The Pediatric Dentist as Amateur Geneticist[1]

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Pediatric dentists treat children with craniofacial anomalies and therefore should have a working knowledge of syndromology and molecular genetics.[1] Often pediatric dentists are the first health care practitioners to document dysmorphic features in a child. It is important for them to have an understanding of molecular genetics because the sensitivity and specificity of molecular-based diagnostics have revolutionized how diseases and disorders are defined.[2] These scientific and technological advances translate into improved health, disease prevention, smarter diagnostics, and innovative therapeutic approaches to craniofacial dysmorphogenesis.[2]

Prior to molecular-based diagnostics, craniofacial disorders were established based on characteristic features (pattern recognition), e.g., a child with malar hypoplasia, mandibular retrognathia, down slanting palpebral fissures, coloboma of the lower eyelid had the clinical appearance of Treacher Collins syndrome. Although the common disorders were identifiable, there was difficulty establishing the diagnosis for all patients. Some had clinical characteristics that did not readily fit into a particular syndrome, or the condition was uncommon and most clinicians were unaware of its existence.

With molecular diagnostics it is now possible to establish the correct diagnosis for most patients with craniofacial anomalies. Using this new tool it has become evident that classification of craniofacial malformations based on clinical features (phenotype) is sometimes quite different from categorization by genetic findings (genotype).[2] Patients with craniofacial syndromes may have similar clinical phenotypes that are caused by different mutations in a gene; identical mutations within a gene can cause widely different clinical phenotypes. Additionally, mutations in different genes can cause similar clinical phenotypes.[2] Molecular-based diagnostics for complex craniofacial anomalies has truly transformed how we define these disorders.

Gene Identification

The entire human genetic lexicon is encoded in approximately 100,000 different genes assembled to constitute the human genome. Every somatic cell nucleus in the human body contains the entire genome packaged into 23 pairs of chromosomes.[2,3] A chromosome is a very long condensed DNA molecule and its associated proteins that contain many genes. A gene is a region of DNA that controls a hereditary characteristic. It usually corresponds to a sequence used in the production of a specific protein. A gene carries biological information in a form that must be copied and transmitted from each cell to its progeny. Genes can be as short as 1,000 base pairs or as long as several hundred thousand base pairs.

The first step in understanding the molecular genetics of a syndrome is to identify and positionally clone the gene associated with the malformation. The story of Boston-type craniosynostosis illustrates how the first gene mutation associated with craniosynostosis was identified. Warman and colleagues, at Children's Hospital, Boston, had been caring for a large family with many members in multiple generations having craniosynosotosis.[4] They believed that this family would be an excellent candidate in which to look for the gene responsible for craniosynostosis and also important in craniofacial development. They obtained an in-depth family history, and thoroughly examined and drew blood from each family member.

The craniosynostosis phenotype within the family was variable with 4 general types identified based on the extent of sutural involvement: 1) fronto-orbital recession; 2) frontal bossing; 3) turribrachycephaly; and 4) clover-leaf skull deformity. None of the family members had midfacial

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hypoplasia, malocclusion, exorbitism, orbital hypertelorism, blepharoptosis, or hand-and-foot anomalies seen in other syndromes. Based on their clinical examinations Warman et al found 19 members with craniosynostosis spanning 3 generations, and the trait was inherited from the grandmother.[4]

For most craniofacial syndromes there are no gross chromosomal abnormalities that identify the specific location of a genetic mutation.[3] Finding the particular gene responsible for a disorder requires the use of a variety of techniques. Linkage mapping identifies the chromosomal location of a disease-causing gene but does not pinpoint the specific mutation. Other strategies are needed to find the responsible gene in the chromosomal region.

Polymorphisms act as gene markers and are essential tools for the technique of linkage analysis. Polymorphisms are variations in the DNA sequence that exist as a stable component of the population's genome. Single-nucleotide polymorphisms (SNP), in which a single base in the DNA sequence is altered, occurs every 1,000 bases along the genome. Some of these SNP's cause a specific disease, some result in normal genetic variation, and others change the sequence of DNA without changing the function of the gene. [5]

Linkage analysis asks the question, Do specific chromosomal markers (SNP's) travel (cosegregate) with the gene responsible for the abnormal phenotype in a family? If so, the locus is said to be "linked" to the gene that causes the disorder – meaning the causative gene is physically close to that marker, within several million base pairs of DNA.[3] Using this technique it is possible to localize a hereditary disorder to a specific chromosomal region.

Müller and colleagues performed linkage analysis on all members (both affected and unaffected) of this family.[6] They tested a randomly chosen short tandem repeat polymorphism (STRP) marker and found linkage with locus D5S211 which had been previously assigned to the distal arm of chromosome 5q.

Although the location of the mutation on the chromosome responsible for craniosynostosis in this family was identified, other strategies were needed to find the specific gene. Positional cloning can be used to look for the gene in the area of the chromosome identified with linkage analysis. However, this method requires searching for all of the genes contained within the region and identifying mutations in each of these, which can be tedious if there are thousands of genes in the interval. Candidate analysis is a more specific method of detecting a mutation but it requires being familiar with the genes in the region and then selecting 1 or more of them based on their known function and how they may relate to the disorder in question. Fortunately, Jabs and colleagues had a likely candidate gene assigned to the region on 5q that had been identified with linkage analysis.[7]

In the mouse, MSX2 expression occurs along calvarial sutures, and has a role in cranial morphogenesis. The human MSX2 gene is similar to the mouse gene and the human MSX2 gene had been mapped to chromosome 5 near the locus responsible for Boston-type craniosynostosis. The chromosomal location of the human MSX2 gene near Boston-type craniosynostosis led Jabs and colleagues to examine MSX2 further as a possible candidate gene for this disorder. They used a marker for the MSX2 gene and found that all affected members of the family inherited this marker (202 bp allele). They demonstrated tight linkage between MSX2 and craniosynostosis, supporting the hypothesis that a mutation in the MSX2 gene was responsible for Boston type craniosynostosis.

To search for the specific mutation in MSX2 that might cause craniosynostosis in this family, Jabs and colleagues sequenced the gene in 2 affected and 2 unaffected family members.[7] They detected a mutation, a single base change from cytosine to adenine resulting in substitution of a histidine (CAC) for a proline (CCC) at amino acid position 7. This mutation occurs in a location that is highly conserved across phylogenetic distances from drosophila to humans – all possess a proline in position 7 of the homeodomain. The conservation of this residue over millions of years of evolution suggests that substitution at this location substantially alters the function of the MSX2 gene. This study showed that a histidine substitutes for a highly conserved proline at position 7 of the MSX2 homeodomain exclusively in affected members. These results provided compelling evidence that the mutation causes this craniosynostosis syndrome.[7]

Although the first mutation to be associated with craniosynostosis was found in MSX2, Boston-type is the only syndrome associated with this gene. Mutations for other craniosynostoses have been identified in 3 of the fibroblast growth factor receptor (FGFR) genes and in the TWIST gene.[8]

Most mutations for the major craniosynostosic disorders are on FGFR2; Crouzon is caused by more than 30 mutations in FGFR2; and Pfeiffer links to 1 mutation in FGR1 and several in FGFR2. Although there are many mutations for Crouzon and Pfeiffer syndromes there are only 2 for Apert syndrome both on FGFR2. Mutations in FGFR3 are responsible for FGFR3 associated coronal synostosis syndrome, or Muenke syndrome, and Crouzonodermoskeletal syndrome. Mutations in the human TWIST gene cause Saethre-Chotzen syndrome, one of the most common autosomal dominant craniosynostotic disorders.[9]

Mutations of FGFR and MSX2 genes are generally described as activating or gain of function mutations. This means that the receptors are turned on even in the absence of their ligand (constitutively activated). Mutations in TWIST are considered loss of function; but the "function" in both gain and loss of function mutations that is affected is unclear.[8] Although the craniosynostotic syndromes occur from mutations in different genes there is evidence to suggest that MSX2, TWIST, and FGFR are part of the same biochemical pathway involved in cranial suture differentiation.[10,11]

Gene Function and Disease

Once the gene responsible for a disorder has been identified and positionally cloned, the next step is to determine the function of the gene and understand how the mutation alters this function and causes disease. This requires multiple experimental strategies, including testing the gene product in biochemical assays in vitro, assays of its function in suitable cells in culture, and whole-animal assays, such as those using transgenic mice.[2] The mouse genome is about the same size as the human genome and comprised of essentially the same genes making it an excellent model for studying the function of human genes and for the pathogenesis and application of new treatments and novel therapies.[5]

Warren and colleagues recently published compelling data as to how a fibroblast growth receptor gene (FGFR) mutation results in craniosynostosis.[12] In the mouse, the posterior frontal cranial suture fuses in the first 45 days of life, whereas the sagittal and coronal sutures remain patent throughout life.[13] These authors asked the question, What is the difference between the fusing and non-fusing sutures? They started by looking to see if there were any differences between the sutures in factors known to promote bone formation, such as bone morphogenic proteins (BMPs).[14,15] Using BMP4 immunolocalization in 18-day-old sagittal and posterior frontal sutures they found abundant BMP's in both patent sagittal and fusing posterior frontal sutures. If BMP is present in both the non-fusing and fusing sutures then why do the sagittal and coronal sutures remain patent?

Noggin, an antagonist of BMP, is expressed postnatally in patent but not fusing cranial sutures. Noggin inhibits BMP activity and keeps sutures patent.[16-18] Warren and colleagues searched for noggin before (day 15), during (days 35 and 42), and after (day 50) the period of predicted suture fusion.[12]They detected noggin in the patent sagittal and coronal sutures. In marked contrast, there was almost no noggin expression in the fusing posterior frontal suture complex as early as day 15. Because BMP4 is present in both fusing and patent sutures, and osteoblasts line the osteogenic fronts of these sutures, they examined the effects of BMP4 on noggin expression in osteoblasts. Primary calvarial osteoblasts from all sutures treated with BMP4 expressed noggin protein in a dose-dependent manner.

These data showed that calvarial osteoblasts lining non-fusing and fusing sutures produced noggin in response to BMP. If BMP4 induces noggin expression, how can the posterior frontal suture fuse? Significantly, only the posterior frontal suture dura mater expresses fibroblast growth factor 2 (FGF2) signifying that FGF2 might regulate BMP4-induced noggin expression in calvarial osteoblasts.[13,19] FGF2 disrupts noggin induction in a dose-dependent fashion suggesting environments with a low FGF2 concentration (ie, the sagittal and coronal sutures) might not suppress BMP-induced noggin expression. However, environments high in FGF2 concentration, (ie, the posterior frontal suture) reduce noggin expression and enable suture fusion. Treatment with FGF2 suppressed noggin production in the coronal and sagittal sutures in a dose dependent fashion. This implies that FGF2 guides suture fate, patency versus fusion, by regulating suture specific noggin production in osteoblasts and, in turn, suture-specific BMP activity. These data indicate a possible mechanism for syndromic craniosynosotoses: the FGFR gain-of-function mutations cause FGF2 receptors to behave as if there is abundant ligand (FGF2), decreasing noggin expression, and leading to suture fusion. Warren and colleagues tested this hypothesis by injecting an FGF2-expressing adenovirus into normally non-fusing coronal dura mater of neonatal (lacZ/noggin) transgenic mice.[12] This led to the suppression of noggin expression in all animals, and pathological coronal suture fusion. Taken together, these cell culture and in vivo data suggest that increased FGFR signaling might lead to suture fusion by suppressing noggin production in the dura mater and osteoblasts of normally patent cranial sutures.

Having demonstrated that noggin is normally expressed in the patent suture complex and that posterior frontal dura mater-derived FGF2 suppresses noggin, they proposed that forced expression of noggin would maintain posterior frontal suture patency. To test the effects of noggin misexpression [overexpression] in vivo, the posterior frontal sutures of 3-day-old CD-1 mice were injected with a noggin adenovirus. After 50 days, the noggin-infected mice had short broad snouts and widely spaced eyes owing to increased frontal bone growth perpendicular to the posterior frontal suture. Histological analysis demonstrated that the posterior frontal sutures of noggin injected mice were widely patent. This result demonstrates that noggin misexpression at an early stage of suture development has profound consequences on cranial suture fate.

In summary, noggin is a high-affinity secreted BMP antagonist that is normally present in patent sutures and enforces patency. Noggin expression is suppressed by FGF2 and syndromic FGFR signaling. Syndromic FGFR-mediated craniosynostosis may result from inappropriate down-regulation of noggin expression. These findings provide an important link between the murine models and the gain-of-function FGFR mutations associated with syndromic forms of human craniosynostosis. FGF2 suppresses noggin expression. The corollary is that forced expression of noggin maintains suture patency, suggesting the possibility that therapeutic noggin could be used to control postnatal cranial suture development.[12]

Summary

Genetic knowledge is becoming critical to the delivery of effective health care. The human genome project has identified and sequenced all of the genes coded in human DNA. Understanding the genetic mutations that interfere with formation of the craniofacial complex will improve diagnosis, prevention, and therapy.

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Abstract of the Scientific Literature

Influence of Movie Stars on the Initiation of Adolescent Smoking

Adolescents watch an average of 3 movies per week. Alarmingly, cigarette smoking among actors in movies has increased in frequency over the past decade. The objective of this study was to determine whether adolescents, whose favorite movie stars smoked onscreen, were at increased risk of tobacco use. The baseline sample of this study included nonsmokers aged 12 to 15 years who were interviewed and who nominated their favorite stars. A review of popular films released during the study period was performed to determine whether stars smoked onscreen in at least 2 films. One third of nonsmokers nominated a star who smoked onscreen, which independently predicted later smoking risk. The effect was strong among girls. Among boys, there was no independent effect after control for receptivity to tobacco industry promotions. These results provide evidence that smoking by movie stars can play an important role in encouraging female adolescents to start smoking. The data suggests that levels of smoking in movies: (1) may undermine other public health tobacco control efforts; and (2) needs to be monitored carefully.

Comments: These findings indicate that smoking by stars in movies significantly increases the risk of future smoking among adolescent girls who have never smoked, independent of the effects arising from other tobacco advertising and promotional practices. Public health efforts to reduce adolescent smoking must confront smoking in films as a tobacco marketing strategy. **FSS**

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