

OROFACIAL CLEFTS AND FACE SHAPE HYPOTHESIS. A BRIEF REVIEW

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ABSTRACT

Cleft lip and palate are common birth defects with complex aetiology, including a definite genetic component as well as environmental factors. Most cases are non-syndromic, meaning they are manifested as isolated deformities not associated with other anatomical or functional abnormalities. Due to the proven role of hereditary factors, parents and siblings of patients with cleft lip and palate run the genetic risk of passing the trait on to their offspring. Furthermore, both individuals with clefts and their unaffected relatives may exhibit distinctive minor facial features that set them apart from the rest of the population. Indeed, numerous studies have highlighted subtle differences in the faces of unaffected relatives from families with children with clefts compared to controls, and such differences have been interpreted as incomplete expression of an underlying genetic predisposition. Hence, these facial characteristics in the unaffected relatives can be seen as a subclinical phenotype and, for the same reason, as indicative of genetic susceptibility to cleft in the offspring of these subjects. The face shape hypothesis assumes that these facial features may constitute a liability factor for orofacial clefts, i.e. unaffected individuals tend to show a pattern in the facial shape that may indicate that they are genetically at risk for orofacial clefting. The most consistent features associated with this predisposition include upper facial width, increased interorbital width, decreased middle and upper facial height, increased lower facial height, and flattening of the facial profile resulting from a combination of excessive midface retrusion and mandibular protrusion. On the other hand, additional changes associated with a history of clefting may include a tendency towards a reduced width of the philtrum, slight alterations of the orbicularis labii muscle and the nasolabial area, as well as a whorled appearance of the lower lip. Thus, the aetiology of orofacial clefts could benefit from the study of these subclinical phenotypes. In this article, the embryological bases of lip and palate morphogenesis and the

developmental pathogenesis of orofacial clefting are briefly reviewed, the concept of endophenotype is introduced, and a general discussion of facial changes associated with non-syndromic clefting is provided according to the face shape hypothesis, with possible implications of this topic as a tool for the study and prevention of these malformations.

Keywords: *orofacial clefting; cleft lip; cleft palate; embryology; developmental pathogenesis; multifactorial threshold concept; face shape hypothesis; endophenotypes*

Throughout the history of humanity, the face has always had a primary importance in the physical constitution of the person as it contains the uniqueness of the individual. It reveals the genetic background, sex, current health status and emotional state as well as possibly some attitudes and psychological characteristics and moods of the subject. The wide morphological variability of human faces derives from a multiplicity of factors that add further complication to the intrinsic complexity of the biological mechanisms underlying the formation of facial structures. We know that the aspects of our facial morphology are largely inherited, and a large number of genes are known to be involved in generating the facial shape [33], but the effects of these genes in determining the normal variation that gives each of us our unique facial appearance are still poorly understood [36]. In fact, it is believed that every feature of human facial appearance is defined by subtle gene- and environment-driven variations whose combinatorial possibilities lead to a virtually infinite diversity of facial morphologies [33, 36]. Face-related aspects provide a means for social interactions, conveying thoughts and emotions to other individuals, thus allowing a communication system common to all human beings. Emotionally charged facial expressions are universally recognised, and the communication of these states is essential for social exchanges [30].

On the other hand, the face is responsible for basic physiological functions such as eating, speaking, breathing, smelling, sighting and hearing. In particular, a child born with an orofacial defect has a significant impairment in fundamental functions such as breathing, sucking, swallowing and feeding, teething and phonation, maintaining good hearing, in addition to determining an altered facial aesthetics [11]. Thus, abnormal facial formation underlies birth defects that severely impact health, social relationships and quality of life.

INTRODUCTION TO OROFACIAL CLEFTING

Orofacial clefting is among the most common congenital defects and the most frequent craniofacial deformity. Classically, orofacial clefts have been divided into two main categories: cleft lip with or without cleft palate (CL/P) and cleft palate alone (CP) (Figure 1), and both can be present either as isolated, non-syndromic forms, or as part of a syndrome associated with other malformations. CL/P is the most common congenital orofacial malformation with a frequency of approximately 1 in 700 live births [3, 4, 10, 11, 22]. Children born with CL/P and CP may have significant physical health problems and psychological difficulties, therefore requiring a multi-specialist medical team [10, 11]. It is known that non-syndromic CL/P has a multifactorial aetiology, being influenced by both genetic and environmental components [3, 11]. The establishment of normal facial patterning is intricately influenced by genes, molecular networks and epigenetic regulatory mechanisms [27], and in recent years evidence has been accumulating that many genes that are thought to play a role in the aetiology of orofacial clefts are also important in normal facial craniofacial morphogenesis and normal-range variation, thus somehow bridging the gap between pathology and normal development [2, 15, 17]. However, knowledge of the mechanisms governing face patterning, the generation of facial variants that are nonetheless considered normal, as well as the disruptive processes that lead to orofacial clefts is still limited [33].

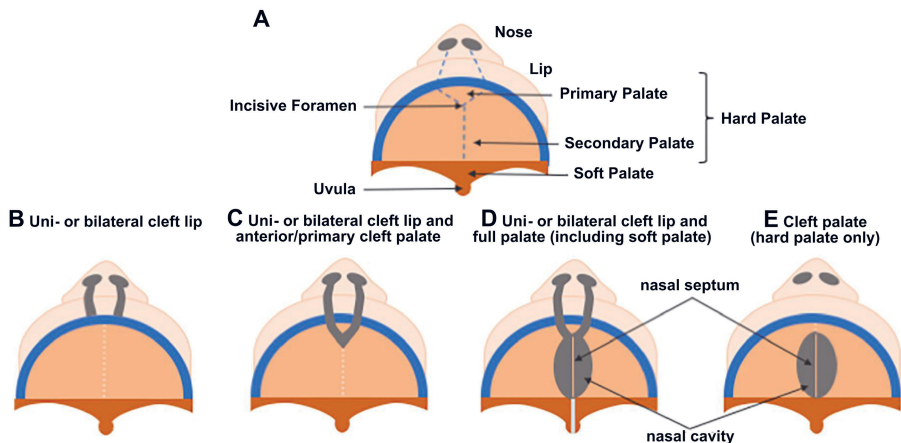


Figure 1. Schematic diagram of some clinical forms of cleft lip and/or palate in humans. Cleft lip can be unilateral or bilateral, and incomplete or complete, and, in addition, the palate can be involved. Cleft palate alone can be unilateral or bilateral, and incomplete or complete. (A) Normal anatomy of lip and palate. (B, C) Cleft lip, without (B), and with involvement of the primary palate (C). (D) Cleft lip with cleft of both the primary and secondary palate. (E) Cleft palate involving only the secondary hard palate. Kindly reprinted from Paiva, Maas, dos Santos, Granjeiro, Letra [22].

The process of lip and palate closure involves a complex interaction between the growth and orientation of embryonic structures, tongue resistance, and mandible and head growth. All these factors must be coordinated with each other to achieve successful lip and palate closure [5]. Although many animal models and a vast literature exist addressing normal development and generation of non-syndromic orofacial clefts [24], many aspects are not yet fully understood.

Attempting to better define the factors involved in orofacial clefting, in this article a brief overview of normal facial development is provided, and the mechanisms that regulate the union of embryonic facial structures and their perturbation to generate the cleft are succinctly presented. Then, these data are evaluated in light of the face shape hypothesis, and how this approach may be useful in estimating cleft risk in family counselling is explained.

CRANIOFACIAL EMBRYOLOGY AND DEVELOPMENTAL PATHOGENESIS

Craniofacial development is one of the most complex processes occurring in the human organism. Most of the craniofacial structures derive from cranial neural crest cells that detach from the epithelium of the closing neural tube and then migrate away to form the primordial facial prominences. Cranial neural crest contributes to the formation of the forehead, the nose and the philtrum (deriving from the frontonasal prominence), the upper jaw (deriving from the maxillary prominences), the lower jaw (deriving from the mandibular prominences), as well as large parts of the skull vault, and ear structures [1].

The first facial primordium that is individualised during the 4th week is the frontonasal prominence placed rostrally to the stomodeum (the primitive mouth) (Figure 2). Subsequently, the paired mandibular prominences merge at the midline caudally to the stomodeum to form the mandible, chin and lower lip. Then, at the 5th week, the frontonasal prominence separates into the paired medial nasal prominences and the lateral nasal prominences, which then fuse with the paired maxillary prominences at the 7th week. This latter critical event leads to the formation of the primary palate, philtrum, and upper lip, and takes place on each side through a fusion process at the three-way seam called the lambdoidal junction where the three facial prominences meet on each side of the face [1, 11].

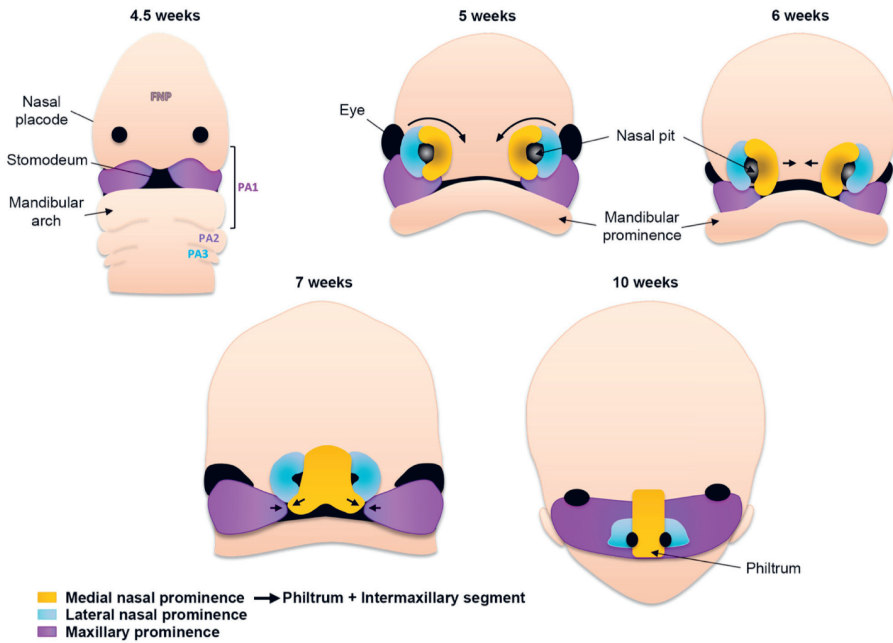


Figure 2. Drawing illustrating human facial development. At 4.5 weeks, the facial primordia include the frontonasal prominence (FNP, *light tan*) with nasal placodes placed rostrally to the stomodeum, the paired maxillary prominences (*dark pink*) flanking the stomodeum, and the mandibular prominences (*light tan*) placed caudally. Derivatives of the first pharyngeal arch (PA1) are indicated. At 5 weeks, the frontonasal prominence divides into medial (*yellow*) and lateral (*blue*) nasal prominences. Medial nasal prominences converge (6 weeks) and merge on the midline (7 weeks), thus giving rise to the intermaxillary segment and, therefore, to the middle of the nose and the philtrum of the lip/primary palate (10 weeks). The lateral parts of the upper lip arise from the maxillary prominences, while the lateral nasal prominences form the nasal alae. From Antiguas, Paul and Dunnwald [1], reprinted by kind courtesy.

Development of the secondary palate occurs at week 6 and ends at week 12, and derives from fusion of the palatal shelves growing intra-orally from each side of the maxillary prominences (Figure 3); the definitive palate is created by the union of the primary palate anteriorly with the fused secondary palate posteriorly. The bilateral palatal shelves initially grow down vertically from the maxillary prominences along the two sides of the tongue. Then, the tongue flattens and moves inferiorly in the oral cavity due to the forward and downward growth of the mandible. The downward displacement of the tongue facilitates the reorientation of the palatal shelves over the dorsum of the tongue where they further grow toward each other and, eventually, adhere at the midline where the medial edge epithelia (MEE) merge on the tip of each shelf to form the single midline edge seam (MES) (Figures 3 and 4) [10, 11, 20].

Developing facial prominences, including the primordia of lip/primary palate and the secondary palatal shelves, are lined by an ectoderm-derived epithelium important for the union between the apposing structures to create a seam which then disintegrates to generate continuity between the opposing parts. The disappearance of the seam is mediated by apoptosis, cell migration and epithelial-mesenchymal migration [1, 10, 11], or a combination of these mechanisms [11, 21]. In secondary palatogenesis, the contribution of both apoptosis and cell migration in epithelial seam disappearance has been confirmed by several studies and has a well-established experimental validity [10].

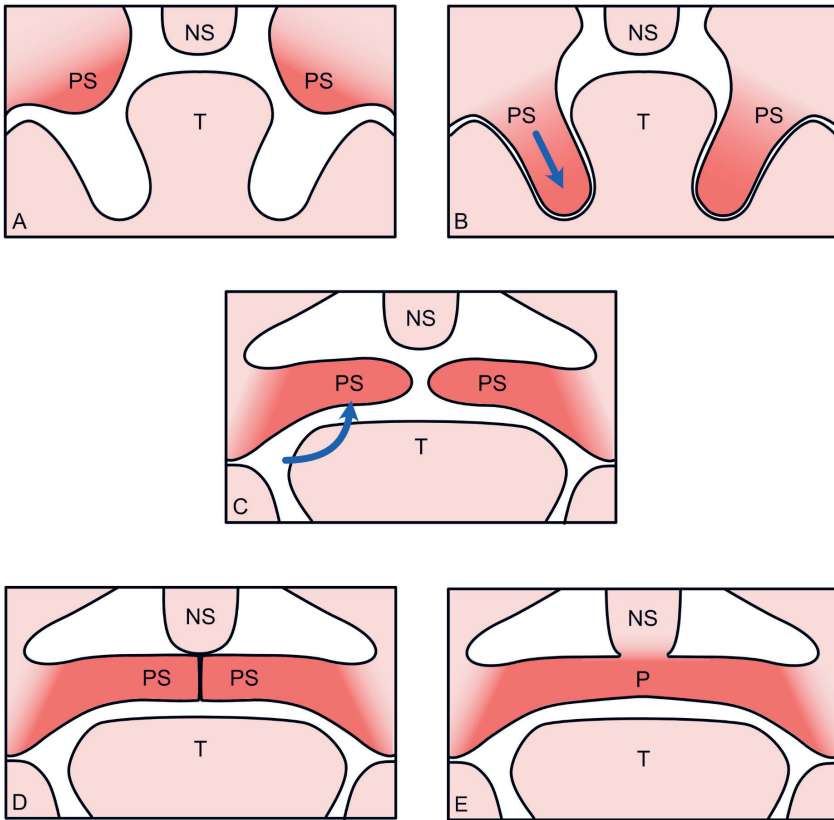


Figure 3. Scheme of secondary palate development. **(A)** At 6 weeks, palatal primordia appear on the intra-oral sides of the maxillary processes and begin their growth downwards. **(B)** The palatal shelves grow down along the sides of the tongue that in this stage is placed high in the oral cavity. **(C)** At 7 weeks, the tongue drops and flattens, thus allowing the palate shelves to elevate above the dorsum of the tongue where they grow further towards each other. **(D)** At 8 weeks, the shelves adhere to each other through their medial edge epithelia (MEE), which then merge to form the medial epithelial seam (MES). **(E)** At 12 weeks, dissolution of the MES leads to complete fusion of the shelves to form a continuous palate, which also fuses with the overlying nasal septum. PS – palate shelves; T – tongue; NS – nasal septum; P – palate.

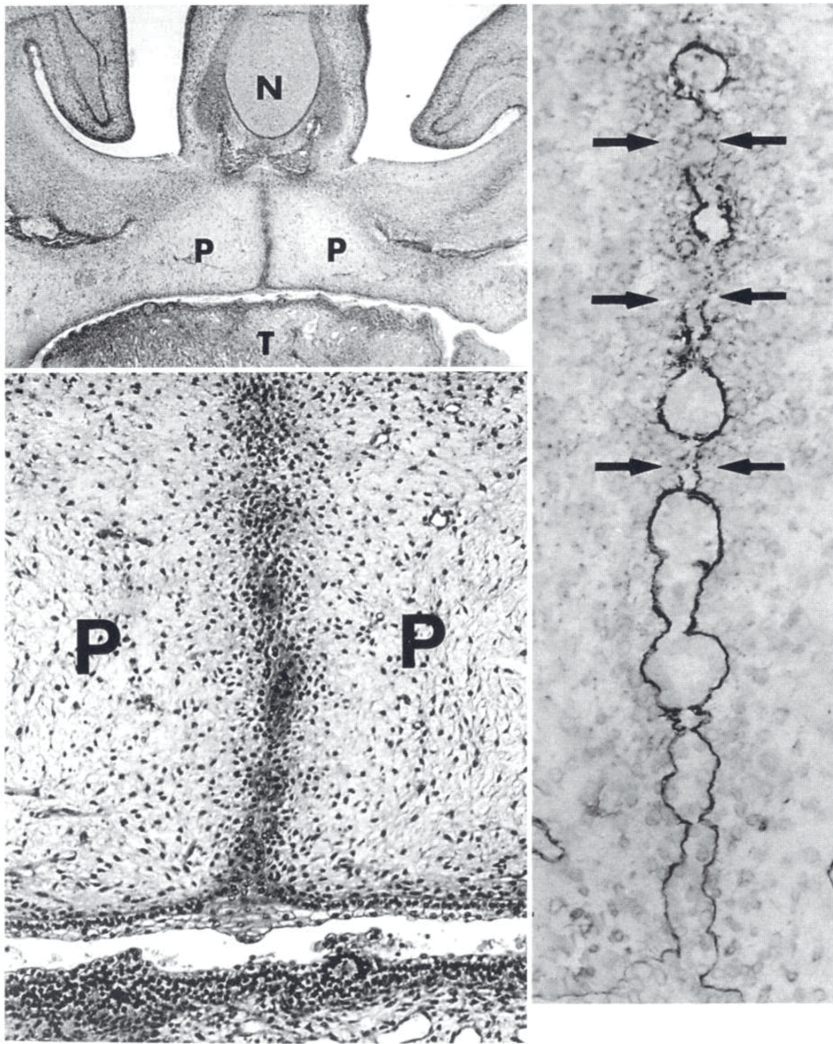


Figure 4. Histological section of the palate of a 9-week-old human foetus. (A – upper left; B – below left) Haematoxylin and eosin-stained coronal section from the anterior palate region, showing fusion between the palate shelves with the intervening MES exhibiting initial breakdown (magnified in B). (C – right) An adjacent section from the same sample stained with antibodies to collagen type IV highlighting the basement membrane which exhibits some interruptions (arrows). In correspondence, the MES shows images of fragmentation/disintegration which foretell its complete disappearance. P – palate shelves; N – nasal septum; T – tongue. From Guarino et al. *Pathol Res Pract*, 1999, 195 (6), 379–389 [14], reprinted with permission of Elsevier.

Epithelial-mesenchymal transition occurs in embryogenesis, wound healing, and tumour formation and progression [12–14], but its actual involvement in palatal seam dissolution is less certain [10].

Failure of any of these morphogenetic steps can lead to the development of an orofacial cleft. As one can see, the upper lip / primary palate develops before the secondary palate, and involves different structures. However, due to their relationship of continuity, and because the development of the former affects that of the latter, cleft lip is frequently associated with cleft of the secondary palate and, therefore, cleft lip and cleft lip with cleft palate (CL/P) are treated together. In contrast, cleft palate alone (CP), which is considerably less common, involves exclusively failure of the palatal shelves to unite (Figure 1E) and, due to biological and epidemiological differences, is usually classified separately from CL/P [11, 22].

Development of clefting

Genes, environmental factors as well as gene-gene and gene-environment interactions, and epigenetic changes are all thought to play an important role in the development of orofacial clefts through different mechanisms. The formation of the lip / primary palate and secondary palate takes place through ordered sequences of complex interactions among epithelial cells, mesenchymal cells and the extracellular matrix, orchestrated by multiple signalling molecules and a large number of genes, some of which are also known to be involved in the aetiology of orofacial cleft [11]. Any interference during morphogenesis of the primary lip/palate or secondary palate, whether at the anatomical, cellular or molecular level can lead to a cleft. Cranial neural crest cell defective migration, proliferation, patterning as well as impaired morphogenetic movement and adhesion of facial primordia can compromise the closure of the lip and palatal buds [11]. Specifically, this can occur as a result of different mechanisms [5]:

- (i) In some cases, the cleft is a result of fusion failure, since the facial prominences grow in a normal way and make contact with each other, but the cellular and molecular mechanisms that mediate the adhesion and fusion of the parts are defective, and, therefore, closure does not occur.
- (ii) In other cases, the facial primordia do not grow enough, and, consequently, they fail to contact each other, or they meet too late for successful fusion to occur.
- (iii) Another circumstance is that cell proliferation is regular, but the growth trajectory of facial buds is anomalous; in this situation, the precise spatial

arrangement required for facial prominences to meet is missing, thereby leading to a cleft.

- (iv) Finally, another possibility is that extrinsic factors/forces in the developing cranium prevent the normal positioning of orofacial buds relative to each other, which is necessary for contact and adhesion [5, 32].

THE MULTIFACTORIAL THRESHOLD CONCEPT

It is known that many genes that are thought to play a role in the aetiology of orofacial clefts are also important in normal craniofacial variation [2, 15, 17]. Therefore, it is believed that an altered function of these genes can disrupt normal patterning in facial morphogenesis, for example by modifying the growth rate and/or orientation of facial primordia. Clefting occurs when these gene-caused deviations somehow decrease the likelihood that adjacent facial primordia will meet and then fuse and, in fact, even subtle changes in these morphogenetic events can push an individual to the critical threshold for clefting to occur. In less severe cases, facial morphology can undergo some minor change without reaching the threshold and, therefore, without the clinical manifestation of clefting [35]. The multifactorial threshold concept refers to a model of inheritance of discrete traits that did not follow strictly Mendelian patterns, and it has found application in orofacial clefting whereby a combination of factors creates a continuous liability to cleft, thus allowing a conceptualisation of the predisposition to the trait as a continuum and, therefore, a prediction of cleft risk [6, 7]. Assuming that the cleft phenotype is multifactorial, it is believed that a number of genetic and environmental factors interact with each other and will contribute to determining whether a given developmental event reaches a certain threshold; if this occurs, the malformation will develop [7]. In other words, the condition occurs only when the combination of genetic and environmental predisposing factors exceeds a critical threshold. The shape of our face is based on morphogenetic events that depend on the degree of the expansion and growth trajectory of primordial facial prominences [11] which, in turn, determine the possibility of fusion with each other during embryonic life. The successful closure of the embryonic buds of the lip and palate depends on various factors; for example, the buds must grow sufficiently and move in the correct directional trajectory before the growth of the head pulls them too far apart to reach each other [7]. If head growth continues, a specific developmental stage will be reached beyond which the buds will no longer be able to meet, and a cleft will occur. On the other hand, the variability

of growth, orientation, and movement of the buds can be considered a continuously distributed variable, and the point beyond which they cannot meet can be considered a threshold separating normality from abnormality, thus making orofacial clefts a threshold trait [5, 6]. As a matter of fact, studies on mouse strains with different susceptibility to cleft lip have suggested that the threshold for development of the malformation may be related to embryonic facial shape [18, 28, 29]. If facial shape is indeed related to the predisposition to cleft lip, and facial shape is at least partly genetically determined, it follows that parents of children with CL/P could have faces that are shaped differently than those of the general population [7]. As such, a model can be envisioned that provides a conceptual framework in which to place individuals at risk for orofacial clefts due to their family history and who show altered facial features. Facial shape might therefore be regarded as one of the factors underlying cleft susceptibility. Therefore, as unaffected family members exhibiting altered facial shape possess similar genetic risk factors as their affected relatives, they should be closer to the threshold compared to the rest of the population [32]. These concepts introduce the hypothesis that facial shape is in itself a predisposing factor for clefting.

THE FACE SHAPE HYPOTHESIS

There are several signs suggesting that among the factors affecting embryonic facial primordia fusion a specific role is played by the shape of the embryonic face [7]. The “face shape hypothesis” of orofacial clefting dates back at least to the 1960s when morphological differences were observed within relatives of the affected patients, and, in the same period, comparative studies on murine strains with different susceptibility to clefting were conducted [32]. The hypothesis supports the concept that some inherited facial shape characteristics may reflect a predisposing factor for the development of non-syndromic orofacial clefts in offspring. In fact, based on the observations of individual physicians studying cleft patients and their families, a familiar pattern of minor facial alterations in relatives of individuals with orofacial clefts has been recognised since at least the 18th century [31, 32].

Some important insights into the relevance of facial shape have come from animal studies. Studies on mouse embryos have documented the critical role played by the changes in the shape and/orientation of the facial primordial prominences, thus establishing that variation in embryonic facial shape participates in cleft development, being the result of the spatial relationship between

the rapidly growing collections of facial primordia. Indeed, since the 1960s, several studies have detected morphological differences of the embryonic face of mouse strains susceptible to clefting compared to non-susceptible strains, showing that cleft-susceptible mice exhibited altered orientation of the medial/nasal prominences and/or underdevelopment of the maxillary prominences [5, 18, 29]. This resulted in an increase in cleft susceptibility due to interference with the normal relationship between the facial primordia, whereby the probability that they may meet and fuse was diminished [32, 35]. In the high-risk strains, these morphological anomalies would persist after birth and would give rise to subtle differences in adult facial shape.

Similar alterations in postnatal facial shape have been observed in human subjects considered at risk for CL/P due to their family history, and studies have focussed on the relation between face shape and cleft predisposition, based on the analysis of the facial phenotype of unaffected relatives from cleft families compared to non-relative controls. The reasoning behind this approach is simple: since CL/P is at least partly an inherited condition, it is believed that relatives of affected individuals carry a higher proportion of genes potentially involved in clefting than the rest of the population, and some of these genes may even confer peculiar face shape characteristics. Consequently, differences in facial morphology between unaffected relatives and controls could be seen as reflecting variations in underlying genetic susceptibility to develop clefting [35]. Subtle, but significant facial differences between “at-risk” individuals and the control population are therefore hypothesised to indicate a subclinical manifestation of CL/P [35]. The factors underlying the distinctive facial features observed in unaffected parents are thought to be closely related to those causing the failure of bud fusion and therefore responsible for the cleft, and thus, alterations in postnatal facial shape in unaffected relatives are similar to these seen in the cleft children [32]. Several quantitative studies have been performed comparing facial morphology between unaffected relatives of individuals with clefts and controls with no family history of clefts. These studies have utilised a variety of methods including cephalometry, direct anthropometry, 2D photogrammetry and more advanced, non-invasive techniques such as 3D surface imaging [31]. Subtle facial differences of unaffected parents were found, including increased interorbital, nasal cavity and upper face width; forward prominence of the orbital-nasal bridge; forward mandibular protrusion with longer mandibles; broader mid-facial width; reduced upper face height coupled to increased lower face height, resulting in an altered proportional relationship between the upper and lower heights of the face; increased midface retrusion with consequent reduced convexity and flattening of the facial profile

(Figure 5) [32, 34, 35]. In addition, a recessed facial contour – caused by under-developed/flat maxillae and less prominent upper lip, and thus in line with the tendency for mid-face retrusion mentioned above – has been observed more frequently than a vertical or convex facial contour pattern [7]. Furthermore, a prevalence of trapezoidal and rectangular face shapes has also been found [7], probably mirroring the prevalence of broad mid/upper face and long lower face, respectively, identified in the above-mentioned studies.

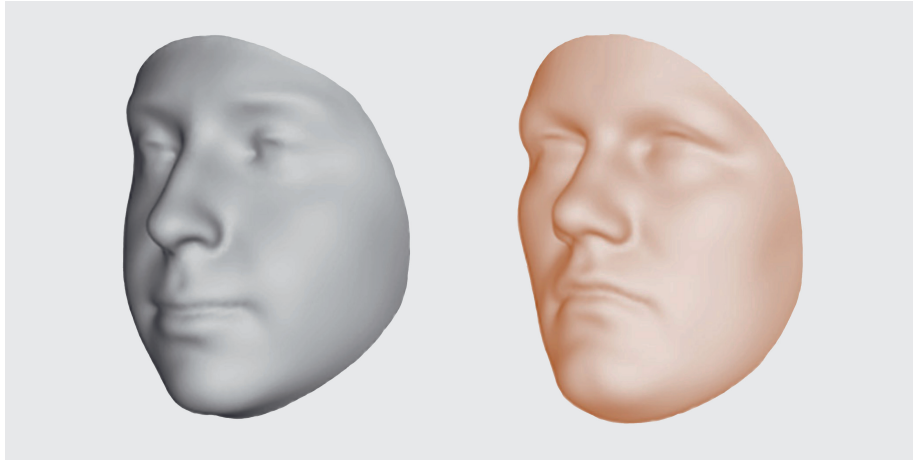


Figure 5. The 3D facial surface differences between a hypothetical control subject (left) and a hypothetical unaffected individual with familial history of non-syndromic clefting (right). The latter shows mid-face flattening with reduced facial convexity, broadening of the upper face, decreased middle and upper facial height, increased prominence of the chin, more upturned nose and more prominent brow ridges. Kindly reprinted from Indencleef et al. [16], modified.

Interestingly, by postulating that the size of the embryonic facial processes affects facial morphology and, therefore, cleft susceptibility, the face shape hypothesis could provide some insight into the different incidence of orofacial clefts between Asians (higher frequency) and Africans (lower frequency) [3, 4], since the size of the prominences could contribute to their ability to contact neighbouring structures. Asians have smaller midfaces and flatter nasal structures, and this could theoretically be the result of inherently smaller medial nasal prominences and, therefore, be related to reduced ability to easily meet the lateral nasal and maxillary prominences, thus partially accounting for their relatively high rates of clefting. In contrast, Africans have a trend to have broad, larger noses, and increased maxillary dentoalveolar protrusion; all these features are indicative of well-developed medial nasal prominences, and this may contribute to a decreased clefting frequency [3, 4].

Research conducted on mice has revealed that the embryonic face of cleft-susceptible strains consistently manifests alterations in the size, shape and orientation of the facial primordia. Indeed, strains of mice with high and low susceptibility to develop clefts show differences in the shape of the face, with cleft-susceptible embryos having underdeveloped and more divergently oriented maxillary prominences compared to low-risk strains [32, 35]. Similarly, one could hypothesise the loss of facial convexity seen in unaffected human parents, largely due to excessive midface retrusion, might be related to reduced growth of the nasal and/or maxillary prominences during embryonic development which, in the most severe cases, achieves the critical threshold for developing a cleft as a consequence of the total inability of the prominences to meet. Similarly, unaffected parents of cleft children also exhibit an increase in interorbital distance, which is a trait observed in individuals with CL/P as well as in cleft animal models, and therefore hypertelorism might be related to changes in the horizontal proportions of the upper face and cranial base [34, 35]. Indeed, excessive upper facial width is one of the most consistently reported features in unaffected relatives [34, 35]. To assess the effects of genes on face shape, Indencleef et al. [17] evaluated an array of single nucleotide polymorphisms (SNPs) known to have a role in human non-syndromic clefting. The authors found a positive correlation for some SNPs near the genes *NOG* (associated with philtrum shape), *THADA* (associated with the morphology of the supraorbital ridge) and *PAX7*, *MSX1*, and *PTCH1* (all related to the shape of the nose), thus providing evidence that genetic variants associated with clefting also affect normal craniofacial morphology, with most notable effects being in the chin, nose, supraorbital ridges, and philtrum area [17]. Several human face shape investigations based on genome-wide association studies (GWAS) found chromosomal regions harbouring genes involved in orofacial clefts that were also associated with the development of the aspects of normal-range face shape. These genes showed a significant connection with the phenotypes and developmental events related to clefting, and included the cleft candidate genes *PRDM16*, *PAX7*, *PKDCC*, *SATB2*, *COL8A1*, *MSX1*, *FAT4*, *FGFR2*, *PAX9*, *BMP4*, *GREM1*, *NOG*, *BMP2*, *PAX1*, and *BMP7* [32]. Taken together, these data indicate the existence of specific genes that determine normal facial conformation as well as its normal-range variation which, however, can push individuals along the susceptibility spectrum toward the cleft threshold, and thereby leading to an increased risk for clefting or actual cleft [32]. In terms of genetic counselling and prevention, increased knowledge in this field could, therefore, potentially lead to greater precision and personalisation in estimating the risk of cleft recurrence and, thus, to improved prevention.

ENDOPHENOTYPES RELATED TO CLEFT LIP/PALATE

The face shape hypothesis is based on the postulate that the same genes that drive face shape variation and then the subtle alterations observed in parents and siblings of cleft-affected patients are also involved in the developmental pathogenesis of orofacial clefting. This is in line with recent studies showing that genes that impinge upon normal facial shape in humans and mice also provide insights into the genetic basis of orofacial clefting, thus indicating a continuum between normal development, small alterations observed in a peculiar shape of the face and a true cleft [2, 15, 17]. Endophenotypes are characteristics associated with a pathological condition or trait, which are also present in unaffected family members [19]. Therefore, regarding the facial shape hypothesis, peculiar facial features present in the child with non-syndromic orofacial cleft as well as in the unaffected relatives constitute a facial endophenotype and represent an expression of underlying genetic susceptibility to the development of the trait. By definition, the endophenotype is heritable, can independently manifest the trait in question, co-segregates with the trait within the family and is more frequent in unaffected family members compared to the general population [8, 19]. Therefore, endophenotypes are features clearly linked with a multifactorial condition and are state-independent, that is, they are present in an individual regardless of the presence or absence of the pathological trait [19]. Endophenotypes can therefore be defined as intermediate phenotypes that are still within the normal range but are associated with the clinically manifested trait and, consequently, can be used as markers of disease risk. In this respect, the study of the endophenotypes can help identify candidate genes potentially responsible for the disease trait [19] and, thereby, decipher the aetiology of orofacial clefts. In addition to altered facial shape, which we have already discussed, other endophenotypes have been identified in unaffected relatives of patients with non-syndromic orofacial clefts: mild disruption of the upper lip musculature, alteration of the continuity of the nasolabial folds, the appearance of “whorls” on the surface of the lower lip, dental anomalies, and reduced olfactory function [16, 23, 25, 26]. As we have seen, numerous studies have indicated that subtle differences in the faces of non-cleft relatives from cleft families constitute endophenotypes. In particular, unaffected relatives can exhibit midfacial retrusion, broadening of the upper face or a tendency to an upturned nose and protruding chin in comparison with subjects with no family history of clefting (Figure 5) [16]. Moreover, unaffected parents of children with non-syndromic CL/P exhibit a tendency to reduced philtrum width [35] – possibly due to smaller medial nasal prominences during embryonic development. In one study, genetic risk

factors for non-syndromic CL/P were indeed associated with a reduction in philtrum width in unaffected individuals. Genetic overlap was found between non-syndromic CL/P and normal variation in philtrum width, and genetic risk for developing CL/P has been consistently associated with philtrum narrowing. This study therefore interprets smaller philtrum width as a sub-phenotypic manifestation of non-syndromic CL/P, thus highlighting the shared genetic influences on normal-range facial variation and cranio-facial anomalies [15, 17]. Another study found evidence of a specific facial phenotype and an increased risk of olfactory dysfunction in unaffected first-degree relatives of subjects with cleft lip/palate, and these phenotypic features can probably be explained by the presence of shared cleft lip/palate-related susceptibility genes. In this survey, non-affected relatives showed midface retrusion, hypertelorism, and olfactory dysfunction compared to controls, and altered olfactory function was correlated to a smaller upper nasal region and, therefore, a smaller central olfactory area [25]. The association between orofacial cleft-linked loci and normal-range facial variation in the central facial area of the nose and the philtrum [17] is not unexpected as the central part of the nose, the philtrum and the primary palate are formed by merging of the medial nasal prominences to create the intermaxillary segment which then fuses with the lateral nasal and maxillary prominences to form the nose and upper lip/primary palate [1]. Thus, it is likely that the same genetic regulatory mechanisms are involved in nasal and philtrum morphology and in lip/palate fusion [17]. Specifically, these findings point to a facial endophenotype characterized by midfacial retrusion and decreased philtrum width, both possibly reflecting a retruded and constricted maxilla [25].

Mouse studies of mutants in *TFAP2A*, a gene implicated in CL/P, have suggested that small changes in gene expression that alter the growth and shape of embryonic facial prominences may be a possible cause of clefts [9]. If fusion of embryological structures takes place, however, the resulting altered shape might constitute the intermediate state of a facial endophenotype. Thus, there is a clear intersection between normal facial development through shape alterations and birth defects such as CL/P [16].

In summary, most studies of cleft-related endophenotypes have focused on subtle variations in facial morphology or minor changes including the anatomy of the orbicularis oris muscle, nasolabial folds and the olfactory function in unaffected relatives in line with the basis of the face shape hypothesis [23, 26].

CONCLUSION

Non-syndromic orofacial clefts have a multifactorial aetiology, with environment-dependent factors and a complex genetic component not yet fully elucidated, thus requiring an approach to the study from different angles and perspectives. Because of their family history, individuals at increased risk for orofacial clefts tend to exhibit a pattern of facial features that seems to correlate with a predisposition to clefting in their offspring. Moreover, such differences in facial pattern may help identify high-risk families and thus improve estimates of recurrence risk [7]. The theoretical basis underlying the distinctive face conformation in unaffected relatives is that the processes causing failure of fusion of nasal/maxillary prominences during embryonic life lead to clefting, while less severe forms may result in altered growth responsible for facial physiognomy “at risk”. The concept that variation in facial shape has some relationship with genetic susceptibility to orofacial clefting is not unexpected, due to the knowledge that candidate genes for orofacial clefting have been shown to play a role in the normal development of the face; so many of the studies on human dysmorphologies have been informative about normal craniofacial development. Studies relating craniofacial morphology to cleft incidence in populations have provided an indirect line of evidence supporting the face shape hypothesis. As mentioned before, epidemiological studies indicate that the prevalence of clefting varies widely in different populations, with Asians having the highest rates, followed by the Europeans and, lastly, Africans who have the lowest risk. It has been hypothesised that the populations with the highest incidence of clefting have facial morphological features similar to those observed in unaffected relatives of individuals with CL/P. Indeed, compared to other populations, some Asian groups seem to be characterised by a broader upper face and smaller facial convexity [31].

In conclusion, orofacial clefts are common birth defects that warrant significant medical care and also place a psychological and social burden on both patients and their families, requiring a long and multidisciplinary follow-up which often includes several surgical procedures. Individuals with clefts and their unaffected family members sometimes manifest some minor craniofacial anomalies and differences in facial morphology compared to the general population. Attempting to shed light on the genetic architecture of non-syndromic clefts, it may be useful to study the phenotype of unaffected first-degree relatives of patients, since they have a high probability of carrying genes for cleft susceptibility and are holders of endophenotypes. Since the study of endophenotypes can lead to the identification of candidate loci for disease-driving genes, it is

therefore hoped that this approach will help improve the prevention and treatment of these disfiguring anomalies.

ACKNOWLEDGEMENTS

I would like to thank Mr Fabio Nangeroni for his impeccable technical assistance and helpful advice.

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