanger K, Martin RA, Mooney MP, Kane AA, Wenger SL, Losee J, Deleyiannis F, Ma L, De Salamanca JE, Czeizel AE, Marazita ML. The Pittsburgh Oral-Facial Cleft study: expanding the cleft phenotype. Background and justification. *Cleft Palate-Craniofacial J Off Publ Am Cleft Palate-Craniofacial Assoc*. 2006;43(1):7-20.
27. Al Chawa T, Ludwig KU, Fier H, Pötzsch B, Reich RH, Schmidt G, Braumann B, Daratsianos N, Böhmer AC, Schuencke H, Alblas M, Fricker N, Hoffmann P, Knapp M, Lange C, Nöthen MM, Mangold E. Nonsyndromic cleft lip with or without cleft palate: Increased burden of rare variants within *Gremlin-1* , a component of the bone morphogenetic protein 4 pathway: Burden Analysis of GREM1 in Orofacial Cleft. *Birt Defects Res A Clin Mol Teratol*. 2014;100(6):493-498.
28. Miller SF, Weinberg SM, Nidley NL, Defay DK, Marazita ML, Wehby GL, Moreno Uribe LM. Exploratory genotype-phenotype correlations of facial form and asymmetry in unaffected relatives of children with non-syndromic cleft lip and/or palate. *J Anat*. 2014;224(6):688-709.
29. Baynam G, Claes P, Craig JM, Goldblatt J, Kung S, Le Souef P, Walters M. Intersections of Epigenetics, Twinning and Developmental Asymmetries: Insights Into Monogenic and Complex Diseases and a Role for 3D Facial Analysis. *Twin Res Hum Genet*. 2012;14(4):305-315.
30. Ben J, Jabs EW, Chong SS. Genomic, cDNA and embryonic expression analysis of zebrafish IRF6, the gene mutated in the human oral clefting disorders Van der Woude and popliteal pterygium syndromes. *Gene Expr Patterns*. 2005;5(5):629-638.
31. Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB, Liang KY, Wu T, Murray T, Fallin MD, Redett RA, Raymond G, Schwender H, Jin SC, Cooper ME, Dunnwald M, Mansilla MA, Leslie E, Bullard S, Lidral AC, Moreno LM, Menezes R, Vieira AR, Petrin A, Wilcox AJ, Lie RT, Jabs EW, Wu-Chou YH, Chen PK, Wang H, Ye X, Huang S, Yeow V, Chong SS, Jee SH, Shi B, Christensen K, Melbye M, Doheny KF, Pugh EW, Ling H, Castilla EE, Czeizel AE, Ma L, Field LL, Brody L, Pangilinan F, Mills JL, Molloy AM, Kirke PN, Scott JM, Arcos-Burgs M, Scott AF. A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nat Genet*. 2010;42(6):525-529.
32. Alappat S, Zhang ZY, Chen YP. Msx homeobox gene family and craniofacial development. *Cell Res*. 2003;13(6): 429-442.
33. Zhang Z, Song Y, Zhoa X, Zhang X, Fermin C, Chen Y. Rescue of cleft palate in Msx1-deficient mice by transgenic Bmp4 reveals a network of BMP and Shh signaling in the regulation of mammalian palatogenesis. *Dev Camb Engl*. 2002;129(17):4135-4146.
34. Jagomägi T, Nikopensius T, Kjrutskov K, Tammekivi V, Viltrop T, Saag M, Metspalu A. MTHFR and MSX1 contribute to the risk of nonsyndromic cleft lip/palate. *Eur J Oral Sci*. 2010;118(3):213-220.
35. Pauws E, Stanier P. FGF signalling and SUMO modification: new players in the aetiology of cleft lip and/or palate. *Trends Genet*. 2007;23(12):631-640.
36. Wang C, Chang JY, Yang C, Huang Y, Liu J, You P, McKeehan WL, Wang F, Li X. Type 1 Fibroblast Growth Factor Receptor in Cranial Neural Crest Cell-derived Mesenchyme Is Required for Palatogenesis. *J Biol Chem*. 2013;288(30):22174-22183.

37. Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, Russo ET, Vieira AR, Dodé C, Mohammadi M, Marazita ML, Murray JC. Impaired FGF signaling contributes to cleft lip and palate. *Proc Natl Acad Sci*. 2007;104(11):4512-4517.
38. Bamforth JS, Hughes IA, Lazarus JH, Weaver CM, Harper PS. Congenital hypothyroidism, spiky hair, and cleft palate. *J Med Genet*. 1989;26(1):49-51.
39. Lee YH Saint-Jeannet JP. Sox9 function in craniofacial development and disease. *genesis*. 2011;49(4):200-208.
40. Benko S, Fantes JA, Amiel J, Kleinjan DJ, Thomas S, Ramsay J, Jamshidi N, Essafi A, Heaney S, Gordon CT, McBride D, Golzio C, Fisher M, Perry P, Abadie V, Ayuso C, Holder-Espinasse M, Kilpatrick N, Lees MM, Picard H, Temple IK, Thomas P, Vazquez MP, Vekemans MP, Roest Crollius H, Hastie ND, Munnich A, Etchevers HC, Pelet A, Farlie PG, Fitzpatrick DR, Lyonnet S. Highly conserved non-coding elements on either side of SOX9 associated with Pierre Robin sequence. *Nat Genet*. 2009; 41(3):359-364.
41. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. *Am J Med Genet C Semin Med Genet*. 2013; 163C(4):246-258.
42. Mansouri A, Stoykova A, Torres M, Gruss P. Dysgenesis of cephalic neural crest derivatives in Pax7-/- mutant mice. *Dev Camb Engl*. 1996;122(3):831-838.
43. Kurosaka H, Iulianella A, Williams T, Trainor PA. Disrupting hedgehog and WNT signaling interactions promotes cleft lip pathogenesis. *J Clin Invest*. 2014;124(4): 1660-1671.
44. Carter TC, Molloy AM, Pangilinan F, Troendle JF, Kirke PN, Conley MR, Orr DJ, Earley M, McKiernan E, Lynn EC, Doyle A, Scott JM, Brody LC, Mills JL. Testing reported associations of genetic risk factors for oral clefts in a large Irish study population. *Birt Defects Res A Clin Mol Teratol*. 2010;88(2):84-93.
45. Maestri NE, Beaty TH, Hetmanski J, Smith EA, McIntosh I, Wyszynski DF, Liang KY, Duffy DL, Vanderkolk C. Application of transmission disequilibrium tests to nonsyndromic oral clefts: including candidate genes and environmental exposures in the models. *Am J Med Genet*. 1997:73(3):337-344.
46. Spritz RA. The genetics and epigenetics of orofacial clefts. *Curr Opin Pediatr*. 2001;13(6):556-560.
47. Claes P, Liberton DK, Daniels K, Rosana KM, Quillen EE, Pearson LN, McEvoy B, Bauchet M, Zaidi AA, Yao W, Tang H, Barsch GS, Absher DM, Puts DA, Rocha J, Beleza S, Pereira RW, Baynam G, Suetens P, Vandermeulen D, Wagner JK, Boster JS, Shriver MD. Modeling 3D facial shape from DNA. *PLoS Genet*. 2014:(10):e1004224.

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