ORIGINAL ARTICLE

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# Syndromic craniosynostosis: from history to hydrogen bonds

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**Structured Abstract**

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The syndromic craniosynostoses, usually involving multiple sutures, are hereditary forms of craniosynostosis associated with extracranial phenotypes such as limb, cardiac, CNS and tracheal malformations. The genetic etiology of syndromic craniosynostosis in humans is only partially understood. Syndromic synostosis has been found to be associated with mutations of the fibroblast growth factor receptor family (*FGFR1, -R2, -R3*), *TWIST1*, *MSX2*, and *EFNB1*. Apert, Pfeiffer, Crouzon, and Jackson-Weiss syndromes are due to gain-of-function mutations of *FGFR2* in either the Ig II–III linker region (Apert) or Ig III domain. Loss of function mutations of *TWIST1* and gain-of-function mutations of *MSX2* lead to Saethre–Chotzen and Boston-type syndromes, respectively. The mutations in Pfeiffer (*FGFR1*), Muenke (*FGFR3*), and Apert syndrome (*FGFR2*) are caused by the same amino acid substitution in a highly conserved region of the Ig II–III linker region of these proteins, which suggests that these receptor tyrosine kinases have an overlapping function in suture biology. In this review we will discuss the historical descriptions, current phenotypes and molecular causes of the more common forms of syndromic craniosynostosis.

**Key words:** Apert; craniosynostosis; crouzon; pfeiffer; saethre-chotzen; TWIST

## Introduction

Craniosynostosis is the premature fusion of the calvarial sutures. Although clinical descriptions of craniosynostosis date back to Hippocrates and Galen, it is generally accepted that the first historical reference to craniosynostosis was by Mestrius Plutarchus (46–127AD). Known in English as Plutarch, this historian and biographer described the Athenian statesman Pericles (495 BC–429 BC) as 'squill headed' in 75AD. Squill is the common name for a plant in the lily family with an elongated bulb. All artistic renditions of Pericles depict him with his head covered by a helmet – with an unusual elongate shape. Approximately 1500 years later in *De Humani Corporis Fabrica*, Andreas Vesalius characterized specific skull shapes associated with the absence of cranial sutures (Fig. 1) (1). The recent identification of two Precolumbian skulls with sagittal synostosis (dated at 6000 and 250 BC) confirm that craniosynostosis is an ancient disorder of humans (2).

**Dates:**
Accepted 23 January 2007

**To cite this article:**
Cunningham ML, Seto ML, Ratisoontorn C, Heike CL, Hing AV:
Syndromic craniosynostosis: from history to hydrogen bonds
*Orthod Craniofacial Res* 10, 2007; 67–81

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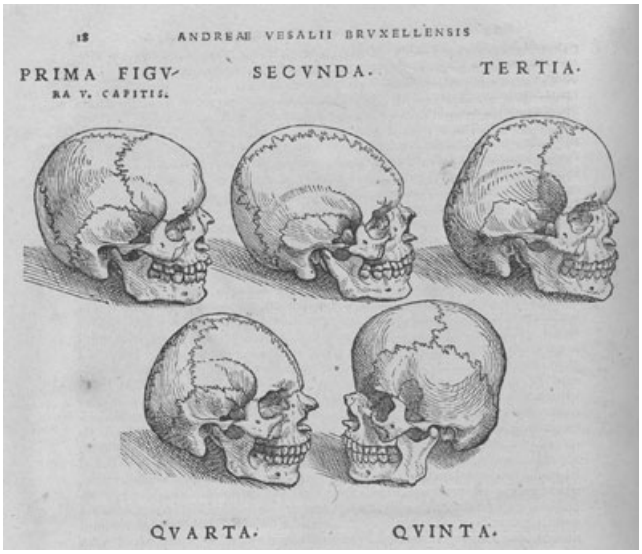


*Fig. 1.* From *De Humani Corporis Fabrica*, Andreas Vesalius, 1543. Skulls depicting absence of cranial sutures and abnormal skull shapes.

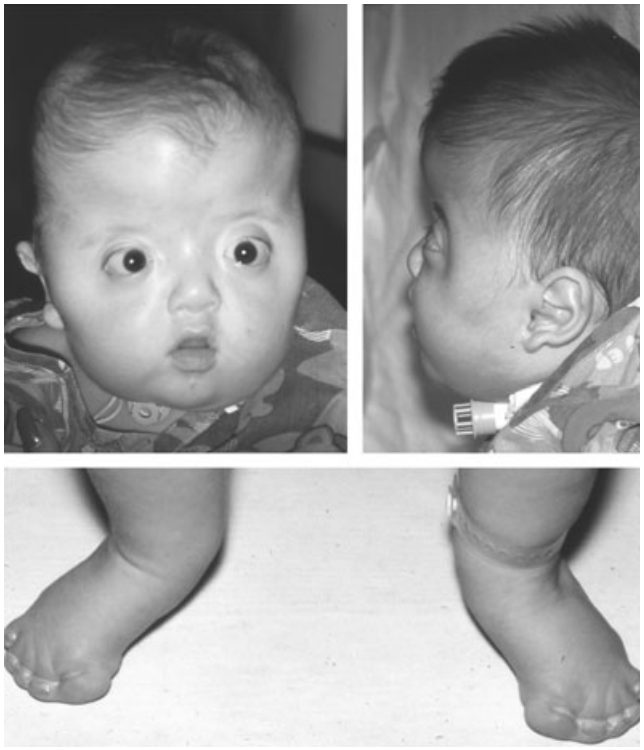

*Fig. 2.* Classic features of Apert syndrome including turribrachycephaly, proptosis, midface hypoplasia, and syndactyly. Airway compromise, because of midface hypoplasia, necessitated tracheostomy.

Craniosynostosis, which occurs in sporadic and hereditary forms, remains a major medical and dental problem with considerable morbidity. In this review, we will discuss the original descriptions, classic phenotypes, molecular genetics, and phenotype–genotype correlations of the major craniosynostosis syndromes. We will review the major clinical implications of these conditions and propose critical questions for future research endeavors.

## Fibroblast growth factor receptor mediated craniosynostoses

### History and heredity

#### Apert syndrome

The first two cases of 'Apert' syndrome were eloquently described by SW Wheaton in 1894 including craniofacial, skull base, and limb findings (3). Unfortunately he attributed the calvarial phenotype and respiratory symptoms to congenital syphilis and the syndactyly to fetal inflammation and *in utero* constraint. Twelve years later, in 1906, the French Pediatrician Dr. Eugène Charles Apert (1868–1940), described nine cases of syndactyly associated with acrocephaly 'De l'acrocephalosyndactylie' (4). Now known as Apert syndrome, this condition is characterized by coronal craniosynostosis, syndactyly, symphalangism (fusion between the phalanges of the digits), radiohumeral fusion and variable mental retardation (Fig. 2). Although usually considered to have a very consistent phenotype, large case series' have demonstrated wide variation in the craniofacial phenotype and neurocognitive outcome (5). Apert syndrome demonstrates an autosomal dominant mode of inheritance and is associated with advanced paternal age (6, 7). Management of children with Apert syndrome includes surgical correction of the craniosynostosis, midfacial hypoplasia and syndactyly. In 1995 Wilkie *et al.* (8) identified $FGFR2^{S252W}$ and $FGFR2^{P253R}$ mutations in each of 40 unrelated cases. To date four distinct mutations in *FGFR2* (S252W, P253R, and two Alu insertions) have been identified as causative in this debilitating condition (9).

#### Crouzon syndrome

Six years following Dr Apert's description of 'acrocephalosyndactyly' a neurologist named Louis Edouard Octave Crouzon (1854–1918) described a 29-year-old woman with prognathism, maxillary hypoplasia, exophthalmos, papilledema, hypermetropia, occipital headaches, and divergent strabismus (Fig. 3) (10). In addition he described her 3-year-old son who had a similar facial appearance with frontal bossing, bilateral

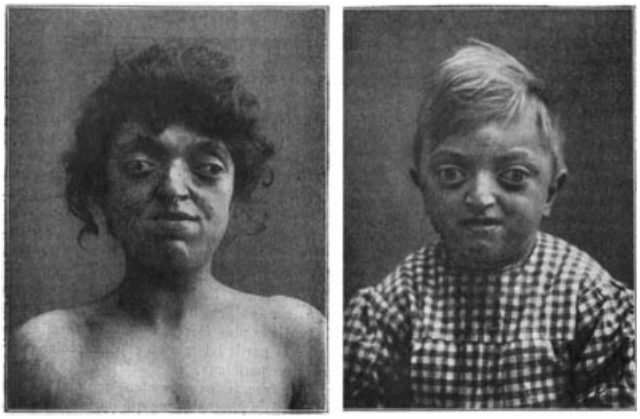

*Fig. 3.* Original cases described by Crouzon demonstrating prognathism, maxillary hypoplasia, exophthalmos, papilledema, and divergent strabismus.

exophthalmia, strabismus, and dull optic discs. Crouzon recognized the hereditary nature and absence of syndactyly as distinguishing features. Now known as Crouzon syndrome, this form of hereditary craniosynostosis also demonstrates wide phenotypic variability. The most frequent manifestations of Crouzon syndrome include coronal craniosynostosis with variable involvement of other calvarial sutures, brachycephaly, frontal bossing, proptosis, hypertelorism, strabismus, maxillary hypoplasia, mandibular prognathism, atresia of the external auditory canals, premature calcification of stylohyoid ligament, Chiari I malformation, hydrocephalus, and mental retardation. As with Apert syndrome, sporadic cases of Crouzon syndrome are associated with advanced paternal age (7, 11). In 1994 Reardon *et al.* (12) described mutations in the third Ig domain of *FGFR2* as the cause of Crouzon syndrome.

### Jackson–Weiss syndrome

In 1976, Drs. Jackson, Weiss and their coauthors described a large Amish kindred in which 138 members were reported to have an unusual spectrum of craniofacial and foot anomalies (13). Like Crouzon syndrome the craniofacial features in the original kindred included hypertelorism, proptosis, midface hypoplasia, and acrocephaly. The clinical foot anomalies included a broad and medially deviated great toe, partial cutaneous syndactyly of second and third toes, and broad and short metatarsals. Radiographs of the feet in affected individuals revealed fusion of the tarsal and metatarsal bones, broad short first metatarsals, and broad proximal phalanges (Fig. 4). As described in their manuscript 'The only consistent manifestation of the gene observed has been some abnormality in the clinical or radiologic appearance of the feet' (13). In 1994 Jabs

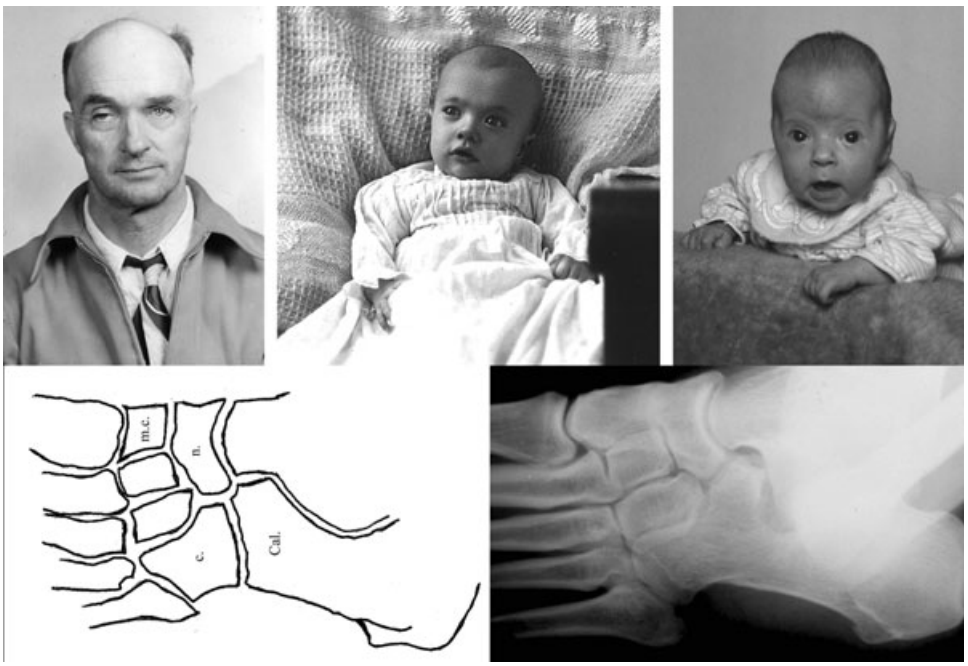


*Fig. 4.* Affected members from four generations from the original Amish kindred demonstrate the wide phenotypic variability of Jackson–Weiss syndrome. Proband (right) demonstrated severe bilateral coronal synostosis while grandfather (middle) and great grandfather (left) have milder craniofacial features. Radiograph from the maternal uncle of the proband demonstrates fusion of the medial cuneiform and navicular.

*Fig. 5.* Original family with autosomal dominant coronal synostosis reported by Glass *et al.* Note the mild midfacial hypoplasia, hypertelorism, and downslanting palpebral fissures. This family later found to harbor the *FGFR3*$^{P250R}$ mutation of 'Muenke' syndrome.

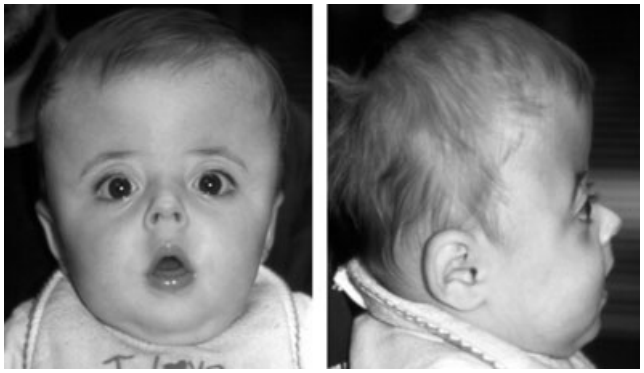

*Fig. 6.* Classic example of *FGFR3*$^{P250R}$ mediated bilateral coronal synostosis. This patient has severe bilateral coronal craniosynostosis and proptosis in the absence of significant midfacial hypoplasia.

*et al.* (14) identified an *FGFR2*$^{A344G}$ mutation in affected individuals in the original Amish kindred. Wide variability in expression of the Jackson–Weiss syndrome phenotype in this kindred was subsequently described by Heike *et al.* (15) in 2001.

### Muenke syndrome

In 1994 Glass *et al.* (16) described a family of five affected individuals with a variable autosomal dominant phenotype including premature coronal sutural synostosis accompanied by a mild midfacial hypoplasia, hypertelorism, downslanting palpebral fissures, beaking of the nose and brachydactyly (Fig. 5). Often inaccurately described as a syndrome that was based on molecular rather than phenotypic findings, we now know that the family described by Glass harbored the FGFR3$^{P250R}$ mutation of what is now commonly known as 'Muenke syndrome' (17). The identification of the P250R mutation in *FGFR3* occurring in 20 unrelated families served as the definition of this craniosynostosis syndrome (18). Unlike all other dominant forms of syndromic craniosynostosis, this syndrome is named after the discovery of the mutation rather than the first description of the phenotype. Perhaps a more appropriate designation would be Glass syndrome. Since its initial description, the phenotype of Muenke syndrome has evolved to include unilateral or bilateral coronal craniosynostosis, brachydactyly, thimble-like middle phalanges, coned epiphyses, carpal and tarsal fusions, sensorineural hearing loss, Klippel–Feil anomaly and infrequent cognitive impairment (Fig. 6) (19). As with Apert and Crouzon syndrome, sporadic cases of Muenke syndrome are associated with advanced paternal age (20).

### Crouzon syndrome with acanthosis nigricans (Crouzonodermoskeletal syndrome)

Acanthosis nigricans associated with a phenotype resembling Crouzon syndrome was first described by Helene Ollendorff Curth in 1971 in an article distinguishing benign, malignant and syndromic acanthosis nigricans (21). This initial account of 'Crouzonodermoskeletal syndrome', a term proposed by Cohen (22), could easily go unnoticed, with only 'Crouzon syndrome' listed in a table of other syndromes associated with acanthosis nigricans. No additional cases were described until 1985 when Lorraine Suslak reported a mother and her son with classic features of Crouzon syndrome (coronal and sagittal craniosynostosis, proptosis, midface hypoplasia, and choanal atresia) associated with acanthosis nigricans and odontogenic tumors (23). She proposed that the association of three rather rare conditions (Crouzon syndrome, acanthosis nigricans, and odontogenic tumors) suggested that this was a single gene disorder but did not address whether this was a rare feature of Crouzon syndrome or a distinct condition. The recognition by Dr Suslak of the constellation of a Crouzon-like craniofacial phenotype and acanthosis nigricans suggests that it may be appropriate, as in the case of other hereditary synostoses, to name this condition in recognition of her contribution (Fig. 7). In 1995 Meyers *et al.* (24) described a novel A391E substitution in the transmembrane domain of *FGFR3* only 11 amino acids from the Gly380Arg mutation of achondroplastic dwarfism. There have been no reports of the *FGFR3*$^{A391E}$ mutation in cases of Crouzon syndrome in

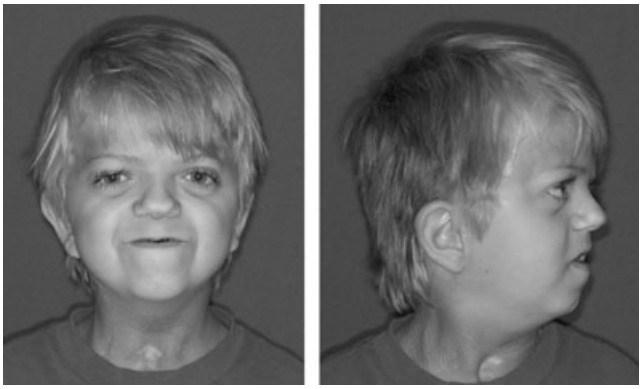

*Fig. 7.* Craniofacial and skin findings in Crouzonodermoskeletal syndrome. After successful cranioplasty to treat Kleeblatschaedel skull deformity he has residual midfacial hypoplasia. Note periorbital and perioral acanthosis nigricans. This patient was found to have the classic *FGFR3*$^{A319E}$ mutation of Crouzonodermoskeletal syndrome.

the absence of acanthosis nigricans. Since the original description by Curth in 1971, approximately 37 additional cases have been described in the literature (25–32). Although this would appear to make it one of the rarest forms of syndromic craniosynostosis the phenotypic similarities with Crouzon syndrome may lead to under diagnosis.

### Pfeiffer syndrome

Pfeiffer syndrome was described by Rudolf Arthur Pfeiffer, a geneticist from the University of Münster, Germany in 1964 as 'Dominant erbliche Akrocephalosyndaktylie' (dominant hereditary acrocephalosyndactyly) (33). Interestingly, this is the identical description used to describe Apert syndrome 60 years earlier. In his original description Pfeiffer described a condition consisting of craniosynostosis, broad thumbs and great toes, and variable soft tissue syndactyly of the hands and feet. Currently Pfeiffer syndrome is defined as the highly variable association of coronal synostosis with or without sagittal synostosis, turribrachycephly, maxillary hypoplasia, prognathism, proptosis, hypertelorism, strabismus, low nasal bridge, choanal stenosis or atresia, tracheal cartilage anomalies, radiohumeral synostosis, broad first digits, partial syndactyly of the fingers and toes, brachymesophalangy, Arnold Chiari malformation, hydrocephalus, and occasional cognitive impairment. Types I, II, and III have been defined principally on the basis of calvarial and midfacial severity with Type I the most mild and type III the most severe. Families with the Pfeiffer phenotype have been described with a mutation of *FGFR1*$^{P252R}$ as well as mutations seen in classic examples of Crouzon syndrome *FGFR2* between residues 252 and 663 (34–36). Unlike other hereditary synostoses, Pfeiffer syndrome is said to be caused by mutations in two distinct genes *FGFR1* and *FGFR2*. This phenotype based classification, in an era in which we have identified molecular pathogenesis, is at the heart of the debate over the modern classification of syndromes. Should we define conditions by phenotype or genotype? We will address this issue below.

## Molecular genetics and developmental pathogenesis of FGFR mediated craniosynostosis

The fibroblast growth factor receptor mutations seen in craniosynostosis syndromes can be divided into four categories: 1) immunoglobulin like domain II–III linker region mutations, 2) immunoglobulin-like domain III mutations, 3) transmembrane domain mutations, and 4) tyrosine kinase domain mutations (Fig. 8).

| Gene | Mutation | FGFR functional domain | Phenotype |
|---|---|---|---|
| *FGFR1* | P252R | Ig II–III linker region | Pfeiffer |
| *FGFR2* | P253R, S252W | Ig II–III linker region | Apert* |
| *FGFR3* | P250R | Ig II–III linker region | Muenke |
| *FGFR2* | Many | Ig III domain | Crouzon, Pfeiffer, Jackson–Weiss |
| *FGFR2* | A344G | Ig III domain | Jackson–Weiss† |
| *FGFR3* | A391E | Transmembrane domain | Crouzanodermoskeletal |
| *FGFR2* | Several | Tyrosine Kinase domains I and II | Pfeiffer, Crouzon |

*A case with a Crouzon phenotype harboring S252L an alternative substitution in the 'Apert' S252W locus has been described (37).
†Although the Jackson–Weiss phenotype has been associated with mutations causing a classic Crouzon phenotype, the A344G mutation has to date been described only in members of the original Amish kindred (13, 15).

### Linker region mutations of FGFR1, FGFR2, and FGFR3 result in similar phenotypes

If one considers the craniofacial phenotype resulting from the linker region mutations of *FGFR1*$^{P252R}$ (Type I Pfeiffer), *FGFR2*$^{P253R,S252W}$ (Apert), and *FGFR3*$^{P250R}$ (Muenke), one can see obvious similarities. Each principally involves the coronal suture and when bilateral, result in a remarkably similar skull phenotype


*Fig. 8.* Simplified model of an FGF receptor. Note alternative splicing of exon 8 (yellow) and exon 9 (green) results in the FGFRIIIb and FGFRIIIc isoforms. TM, transmembrane domain; TK1/TK2, tyrosine kinase domains.

(turribrachycephaly) that is distinct from the skull phenotype of classic Crouzon syndrome. The distinct difference between Muenke, Apert, and the *FGFR1*$^{P252R}$ mediated form of Pfeiffer syndrome is the severity of limb and midfacial phenotype. While both Type I Pfeiffer and Apert syndrome have a combination of syndactyly, symphalangism, and significant midfacial hypoplasia, the limb and midfacial involvement of Muenke is mild.

Kinetic ligand-binding studies and X-ray crystallography of linker region mutations in *FGFR1*$^{P252R}$, *FGFR2*$^{P253R,S252W}$, and *FGFR3*$^{P250R}$ demonstrate that these mutations result in increased ligand affinity and altered specificity (38–41). Interestingly, the *FGFR2*c linker domain mutations of Apert syndrome result in enhanced binding of FGF7 and FGF10, both mesenchymally expressed ligands of the *FGFR2*b isoform and important in limb development (Fig. 9). The *FGFR1*c and *FGFR3*c mutations do not enhance binding of either FGF7 or FGF10 but rather enhance the binding affinity of FGF9 which is abundantly expressed in the calvaria (42). Taken together these data would suggest that the mutations associated with Apert syndrome affect affinity for the mesodermally expressed FGF7 and FGF10 resulting in autocrine signaling. In contrast, the

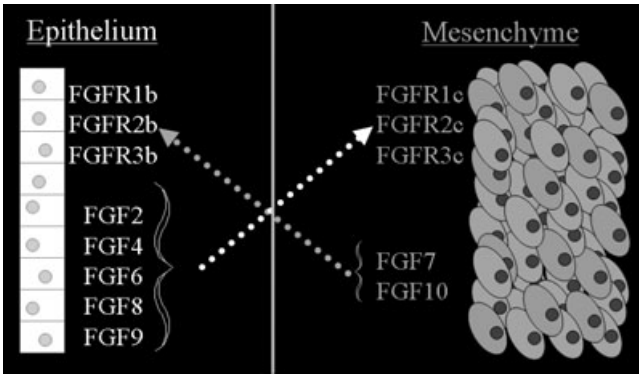


*Fig. 9.* Model of epithelial–mesenchymal signaling of the FGF/FGFR family. Note that in the normal state epithelial ligands bind to mesenchymal receptors and vise versa.

mutations of Type I Pfeiffer and Muenke syndrome enhance the affinity for FGF9 a natural ligand for both *FGFR1*c and *FGFR3*c expressed in the epithelium. During early human limb bud development (26–32) *FGFR1* and *FGFR2* are expressed in both the ectoderm and mesoderm while *FGFR3* is undetectable (43). At later stages (35–38 days gestation), *FGFR2* appears as the first marker of prechondrogenic condensations. In the growing long bones at 40–42 days gestation, FGFR1 and FGFR2 transcripts are restricted to the perichondrium and periosteum, while FGFR3 is mainly expressed in mature chondrocytes of the cartilage growth plate. These data combined with the knowledge that the *FGFR2* mutations affect ligand-binding characteristics may explain the range of limb anomalies seen in Apert, Type I Pfeiffer, and Muenke syndrome. The cellular sequelae of these molecular interactions remains unclear, however alteration in cellular proliferation, differentiation and apoptosis have been proposed (44–49).

Recently two patients with an Apert phenotype have been found to harbor unique Alu insertions (390 base insertion) within or just upstream of exon 9 of *FGFR2* (9). The identification of these cases would appear to challenge the hypothesis that Apert syndrome is a result of altered FGF ligand affinity. Expression analysis in these cases demonstrated ectopic expression of *FGFR2*b in mesenchymal cells allowing for autocrine signaling through binding of FGF7 and 10. The Alu insertions affected splicing of exon 9 leading to ectopic expression of *FGFR2*b while the classic Apert mutations altered ligand-binding affinity, each having the same ultimate effect of autocrine mesenchymal signaling of *FGFR2* in response to FGF7 and FGF10 signaling. Thus the Alu insertions represent a molecular mimic of the classic Apert mutation through a ligand affinity switch.

### Ig-III domain mutations in Crouzon, Pfeiffer, and Jackson–Weiss syndromes

With the exception of the FGFR1 mutations, as described above for Pfeiffer syndrome type I, virtually all of the mutations associated with the phenotypes of Crouzon, Pfeiffer, and Jackson–Weiss syndrome are within the Ig-III domain of FGFR2c. To date 76 distinct mutations have been described (36). While the vast majority represent missense mutations and those effecting splicing, a small number of deletions and insertions have also been described. Twenty-one of the 52 missense mutations reported in the Ig III domain of FGFR2c result

in either a gain or loss of a cysteine residue. The loss of a cysteine residue at position 342 (C342Y), associated with a classic Crouzon phenotype, results in constitutive activation of the receptor (50), presumably secondary to intermolecular disulfide bonding. These results supported previous data demonstrating covalent homodimer formation and mesoderm induction in response to microinjection of FGFR2$^{C332Y}$ in a *Xenopus* embryo model system (51). While similar mechanisms can explain this and other missense mutations resulting in unpaired cysteines, it was not immediately clear how other mutations in the Ig III domain led to the same phenotype. Analysis of classic mutations not involving cysteine substitutions (e.g. W290G, T341P) demonstrated constitutive activation (52). Additional analysis revealed that activation induced by W290G or T341P mutations required cysteine residues in the IgIII domain. Molecular modeling suggested that these mutations disrupt intramolecular disulfide bonds in the IgIII domain allowing for intermolecular disulfide bonding and constitutive activation. Taken together these data provide strong evidence of aberrant intermolecular disulfide bonds between unpaired cysteine residues as the molecular consequence of FGFR2 mutations resulting in Crouzon, Jackson-Weiss and Pfeiffer phenotypes regardless of the involvement of a cysteine residue. These molecular findings place these disorders in an entirely different mechanistic category from the FGFR1$^{P252R}$, FGFR2$^{S252W\ and\ P253R}$, and FGFR3$^{P250R}$ mutations of Type I Pfeiffer, Apert, and Muenke syndromes each associated with altered ligand-binding specificity and/or affinity.

### Transmembrane domain mutations in Crouzonodermoskeletal syndrome

The transmembrane mutation *FGFR3*$^{A391E}$ associated with Crouzonodermoskeletal syndrome is unique among the classic craniosynostoses. Most notably, the craniofacial phenotype closely resembles Crouzon syndrome and yet the mutation resides just eleven amino acids from the *FGFR3*$^{G380R}$ mutation of achondroplastic dwarfism. Furthermore, a mutation in the transmembrane domain suggests an alternative molecular mechanism to the more common extracellular domain mutations of Apert, Crouzon, Jackson–Weiss, and Pfeiffer syndromes. It has been proposed that the A391E transmembrane mutation of *FGFR3* results in altered hydrogen bonding between the glutamic acid residue and an adjacent transmembrane domain of another receptor (53). Exploration of the energetics of this mutant receptor suggests that single and double hydrogen bonds form between *FGFR3*$^{A391E}$ and adjacent wild type and mutant *FGFR3* receptors, respectively (54). This hydrogen bonding enhances both homo and heterodimers and could result in significant alteration in receptor functioning. Interestingly, the *FGFR3*$^{G380R}$ mutation of achondroplasia does not appear to alter dimerization energetics (55), perhaps explaining the lack of phenotypic overlap between Crouzonodermoskeletal syndrome and achondroplasia. It seems reasonable that the phenotypic similarities of Crouzonodermoskeletal syndrome and Crouzon syndrome lies in the basic molecular ramifications of their mutations, namely enhanced intramolecular hydrogen and disulfide bonding resulting in ligand independent constitutive activation rather than the ligand affinity alterations suggested for Type I Pfeiffer, Apert, and Muenke syndromes. It is interesting to consider the craniosynostosis and acanthosis nigricans phenotype of Beare-Stevenson cutis gyrata caused by transmembrane domain mutations in *FGFR2* (56). Perhaps the molecular events associated with transmembrane mediated constitutive activation of fibroblast growth factor receptors have a specific effect on skin development.

### FGF receptor mutation: variable midfacial phenotypes

In addition to considering the molecular impact of craniosynostosis, it is important to consider embryonic expression patterns in disease pathogenesis. Several studies have demonstrated the expression of FGF receptors in craniofacial tissues during human, mouse and chick development. *FGFR1* and *FGFR2* are highly expressed in midfacial membranous ossification sites while *FGFR3* is poorly expressed (57–60). These findings strongly correlate with variations in the severity of midface retrusion seen in analogous mutations of *FGFR1*, *FGFR2* and *FGFR3*. The *FGFR1*$^{P252R}$ mutation of Pfeiffer syndrome and the *FGFR2* mutations of Apert, Crouzon, and Pfeiffer syndrome display significant midface retrusion. In contrast, the *FGFR3*$^{P250R}$ mutation of Muenke syndrome does not have a severe midface retrusion phenotype, perhaps due to the relative lack of *FGFR3* expression in midfacial ossification (57). Consideration of the developmental expression patterns of the FGFRs will be an important component of future investigation into disease pathogenesis and phenotypic variability.

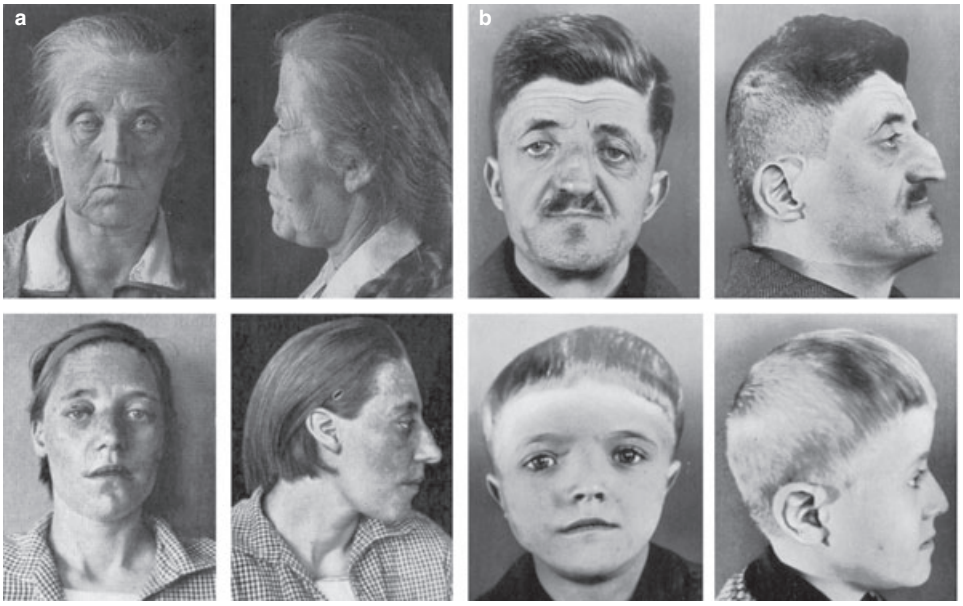


*Fig. 10.* Original cases described by (a) Saethre and (b) Chotzen in 1931 and 1932, respectively. Note the low frontal hairline, ptosis, facial asymmetry, and deviated nasal septum.

## *TWIST1* mediated craniosynostosis

### History and heredity

#### Saethre–Chotzen syndrome

In 1931 Haakon Saethre, a Norwegian psychiatrist at the University of Oslo, described a woman with craniosynostosis, a low frontal hairline, facial asymmetry, deviated nasal septum, defects of the vertebral column, brachydactyly, fifth finger clinodactyly and partial soft tissue syndactyly of the second to third fingers and second to fourth toes (Fig. 10a) (61). Her half sister was similarly affected with a low frontal hairline, ptosis and similar limb anomalies (61, 62). One year later a German psychiatrist Dr F. Chotzen of Breslau, described similar craniofacial malformations in a father and two sons (Fig. 10b) (63). The combination of these reports suggested a specific phenotype and heritability.

Previously called acrocephalosyndactyly type III, classic Saethre–Chotzen syndrome (SCS) is characterized by unilateral or bilateral coronal synostosis, facial asymmetry (particularly in individuals with unicoronal synostosis), ptosis, ocular hypertelorism, a low frontal hairline, maxillary hypoplasia, a characteristic appearance of the ear (small pinna with a prominent crus) and short stature (62, 64, 65). Syndactyly of digits two and three of the hand and hallux valgus are variably present. Although mild-to-moderate developmental delay and mental retardation have been reported, the vast majority of individuals with point mutations are of normal intelligence. The risk for developmental delay in individuals with deletions involving the *TWIST1* gene is approximately 90%, or eightfold greater than in individuals with intragenic mutations (66). Less common manifestations of SCS include parietal foramina, vertebral fusions, radioulnar synostosis, cleft palate, duplicated distal hallucal phalanx, and congenital heart malformations. Although phenotypic similarities between SCS and Muenke syndrome have been noted (67), the classic features of low hairline, ptosis, small ears, and two to three syndactyly make the SCS phenotype quite distinct (Kress 2006).

### Molecular genetics and developmental pathogenesis

Saethre–Chotzen syndrome is an autosomal dominant condition caused by a variety of loss of function mutations leading to functional haploinsufficiency of the basic helix-loop-helix transcription factor *TWIST1*. Missense mutations cluster in the DNA binding and dimerization domains of the protein. To date over 100 distinct mutations in the *TWIST1* gene have been found to cause SCS. These include nucleotide substitutions (missense and nonsense), deletions, insertions, duplications, and complex rearrangements (36, 68–72). All of the point mutations are located within the coding region; no splice mutations, intronic mutations, or changes within the second exon have been reported. Nonsense mutations that preclude translation of the DNA binding domain and the HLH domain have been identified from

the 5′ end of the coding sequence to the end of the HLH motif. Functional haploinsufficiency, either through deletion, inhibition of dimerization, or impaired DNA binding appears to be a unifying molecular mechanism underlying the pathogenesis of SCS (73).

During mouse development, *Twist1* is expressed in neural crest cells populating the cephalic region and branchial arches that differentiate into connective tissue, muscle, cartilage, and bone (74–76). The migratory populations of cephalic neural crest cells are the origin of the membranous bones of the skull (frontal, parietal, and squamosal), their intervening sutures, overlying dermis, and underlying dura mater (77–80). Suture mesenchyme (intrasutural mesenchyme) and the osteogenic fronts demonstrate high levels of *Twist1* expression (81). Like other bHLH transcription factors, *Twist* is thought to play a central role in specifying and maintaining cell identity (82, 83). With regard to osteoblast development, the Twist-box domain of *Twist1* binds to the DNA-binding domain of *Runx2*, reversibly inhibiting its function (84). *Runx2* is a major bone regulatory transcription factor that increases the expression of osteocalcin through interaction with the vitamin D receptor (85–87). It is presumed that the derepression of *RUNX2* in the presence of *TWIST1* mutations is directly related to the pathogenesis of SCS. *In vitro* culture of human osteoblasts harboring naturally occurring *TWIST1* mutations demonstrated significant reduction in proliferation, alkaline phosphotase activity, *RUNX2* expression and a trend toward increased mineralization suggesting that loss-of-function mutations of *TWIST1* lead to reduced proliferation and enhanced differentiation (88).

## Phenotype–genotype correlations: value and limitations

Increasingly the genetics community asks whether a condition should be defined by its phenotype or its molecular cause. The recommendation that clinical and molecular diagnoses should be in agreement (89) may be too simplistic. We would like to suggest that these are not exclusive goals and that both molecular cause and phenotypic presentation are important components of clinical management and scientific investigation. Molecular data will enhance our ability to counsel our patients about the phenotypic variability identified in historical cases while the scientific investigation of highly variable phenotypes may identify environmental and genetic modifiers of disease presentation. With this as a backdrop we must be careful in our description of specific disorders. Two mutations, $FGFR3^{A319E}$ and $FGFR2^{A344G}$ are highly restricted in their presentation. The $FGFR2^{A344G}$ has to date been seen only in the original kindred of Jackson–Weiss syndrome and the $FGFR3^{A319E}$ of Crouzonodermoskeletal syndrome (described by Suslak) has a highly consistent phenotype of acanthosis nigricans and Crouzonoid craniofacial features. We would suggest that the terms ‘Jackson–Weiss’ and ‘Crouzonodermoskeletal’ syndrome should be restricted to those individuals found to harbor these specific mutations. Although somewhat more controversial, we would suggest that the term ‘Pfeiffer syndrome’ be restricted to cases with the $FGFR1^{P252R}$ mutations. It is our opinion that cases of ‘Pfeiffer’ syndrome found to have *FGFR2* mutations would more appropriately be designated as Crouzon syndrome. The marriage of careful phenotypic descriptions and modern molecular genetics will allow us to advance the increasingly important fields of gene–gene and gene–environment interaction. Our ultimate goal in the classification of these disorders should be the identification of phenotype modifiers that will enhance our ability to counsel our patients and understand the basic biology for the development of new diagnostic and treatment strategies. Below are illustrative examples of cases with syndromic craniosynostosis that challenge our current method of classification.

### Challenging craniosynostosis phenotypes

#### Late onset pan-synostosis and hypertelorism

We previously described a family with four affected members in three generations that manifest either isolated hypertelorism or hypertelorism associated with late onset pan-synostosis, and mild midfacial hypoplasia (Fig. 11) (90). Mutational analysis revealed a novel $Fgfr2^{L3575}$ mutation distal to Ig loop III and located between two previously identified missense mutations ($Fgfr2^{s354c}$ and $Fgfr2^{v359f}$) associated with crouzon (12) and Jackson–Weiss (91) phenotypes, respectively. To date this $Fgfr2^{L3575}$ mutation has not been described in any other cases. While it is true that we may have merely found a novel Crouzon mutation, the phenotypic similarities among members of this family is quite

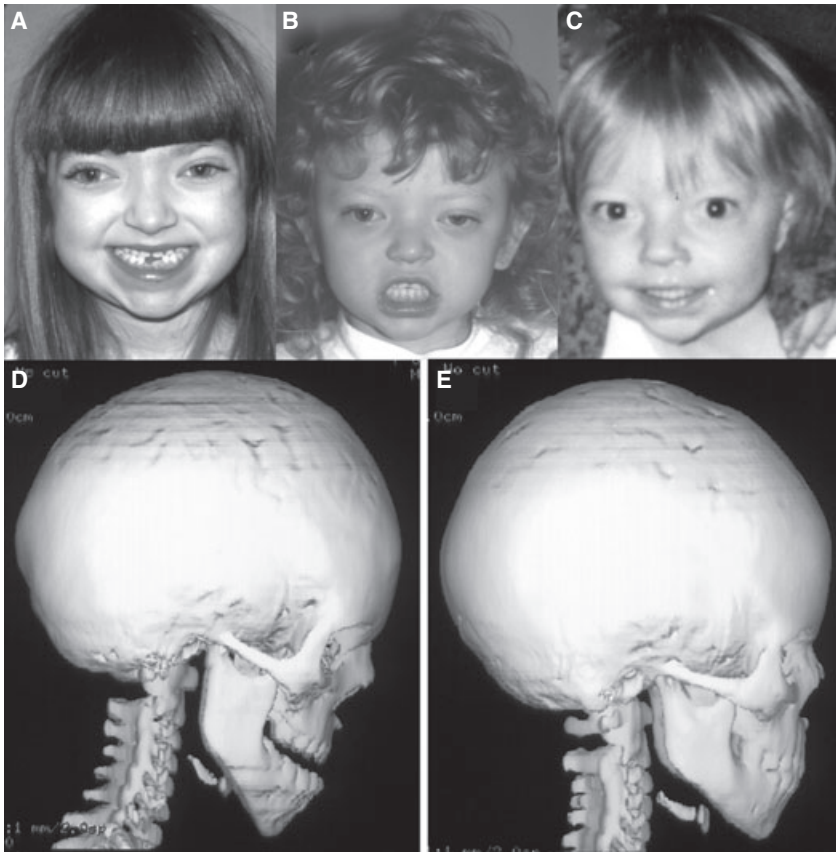


*Fig. 11.* A family reported with late onset pan-synostosis, hypertelorism and mild midfacial hypoplasia associated with a novel *FGFR2*$^{L357S}$ mutation. 3D CT scans D and E representing cases depicted in panels A and B, respectively.

striking. Have we defined a novel hypertelorism syndrome? These findings raise the question asked by many authors in previous publications: are we studying clinically distinct syndromes or is this a 'spectrum of Fgfr2 craniosynostotic syndromes'? (13, 91, 92). The identification of another family with a classic Crouzon phenotype associated with this Fgfr2$^{L357S}$ would answer this question. Until such time, the possibility remains that this, much like the Fgfr2$^{A344g}$ mutation of Jackson–Weiss syndrome, may represent a unique genotype–phenotype association.

### Coronal synostosis with fibular hemimelia

We have reported a case born with bilateral coronal synostosis, midfacial hypoplasia, unilateral shortening of the right fibula and tibia, and oligodactyly (absence of the fifth digital ray on the right foot) (Fig. 12) (15). A careful family history revealed that several family members had undergone foot surgery and subsequent review of their records revealed variable tarsal/metatarsal coalitions, broad and medially deviated first metatarsals. A tentative diagnosis of Jackson–Weiss syndrome was made and subsequent mutational analysis revealed the *FGFR2*$^{A344G}$ mutation described in the original Amish kindred. This mutation has been seen only in the original kindred and subsequent review of this family's genealogy revealed that they were a long lost branch of the original kindred having left Indiana four generations prior to our identification of the proband. As this family demonstrates, there have still been no cases of this mutation in any but the original kindred suggesting that despite the now widened phenotypic spectrum, Jackson–Wiess syndrome is caused by a single *FGFR2*$^{A344G}$ mutation.

### Radial ray hypoplasia and multiple sutural craniosynostosis

We recently reported a male child born with synostosis of the metopic, sagittal, and coronal sutures, increased intracranial pressure, radial ray hypoplasia with a pedunculated thumb, prominent crus helix, hypotelorism masked by prominent epicanthic folds, a low anterior hairline, and cervical vertebral anomalies suggesting the diagnosis of Baller–Gerold syndrome (Fig. 13) (93). Subsequent evaluation of his father demonstrated features consistent with mild SCS including a low frontal hairline and very mild two-three syndactyly of his hands. Mutational analysis of the proband and his father revealed a novel *TWIST1* mutation (*TWIST1*$^{I156V}$)

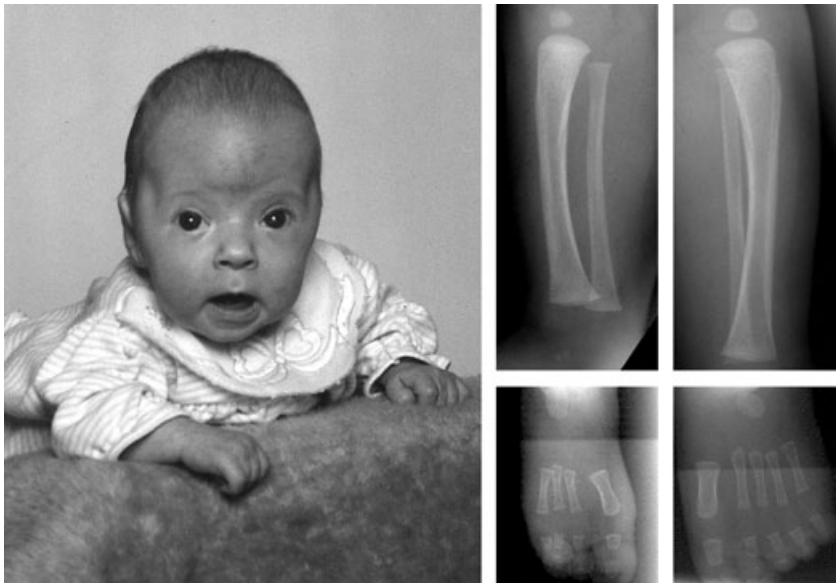

*Fig. 12.* Atypical presentation of Jackson-Weiss syndrome with bilateral coronal craniosynostosis and unilateral absence of the fifth digital ray associated with a FGFR2$^{A344G}$ mutation. This child is a descendant of the original kindred.

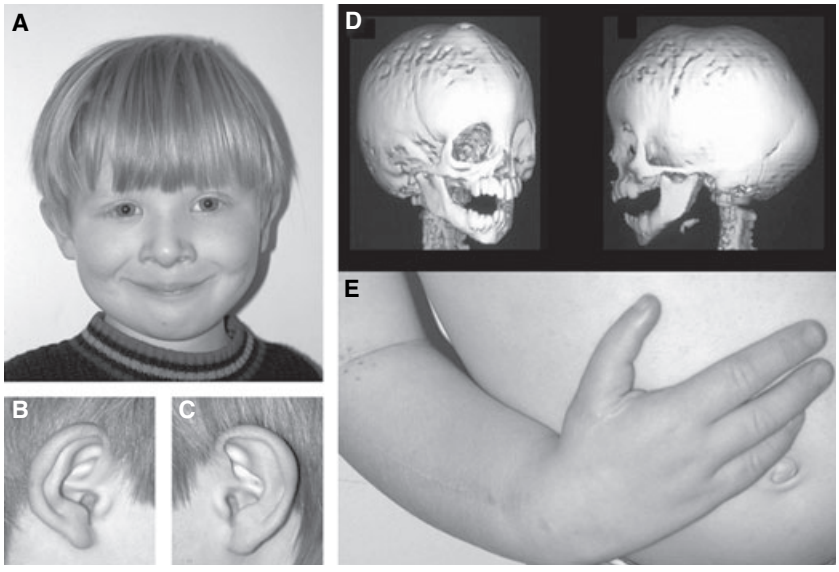


*Fig. 13.* Phenocopy of Baller–Gerold syndrome with synostosis, vertebral and radial ray anomalies. Mutational analysis revealed a novel *TWIST1*$^{I156V}$ mutation.

in the conserved Helix II domain confirming direct paternal transmission. A similar phenotype associated with a nonsense *TWIST1*$^{E181X}$ mutation was reported by Gripp *et al.* (94). More recently two unrelated families were found to have features of Baller–Gerold syndrome associated with mutations in the RECQ-like DNA Helicase, Type 4 (RECQ4) (95). Does this suggest that Baller–Gerold is caused by mutations of both *TWIST1* and RECQ4? Are *TWIST1* and RECQ4 linked through biologic pathways explaining these apparently divergent causes? Is the radial ray hypoplasia merely a severe manifestation of the mild radial ray anomalies of SCS? Because of the phenotype of the father in our report we would favor the later interpretation that these cases represent an extreme of the classic Saethre–Chotzen phenotype. Had the father's mild phenotype gone unrecognized, we may have inappropriately suggested *TWIST1*$^{I156V}$ as a unique molecular cause of Baller–Gerold syndrome. On the opposite extreme, we recently reported two cases of isolated single suture craniosynostosis (one coronal and one sagittal) associated with *TWIST1* mutations in the highly conserved Twist-box (96). As described previously, the Twist-box domain interacts with the DNA binding domain of *Runx2*, potentially giving these mutations a unique effect on downstream gene expression through select-

ive derepression of *RUNX2*. While to date only these and one additional case of scaphocephaly have been described with Twist-box domain mutations, it remains unclear if Twist-box mutations will represent a distinct genotype-phenotype association (97).

## Clinical utility and implications

There is obvious clinical value to the understanding of molecular cause and phenotypic variability in the syndromic craniosynostoses. Appropriate genetic counseling is dependant on careful phenotypic evaluation and genetic screening to define medical needs and recurrence risks. We are now in a position to move forward on initiatives to better understand the molecular basis of phenotypic variability through the investigation of gene–gene and gene–environment interactions that modify phenotypic outcome.

At the same time we must remember that we remain relatively ignorant about the developmental pathogenesis of these disorders. While most developmental research has focused on the impact of these mutations on the development of the calvaria, the most challenging clinical manifestation of the FGFR mediated craniosynostosis may be midfacial hypoplasia. Relative to the management of craniosynostosis, the clinical impact of midfacial hypoplasia on oral health and airway function make this an important focus of study. Investigation of the impact of these mutations on skull base and facial development will likely lead to discoveries with clinical implications. The past 12 years of molecular discovery and development of animal models have put us in an excellent position to begin to unravel these more complex components of syndromic craniosynostosis.

As with most biomedical research we have begun our investigation into the pathogenesis of craniosynostosis through examination of the rare and most extreme phenotypes. Non-syndromic single suture craniosynostosis is 10–100 times more common than any syndromic form. Although metopic and sagittal synostosis may have relatively little impact on other aspects of facial growth, coronal synostosis can have dramatic impact on midface symmetry. Furthermore there is increasing evidence that isolated single suture craniosynostosis can impact neurocognitive development (97, 98). Although most single suture synostosis is sporadic, familial recurrence is relatively common. With the knowledge gained from the investigation of the syndromic forms and the tools afforded by years of molecular genetic research, we are in an excellent position to attack these 'simple', more common forms of human craniosynostosis.

In 100 years we have advanced from the first clinical description of Apert syndrome to the knowledge that the causative *FGFR2* mutations alter FGF ligand binding affinity and specificity. The speed with which we are able to ask the next important questions will continue to accelerate. What then are the most important questions to ask? Keeping in mind that our long-term goal is to positively impact children with these often devastating disorders, we would suggest that future research should focus on the molecular and environmental basis of phenotypic variability, the developmental causes of midface hypoplasia and the molecular causes of isolated single suture craniosynostosis. The basic science that has driven the molecular discoveries of the past 12 years will continue to advance our basic understanding of the molecular causes of these and related conditions, however, it becomes increasingly important to focus on research that will impact the patients we treat.

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