Genetic Screening in Patients with Craniofacial Malformations

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Abstract

Craniofacial malformations include a variety of anomalies, including cleft lip with or without cleft palate, craniosynostosis, microtia, and hemifacial microsomia. All of these anomalies can be either isolated or part of a defined genetic syndrome. A clinical geneticist or genetic counselor should be a member of the craniofacial team to help determine which patients have isolated anomalies and which are likely to have a syndrome. They would then arrange for the appropriate genetic testing to confirm the diagnosis of the specific syndrome. The identification of the specific syndrome is important for the overall care of the patient (as it identifies risk for other medical problems such as congenital heart defect) that will have to be taken into account in the care of the craniofacial malformation. In addition, knowing the specific syndrome will allow the family to understand how this happened to their child and the recurrence risk for future pregnancies. With the advent of new technologies, there are now many types of genetic testing available (including, karyotype, fluorescence in situ hybridization, chromosomal microarrays, and next generation sequencing) and the medical geneticist and genetic counselor can determine which specific testing is needed for a given patient.

Keywords
- craniofacial anomalies
- chromosomal microarrays
- karyotype
- next generation sequencing

Introduction

Craniofacial malformations are some of the most common birth defects and encompass a variety of disorders, including cleft lip with or without cleft palate (CL/P), cleft palate alone (CP), craniosynostosis, microtia, hemifacial microsomia, and many other well-described craniofacial syndromes. All of these craniofacial anomalies can be isolated or part of a genetic syndrome. The role of the clinical geneticist and the genetic counselor in the craniofacial/cleft palate team is to identify which patients have isolated anomalies and which are likely to have a syndrome. This is done by taking a careful history, doing a three-generation pedigree, and a complete physical examination. If a syndrome is suspected, the geneticist or counselor can determine and arrange for the appropriate genetic testing and additional evaluations as needed. This helps families understand how the craniofacial malformation happened and what the recurrence risk will be for future pregnancies for them as well as for other family members. In addition, when a syndrome is diagnosed, there are often other medical issues and so it is the genetics professionals' duty to educate the family and craniofacial/cleft palate team members about other medical issues for which the child is at risk for (i.e., developmental delay and neuropsychiatric disorders in 22q11 deletion syndrome).

Craniofacial Development

The embryonic development of the head involves a highly complex and delicate process guided by a large array of genetic components. Because proper development of the face requires such complex coordination of cell growth, migration, differentiation, and apoptosis, any genetic changes in the genes encoding these areas as well as environmental influences disturbing these

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factors may result in abnormalities involving a wide spectrum of anomalies.[1] Craniofacial development begins in the fourth week of development with the migration of neural crest cells forming the five facial primordia. Formation of the facial prominences is followed by the development of the upper lip and primary palate during 6 and 7 weeks of gestation. Failure of growth or fusion of any processes during this time results in deformities involving the upper lip, alveolus, and/or primary palate.[1] The secondary palate begins to form during the seventh week of embryogenesis, with the emergence of the palatal shelves. Continued growth results in the fusion of the palatal shelves at the midline along the medial edge epithelia, where the nasal cavity becomes completely separated from the oral cavity. A failure at any of these steps may result in clefts of the palate.[1] Unfortunately, much of our knowledge on the role of specific genes in the formation of the human head remains unknown due to the multifactorial nature of these disorders. Scientists have traditionally used a wide range of statistical tools for genetic analysis in families, including segregation and linkage analyses, but not until the recent advances in molecular genetics have they been able to come closer to elucidating the pathogenesis of many of these craniofacial malformations.[2]

Advances in Genetic Testing

There have been a lot of advances in genetic testing in the last few decades. Karyotype analysis was historically used, which looks at chromosome number and searches for deletions or duplications. This was then followed by fluorescent in situ hybridization (FISH), which looks for specific smaller deletions such as 22q11 deletion syndrome. More recently, chromosomal microarray/comparative genomic hybridization became available which looks at the entire genetic material at finer detail compared with karyotype and FISH. The newest technologies are based on next generation sequencing that looks at the individual base pairs of the DNA encoding proteins. The testing available includes either doing a panel of genes for a specific disorder or whole exome sequencing (WES). Many genetic testing companies and academic medical center laboratories offer panels of genes for specific disorders as well as WES.

Orofacial Clefts

Orofacial clefts (OFCs) are a heterogeneous group of disorders that are traditionally categorized as either CL/P or CP. Clefts affect approximately 1/700 live births, with a wide varying incidence depending on geographic origin, racial, and ethnic background, environmental exposure, and socioeconomic status.[3] Although not a major cause of mortality in developed countries, OFCs are among the most common birth defects worldwide, with considerable morbidity to affected children and substantial financial risk for families. Individuals may experience problems with feeding, speaking, hearing, and social integration, often requiring multidisciplinary and long-term treatment, including surgery, dental treatment, speech therapy, and psychosocial intervention to varying degrees. Clinical manifestations of these defects are diverse, as they can occur as isolated, nonsyndromic anomalies or as part of the Mendelian syndromes.

Cleft Lip with or without Cleft Palate

Approximately 70% of CL/P cases are isolated meaning that they are nonsyndromic and are not associated with any other recognizable anomalies. The remaining 30% of cases of CL/P are classified as "syndromic," and present in association with other deficits or structural malformations. It is the role of the medical geneticist and genetic counselor to determine which cases are isolated and which are syndromic. There are over 500 Mendelian syndromes, including those arising secondarily to chromosomal or teratogenic effects associated with CL/P.[4]

Nonsyndromic Cleft Lip with or without Cleft Palate

Understanding the genetic etiology of nonsyndromic CL/P has been traditionally difficult, as this complex multifactorial disorder displays varying levels of penetrance, sex differences, and environmental overlays.[3] Past genetic approaches to causative gene identification include linkage analyses and identification of chromosomal anomalies, while newer methods involve direct sequencing and genome-wide association studies (GWAS). Studies showed strong evidence for the linkage of nonsyndromic CL/P to a region on chromosome 9q21, which after subsequent fine-mapping, suggested the significance of forkhead box protein E1 (FOXE1) as a susceptibility gene for nonsyndromic CL/P.[1] FOXE1 is a forkhead-containing transcription factor, genetically expressed at the point of fusion between maxillary and nasal processes during palatogenesis.[5] Mutations in FOXE1 resulted in the occurrence of CL/P, and a null mutation of FOXE1 specifically in mice studies showed cleft palates.[5]

Other genes identified as significant candidate genes for nonsyndromic CL/P include interferon regulatory factor 6 (IRF6) and transforming growth factor α (TGFA). IRF6 was first implicated as the gene underlying van der Woude syndrome (VWS) and popliteal pterygium syndrome, and is the first causal gene identified for syndromic CL/P that has contributed to the pathogenesis of clefting.[6] As a member of a large family of transcription factors that bind to specific DNA sequences, IRF6 regulates gene expression, and its variants also contribute to nonsyndromic familial clefts.[7] TGFA is involved in intercellular signaling in mice, and has been localized in the epithelium of the palatal shelves before fusion.[8] Positive associations were reported between nonsyndromic CL/P and the TGFA locus with inconsistent replication studies, however, a meta-analysis of past TGFA studies has concluded the significance of this gene as a risk factor for orofacial clefting.[6] GWASs, specifically, have confirmed and strengthened the significance of IRF6 and FOXE1, but have also led to the discovery of a new region of significance on chromosome 8q24. Subsequent extension of this study identified two additional significant loci on chromosomes 10q25 and 17q22.[6]

Syndromic Cleft Lip with or without Cleft Palate

The classification of syndromic CL/P is primarily based on the presence of additional physical or cognitive abnormalities. At least 275 syndromes have been identified in which a mutation of a single genetic locus, chromosomal abnormality or teratogen has been the cause.[1,4] Around 75% of these syndromes have a known genetic cause, including hundreds of

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Mendelian disorders, which result from a single gene defect.[1] The common syndromes that are associated with CL/P include chromosomal abnormalities (trisomy 13, trisomy 18, and trisomy 21), microdeletion syndromes (22q11 deletion syndrome), and single gene disorders.

One of the most common human autosomal dominant disorders associated with CL/P is VWS, which accounts for approximately 2% of all CL/P cases.[1] VWS occurs 1/34,000 live births and is caused by mutations in the *IRF6* gene.[9,10] DNA sequencing has also identified causative genes in Kabuki syndrome, Miller syndrome, CHARGE syndrome, and Bartsocas–Papas syndrome.[1]

Cleft Palate Alone

CP is embryonically a completely separate entity from CL/P and is another genetically distinct subgroup of orofacial clefting. CP occurs in 1/1,500 live births and is associated with at least 370 different malformation syndromes.[11] Approximately 50% of cases of CP are isolated while the other 50% are syndromic.[4] Syndromic causes of CP include trisomies (13, 18, and 21), microdeletion syndromes such as 22q11 deletion syndrome as well as single gene disorders. Other syndromes associated with CP include Pierre–Robin sequence, Schilbach–Rott syndrome, and Glass syndrome.[12–14]

Several genes associated with the cleft palate phenotype have been identified, however, the etiology of the majority of cases remain vague. Past studies of X-linked cleft palate localized the causative gene to be located on chromosome Xq21, pinpointing a variety of mutations in the *TBX22* gene significant. *TBX22* is expressed in the developing palate and is the first gene identified for a major CP syndrome. Mouse studies show that targeted disruption of Tbx1 results in a wide range of developmental anomalies which encompass almost all of the common features of the DiGeorge/velocardiofacial syndromes.[8] FitzPatrick et al, identified *SATB2*, a gene with undefined function, as the cleft palate gene on 2q32-q33. Further studies suggest its role in transcriptional regulation and disruption of SATB2 function appears to be the cause of the isolated cleft palate displayed, though further mutation analysis studies are needed to confirm its role.[15]

Craniosynostosis

The human skull is made up of several bones (frontal, parietal, occipital, and squamosal) which are held together by fibrous-like hinges called sutures. Premature closure of one or more of these sutures is craniosynostosis, a relatively common birth defect that occurs in 1/1,000 births.[16] Craniosynostosis can be presented in either a nonsyndromic (isolated) form or syndromic form, and can be further classified depending on affected suture/sutures as well as other clinical findings. Isolated craniosynostosis usually only involves one suture and there is little to no recurrence risk for families. The most common craniosynostosis syndromes are due to mutations in the fibroblast growth factor receptor 2 (*FGFR2*) gene, which is involved in the signaling of immature cells to become bone cells during embryonic development.[17] A mutation in a specific part of the *FGFR2* gene alters the protein to prolong signaling, which can promote the premature fusion of bones in the skull, hands, and feet.

The eight known FGFR-related craniosynostosis include Crouzon syndrome, Crouzon syndrome with acanthosis nigricans, Apert syndrome, Pfeiffer syndrome, Muenke syndrome, Jackson–Weiss syndrome, Beare–Stevenson syndrome, and FGFR2-related isolated coronal synostosis. The majority of these syndromes can be diagnosed primarily on clinical findings, however, Muenke syndrome and FGFR2-related isolated coronal synostosis require further molecular genetic testing. Diagnosis of Muenke syndrome is based on the identification of the p.Pro250Arg mutation in FGFR3 and diagnosis of FGFR2-related isolated coronal synostosis is based on the identification of a disease-causing mutation in FGFR2.[17] Muenke syndrome is the most common craniosynostosis syndrome, with a prevalence of 1 in 30,000 live births.[4] It is autosomal dominant, and is caused by a point mutation in the FGFR3 gene, which results in p.P250R (proline to arginine substitution at amino acid 250).[4] Common characteristics include unilateral or bilateral coronal craniosynostosis, carpal and/or tarsal bone fusion, developmental delay, and sensorineural hearing loss. Associated anomalies may include mild-to-significant midface hypoplasia, ocular hypertelorism, and high-arched palate.[4]

Microtia

Ranging in severity from mild structural abnormalities to complete absence of the ear, microtia is a congenital anomaly resulting from a developmental malformation of the external ear. The prevalence of microtia varies among regions, from 0.8 to 17.4 per 10,000 live births, more frequently in males, with a particular increase in Hispanic, Asian, Native American, and Andean populations.[18] Microtia can occur unilaterally or bilaterally, and can present in an isolated form or syndromic form. The unilateral form is more common, seen in 79 to 93% of cases, and is associated with normal hearing in the other ear with normal speech and language development.[19] These unilateral forms are usually isolated. Approximately, 15 to 60% of patients with microtia have additional anomalies, and seen more commonly in cases of bilateral microtia. The most common associated malformations include facial cleft, facial asymmetry (hemifacial microsomia), renal abnormalities, cardiac defects, microphthalmia, polydactyly, and vertebral anomalies.[19] As with most craniofacial anomalies, the etiology of microtia is still poorly understood. There is strong evidence confirming the importance of environmental causes for microtia, however, the genetics of this disorder is less understood as a wide variety of chromosomal abnormalities, complex chromosomal rearrangements, microdeletions, and even distinct hereditary genomic copy number variants have been reported.[19] Some studies have identified candidate genes, however, no causal genetic mutation has been confirmed.

Developmental defects for some microtia-associated syndromes are more clearly understood as they reveal novel information on the molecular mechanisms involved in human craniofacial and ear development. Mutations in the Treacher Collins–Franceschetti 1 (*TCOF1*) gene have been identified as the cause of Treacher Collins syndrome (TCS), an

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autosomal dominant disorder of craniofacial development. This syndrome is characterized by hypoplastic facial bones, microtia, micrognathia, and other deformities of the external and mid-ears, auditory pits, hearing loss, and cleft palate.[19] *TCOF1* is involved in the development of the craniofacial complex, as mouse studies suggest its role as a regulator of ribosome biogenesis.[19] Recently two additional genes, *POLR1C* and *POLR1D*, have been shown to cause TCS.[20] Deletions on chromosome 22q11 cause DiGeorge syndrome and velocardiofacial syndrome and are now known as 22q11 deletion syndrome.[21] Clinical manifestations include craniofacial abnormalities (CL/P, CP), ear defects, hearing impairment, thymus and parathyroid gland hypoplasia, and cardiac malformations. Conductive hearing loss is prominent in most cases, as is middle and outer ear anomalies, as well as inner ear malformations. In 22q11 deletion syndrome, up to 30 genes can be deleted, including *TBX1*, a member of the T-box gene family of transcription factors that is required for normal ear development during embryogenesis. The deletion of this single gene is sufficient to cause most of the abnormalities seen in this syndrome.

Another syndrome commonly associated with microtia is Nager syndrome. Patients with Nager syndrome present with micrognathia, external ear defects, external auditory canal stenosis, bilateral conductive hearing loss, cleft palate, downslanting palpebral fissures, high nasal bridge, hypoplastic or absent thumbs, and variable lower limb and toe defects. Through exome sequencing, 18 different mutations in the *SF3B4* gene, most of which are frameshift mutations, have been attributed to causing Nager syndrome.[22] Other genes implicated with this syndrome include the homeobox genes *PRX1* and *PRX2*. Double-mutant mouse studies suggest *PRX2*'s role in external, middle and inner ear deficiencies, while inactivation of both genes result in additional defects in the craniofacial, skull, and vertebral structures. However, no *PRX1*or *PRX2* mutations have been found in Nager syndrome patients to date.[19]

In addition to isolated cases of craniofacial malformations, many associations between craniofacial malformations and genetic syndromes have been observed and studied. With this in mind, it is greatly beneficial for individuals with anomalies such as OFCs, craniosynostosis, and microtia to consult with a clinical geneticist or genetic counselor. With the advancement of scientific technology, genetic testing allows for the determination and confirmation of an expanding number of genetic syndromes with known causes. As a result of identifying genetic syndromes, patients will be able to better understand the origin of their craniofacial malformations, other medical problems that they may be at risk for, and the recurrence risk for future pregnancies.

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