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Craniosynostosis
Molecular pathways and future pharmacologic therapy

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Craniosynostosis describes the premature fusion of one or more cranial sutures and can lead to dramatic manifestations in terms of appearance and functional impairment. Contemporary approaches for this condition are primarily surgical and are associated with considerable morbidity and mortality. The additional post-operative problems of suture refusion and bony relapse may also necessitate repeated surgeries with their own attendant risks. Therefore, a need exists to not only optimize current strategies but also to develop novel biological therapies which could obviate the need for surgery and potentially treat or even prevent premature suture fusion. Clinical studies of patients with syndromic craniosynostosis have provided some useful insights into the important signaling pathways and molecular events guiding suture fate. Furthermore, the highly conserved nature of craniofacial development between humans and other species have permitted more focused and step-wise elucidation of the molecular underpinnings of craniosynostosis. This review will describe the clinical manifestations of craniosynostosis, reflect on our understanding of syndromic and non-syndromic craniosynostoses and outline the different approaches that have been adopted in our laboratory and elsewhere to better understand the pathogenesis of premature suture fusion. Finally, we will assess to what extent our improved understanding of the pathogenesis of craniosynostosis, achieved through laboratory-based and clinical studies, have made the possibility of a non-surgical pharmacological approach both realistic and tangible.

Introduction

Craniosynostosis, a term first coined by Otto in 1830, describes the premature fusion of cranial sutures, the fibrous joints between calvarial bones.[1] Craniosynostosis can lead to dramatic clinical manifestations in terms of cosmesis and functional impairment and unsurprisingly therefore, literary and pictorial representations of this condition span history.[2,3] Moreover, craniosynostosis has captivated the interest of both basic scientists and clinicians alike, by posing unique challenges to understanding its pathogenesis and to identifying appropriate and timely clinical approaches which are capable of providing safe, satisfactory, and sustainable long-term outcomes. Virchow, in the 1850s, was the first to state that following premature fusion of a calvarial suture, compensatory growth occurs in a plane parallel to the fused suture with minimal growth in a perpendicular plane.[4] Furthermore, he proposed that premature fusion was associated with inflammation of the meninges. In 1912, Crouzon made the key observation that there may be a genetic contribution to the pathogenesis of craniosynostosis, and in 1959, Moss proposed that craniosynostosis resulted from abnormal development of the cranial base leading to transmission of altered mechanical forces to the overlying suture.[5,6] These accounts eventually resulted in a paradigm shift in clinical approach to craniosynostosis, with the acceptance that suturectomy alone was insufficient. Paul Tessier subsequently ushered in the modern era of craniofacial surgery with his description of total calvarial vault remodelling to increase intracranial volume and create a more normal appearance.[7,8]

While contemporary approaches to the treatment of craniosynostosis are based on these early pioneering reports, procedures used today still carry a mortality rate of 1.5–2% and are associated with considerable morbidity including blood loss, infection, CSF leak, and lengthy hospital stays as well as post-operative monitoring in an intensive care unit.[9-11] Furthermore, additional post-operative problems include suture refusion and bony relapse necessitating repeated surgeries with their own attendant risks.[12,13] Clearly, a need exists not only to optimize current strategies but to also develop novel biological therapies which may successfully treat or even prevent craniosynostosis, thereby obviating the need for surgery and its potentially deleterious consequences. In this review we will describe the clinical manifestations of craniosynostosis, reflect on our understanding of syndromic and non-syndromic craniosynostoses and outline the different approaches which have been adopted in our laboratory and elsewhere to better understand the pathogenesis of craniosynostosis. Finally, we will assess to what extent our improved understanding of the pathogenesis of craniosynostosis achieved through laboratory-based and clinical studies have made the possibility of a non-surgical pharmacological approach both realistic and tangible.

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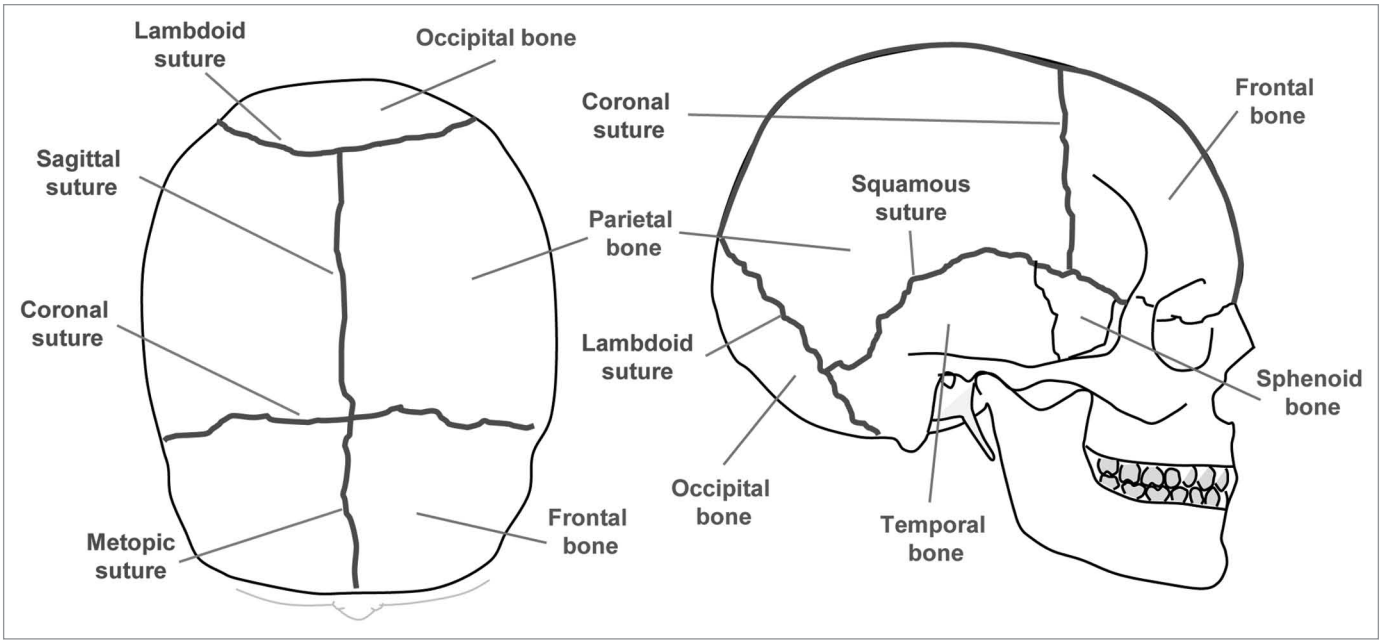

Figure 1. Schematic representation of the major bones and sutures of the adult human cranium. Top view (left) and lateral view (right) of the calvarium, showing bones (blue) and sutures (brown). By the second year of life the metopic suture is normally closed.

Suture Morphogenesis and Development of the Cranial Vault

In order to understand the aberrations in suture biology which may lead to craniosynostosis, sutures should not simply be viewed as articulations between two bones.[2] Rather, they are sites of osteogenesis developed from embryonic mesenchyme. They constitute not only the advancing osteogenic fronts of the flat calvarial bones but also act as a niche for osteogenic progenitors which may proliferate or differentiate in a tightly regulated program orchestrated through appropriate molecular cues. The major sutures in humans are the metopic suture which separates the two frontal bones, the sagittal suture which separates the two parietal bones, the coronal suture where the parietal and frontal bones meet, and the lambdoid suture where parietal and occipital bones meet. While sutures form at the approximations of advancing fronts of the developing bones, what governs their location remains largely unknown.[14] Sutures not only allow for necessary deformation of the skull during passage though the birth canal but also subsequently act as important growth centers of the skull during early years of life.[3] The orchestration of this complex program is tightly controlled, and minor perturbations between developing tissues such as the brain, dura mater, osteogenic fronts, and suture mesenchyme may potentially lead to premature fusion of the sutures.

The membranous cranial vault consists of paired frontal and parietal bones as well as a portion of the occipital bone. These form through intramembranous ossification in which mesenchymal cells condense and differentiate into osteogenic cells without a prior chondrogenic anlagen.[2] Early studies provided conflicting evidence as to the embryonic origin of parietal and frontal bones with Noden and Le Lievre proposing a mixed origin hypothesis of neural crest and mesodermal tissues.[15,16] In contrast, Couly et al. purported that the cranial vault was composed entirely of neural crest cells.[17] More recently, Jiang et al. were able to define the disparate origins of the calvarium by using a transgenic mouse with a Wnt-1-Cre construct and a R26R β-galactosidase reporter.[18] Employing this approach, the frontal bone was found to be of neural crest origin while the parietal bone was noted to be from paraxial mesoderm. The posterior frontal (PF) suture, analogous to the metopic suture in humans, is thus bounded by two neural crest-derived osteogenic fronts and the sagittal suture (SAG) is bounded by two paraxial mesoderm fronts. The coronal suture (COR) located between the frontal and parietal bones lies between osteogenic fronts of disparate embryonic origin. Importantly, while suture mesenchyme from PF, SAG, and coronal sutures all share a common neural crest origin, only the PF suture in mice and rats fuses in a predictable manner.[3,19]

Craniosynostosis: Clues from Clinical Genetics

Craniosynostosis is a heterogeneous condition which can involve the partial or complete premature fusion of one or more cranial sutures. With an incidence of 1:2,500 live births, it can present as part of a syndrome or more commonly as an isolated finding in non-syndromic craniosynostoses.[2,14] Though each of the major sutures including the sagittal, coronal, metopic, and lambdoid sutures may be involved (**Fig. 1**), sagittal synostosis is the most common (40–55%), followed by coronal (20–25%), metopic (5–15%), multiple suture synostosis (5–15%) and lambdoid synostosis (0–5%).[20,21] While a comprehensive description of the clinical phenotypes in syndromic and non-syndromic craniosynostoses is beyond the scope of this review, a brief outline of some

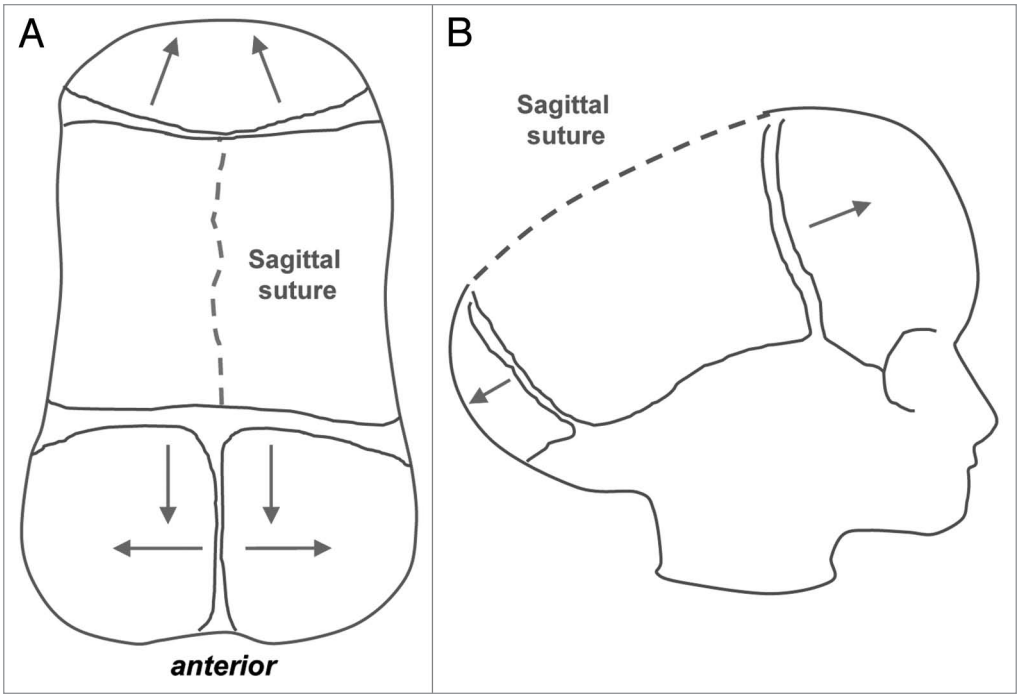

Figure 2. Sagittal synostosis (Scaphocepaly). Top view (**A**) and lateral view (**B**) schematics showing the direction of aberrant compensatory bone growth (red lines) following premature sagittal suture fusion leading to an elongated, scaphocephalic skull, derived from the Greek word scaphos for boat and cephalos for head.

of the expected deformities resulting from premature fusion of each of the individual sutures and some of the more notable syndromes will be highlighted.

Sagittal synostosis is the most common type of craniosynostosis and is most often seen in non-syndromic forms. Compensatory calvarial growth parallel to the suture can lead to an elongated, scaphocephalic skull, derived from the Greek word scaphos for boat and cephalos for head (**Fig. 2**). This "typical phenotype," however, is not always seen and depends on the extent of sagittal suture which is affected. A palpable ridge and narrow skull are more common features.[21] Coronal synostosis can be unilateral or bilateral and is less common than sagittal synostosis. Unilateral synostosis leads to ipsilateral flattening of the forehead and compensatory contralateral frontal bossing. In addition, failure of the greater wing of the sphenoid to descend results in a "harlequin" deformity with elongation of the orbit superiorly and laterally. Bilateral coronal synostosis (**Fig. 3**) leads to shortening in the anteroposterior direction and a brachycephalic deformity.[21] Metopic synostosis (**Fig. 4**) is associated with trigonocephaly, or a triangular keel-shaped head. Widening and increased height in the parietal region can occur due to compensatory growth posteriorly.[21] Finally, lambdoid synostosis (**Fig. 5**) is rare and often difficult to distinguish clinically from positional plagiocephaly. Premature fusion of the lambdoid suture leads to ipsilateral flattening of the occipital region with a compensatory mastoid bulge. Contralateral growth in the parietal region is also noted and the cranial base becomes tilted.

While non-syndromic craniosynostoses are most frequently encountered, their genetic etiology remains poorly understood. EphrinA-4 (*EFNA-4*) is one of the first genes that when mutated has been associated with non-syndromic craniosynostosis.[22] Mutational analysis on cohorts of patients have also demonstrated a possible role for *FGFRs-1, -2, -3* and *TWIST-1*.[23-25] In contrast to non-syndromic craniosynostosis, genetic analysis of syndromic craniosynostosis has contributed a wealth of information, elucidating some of the important pathways for suture development and closure.[26] This has allowed for genetic screening and molecular diagnosis in patients with syndromic craniosynostosis which can be a useful adjunct to clinical diagnosis given the phenotypic heterogeneity. Importantly, it has also helped to direct studies on the molecular etiopathology of craniosynostosis. There are at least 150 syndromes associated with craniosynostosis as a major clinical feature. Linkage analysis in familial cases and molecular analysis of chromosomal alterations have identified several genes that when mutated are closely associated with syndromic craniosynostosis: *FGFR-1, FGFR-2, FGFR-3, TWIST-1, EFNB-1, MSX-2* and more recently, *RAB-23* (**Table 1**).[21]

The FGFR family, which consists of four signal transduction receptor kinases, has drawn the most attention, as three of them have been associated with the majority of cases of syndromic craniosynostosis.[21] Upon dimerization, these receptors autophosphorylate and initiate signal transduction via a range of pathways to control key physiological processes including cell proliferation, differentiation, migration and apoptosis.[21,27,28] While a detailed account of the eponymous syndromes associated with FGFR mutations is beyond the scope of this review, it is worth highlighting some of the different biomolecular mechanisms, which can lead to premature suture fusion. Mutations

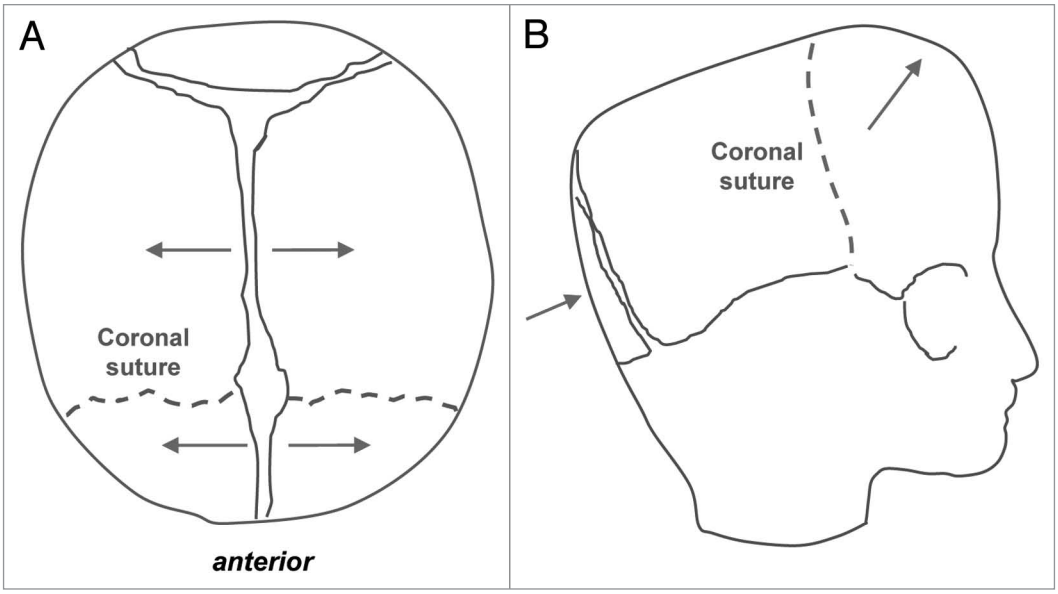

Figure 3. Bilateral coronal craniosynostosis. Top view (**A**) and lateral view (**B**) schematics showing the direction of compensatory calvarial growth (red lines) secondary to premature fusion of both coronal sutures (blue dashed lines) leading to shortening in the anteroposterior direction, increased height of the vault and therefore a turribrachycephalic deformity.

in *FGFRs-1, -2* and *-3* are invariably gain of function mutations, primarily localized to the IgII-IgIII linker regions. This results in enhanced ligand affinity, more promiscuous ligand-receptor binding, or ligand-independent activation. Well documented mutations include the point substitution in *FGFR-2* (Cys342Tyr) associated with Crouzon syndrome and Ser252Trp in Apert syndrome, *FGFR-1* Pro252Arg in Pfeiffer syndrome, and analogous *FGFR-3* mutations in Muenke syndrome.[29-31] The prominent role played by FGF pathway gain of function mutations in syndromic craniosynostoses have prompted development of animal models and highlight potential targets for future biological intervention.

Transcription factors have also been implicated in the pathogenesis of craniosynostosis. TWIST-1, a basic helix-loop-helix transcription factor, is one such example that has drawn attention. Mutations in this gene are associated with Saethre-Chotzen syndrome, or acrocephalosyndactyly type III, an autosomal dominant condition characterized by unilateral or bilateral coronal synostosis.[32] TWIST-1 protein contains two highly conserved regions, a DNA binding domain and a basic helix-loop-helix motif. The majority of the mutations which have been described result in loss-of-function, leading to functional haploinsufficiency of *TWIST-1*.[33-35] To date, over 100 distinct mutations in the *TWIST-1* gene have been found to cause Saethre-Chotzen syndrome, including nucleotide substitutions (missense and nonsense), deletions, insertions, duplications and complex rearrangements.[36] Interestingly, genetic screening of patients with Saethre-Chotzen syndrome has also identified FGFR mutations, suggesting that *TWIST-1* and *FGFRs* may be active in the same signaling network.[37] Experiments with human osteoblasts have highlighted a potential role for TWIST-1 to attenuate cellular response to FGF ligand, with overexpression of TWIST-1 leading to downregulation Early Growth Response Element-1, a known mediator of FGF signaling, and maintenance of an undifferentiated state.[38] In such a manner, TWIST-1 may thus contribute to continued suture patency.

MSX-2, a homeobox containing gene and potential modulator of downstream FGF activity, has also been associated with syndromic craniosynostosis.[39-41] *MSX-2* mutation has been indentified in patients with Boston-type craniosynostosis, an autosomal dominant form of craniosynostosis.[32] *MSX-2* has been shown to be upregulated in response to increased FGF signaling and exogenous FGF-2 promotes *MSX-2* expression and upregulation of markers for osteogenic differentiation.[39] Boston-type craniosynostosis involves a cytosine to adenosine base substitution resulting in the replacement of the amino acid proline with histidine (Pro7His). This amino acid change leads to premature suture ossification and other secondary deformities.[42]

Ephrins, which are membrane-bound ligands for Eph family receptor tyrosine kinases, regulate cell adhesion and migration during development.[21] Mutations in *EFNB-1* have been associated with craniofrontonasal syndrome, a developmental disorder that exhibits a very unusual pattern of X-linked inheritance with a paradoxically greater severity in heterozygous females than in hemizygous males.[26,43,44] Missense mutations constitute about 42% of *EFNB-1* mutations.[45] The other major types of mutations include nonsense, frame-shift, and splice site, and lead to premature termination codons. Missense mutations change highly conserved amino acid residues in the extracellular ephrin domain and are expected to disrupt protein folding or interaction sites for EFNB-1 interacting partners resulting in loss-of-function. Craniofrontonasal syndrome is characterized by craniosynostosis, frontonasal dysplasia, orbital hypertelorism, a broad nasal tip, central nasal groove, and an anterior open bite.[21]

While many of the genes so far identified with syndromic craniosynostosis have been shown to demonstrate autosomal

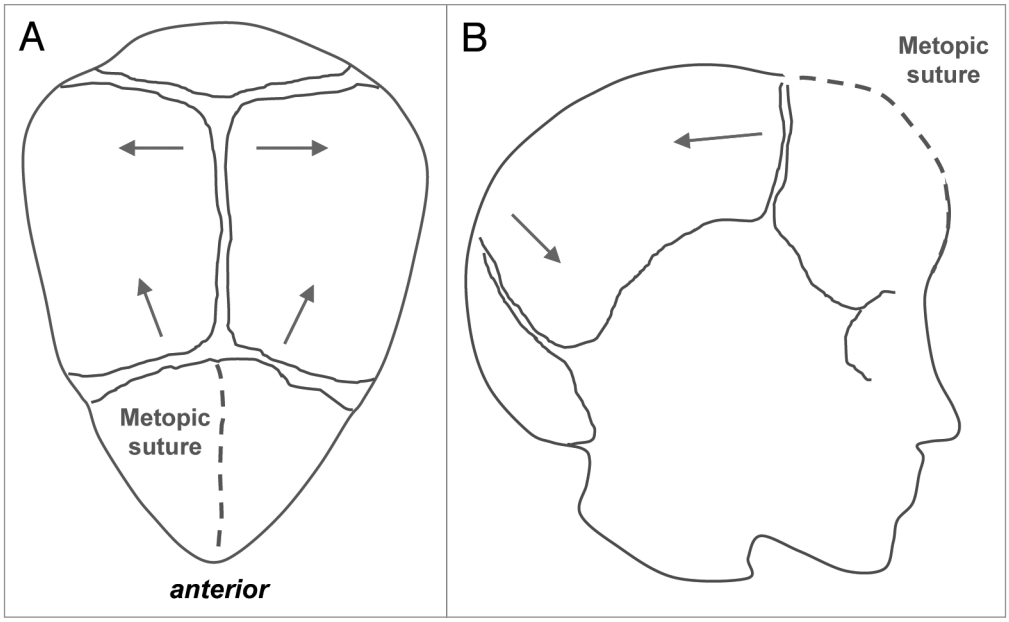

Figure 4. Metopic Synostosis. Top view (**A**) and lateral view (**B**) schematics demonstrating trigonocephaly, or a triangular keel-shaped head. Compensatory calvarial growth (red lines) can lead to widening and increased height in the parietal region due to compensatory growth in a posterior direction following premature fusion of the metopic suture (blue dashed line).

dominant inheritance, *RAB-23* mutations instead result in autosomal recessive transmission for craniosynostosis with Carpenter syndrome. Until 2001, only 40 cases of this condition had been reported.[21] Using homozygosity mapping, Jenkins et al.[46] identified five different mutations (four truncating and one missense) in *RAB-23* which encodes a member of the RAB guanosine triphosphatase (GTPase) family of vesicle transport proteins involved in negatively regulating hedgehog singaling. Fusion of the midline (metopic and sagittal) sutures is typical in Carpenter syndrome and head shape can be variable. In severe cases, cloverleaf-shaped deformities have been described. Other well-recognized features include soft tissue syndactyly, short or missing middle phalanges of the hands and feet and clinodactyly.

Lastly, though the role of TGF-β in bone biology has been well established, mutations in the TGF-β pathway, unlike the FGF signaling pathway, have not been closely associated with craniosynostosis. Hunter-Thompson Chondrodysplasia constituted the first clinical description of a TGF-β superfamily mutation in craniosynostosis.[47] Recently, an autosomal dominant gain of function mutation in TGF-β receptors has also been documented.[48] Despite the paucity of evidence for its role in clinical syndromes, both human and mouse studies have supported the importance of TGF-β signaling in suture morphogenesis and the maintenance of patency. Increased immunoreactivity for TGF-β2 was found on analysis of synostotic suture samples from 10 infants when compared with normal controls.[49] Murine studies have also documented upregulation of TGF-β2 within the PF suture during physiological fusion while TGF-β3 has been localized to the osteogenic fronts in patent sutures. A possible mechanism for these findings is that TGF-β3 may compete for receptors such as TGF-βR1 and in so doing, downregulate the pro-osteogenic effect of TGF-β2.[50] While clinical studies continue to define a role for TGF-β in the pathogenesis of craniosynostosis, available data nonetheless point to this pathway as a potentially important therapeutic target.

The Role of Animal Models in Cranial Suture Biology Research

With the description of clinical syndromes and an improved understanding of the genetics of craniosynostosis, some insights have been made into possible molecular events guiding suture fate. More in-depth definition of the complex pathways involved in premature pathologic suture fusion, however, has necessitated the development of animal models to study cranial suture development and craniosynostosis. These have included both mammalian models (e.g., mouse, rat and rabbit) and non-mammalian models (e.g., zebrafish and *Xenopus laevis*).

The murine model has proven to be an invaluable tool with which to study cranial sutures given the highly conserved nature of craniofacial development.[51,52] The PF suture in both mice and rats undergo predictable physiological fusion in early postnatal life, affording investigators with the opportunity to study the process of normal suture fusion, while other sutures remain patent.[2] Our laboratory, among others, have chosen to study this elegant model to elucidate differences in the molecular signaling pathways and behavior of osteoblastic cells that differentiate between physiologic suture fusion and maintenance of suture patency. Differences in FGF signaling, TGF-β signaling, and Hedgehog signaling have been identified using the murine model.[53-57] In addition, Warren et al. demonstrated that inhibitors of BMP signaling such as Noggin play a pivotal role in the differential fate of the murine PF suture. High FGF-2 activity in the PF suture was found to downregulate *noggin* expression at

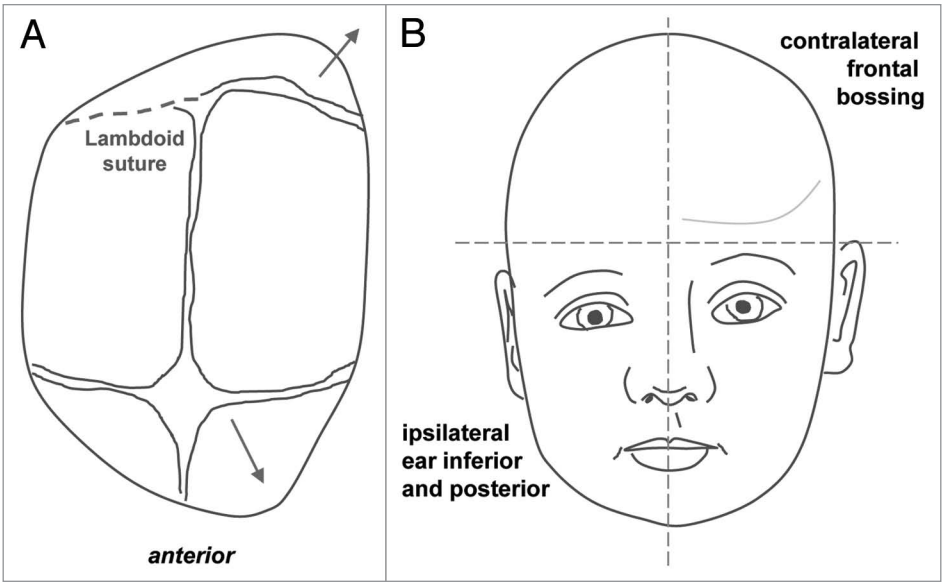

Figure 5. Lambdoid synostosis. Top view (**A**) and antero-posterior view (**B**) showing the direction of compensatory bone growth (red lines) following premature fusion of the lambdoid suture (blue dashed line). Lambdoid synostosis leads to ipsilateral flattening of the occipital region with a compensatory mastoid bulge which is a key distinguishing feature from positional (sometimes called positional or deformational) plagiocephaly from which it can be clinically difficult to differentiate. Contralateral growth in the parietal region is also noted and the cranial base becomes tilted.

Table 1. Common mendelian causes of craniosynostosis

Gene	Chromosome	Phenotype	Reference
FGFR1	8p	Pfeiffer syndrome	87–89
		Jackson Weiss syndrome	87
FGFR2	10q	Crouzon syndrome	87, 90
		Jackson Weiss syndrome	87
		Apert syndrome	29, 91–93
		Pfeiffer syndrome	94, 95
		Beare Stevenson syndrome	96
FGFR3	4p	Crouzon syndrome with Acanthosis	97
		Muenke syndrome	98, 99
		Thantophoric Dysplasia	100
EFNB1	Xq	Craniofrontonasal syndrome	101
TWIST1	7p	Saethre-Chotzen syndrome	102, 103
MSX2	5q	Craniosynostosis (Boston-type)	102
RAB23	6p	Carpenter syndrome	46

key time points thereby permitting unfettered endogenous BMP signaling and subsequent fusion of the suture.[58] More recently, Behr et al. showed that Wnt signaling is also likely to play a key role in suture fate.[59] Closure of the PF suture was found to be accompanied by a downregulation of canonical Wnt signaling, whereas patency of the SAG suture was associated with constitutively activated canonical Wnt signaling. Importantly, the fate of PF and SAG sutures could be reversed by manipulating Wnt signaling. Continuous activation of canonical Wnt signaling in the PF suture prevented endochondral ossification and suture closure whereas inhibition of canonical Wnt signaling in the SAG suture resulted in endochondral ossification and abnormal fusion.[59] Finally, the murine model has allowed for study of the stress induced system of intrauterine constraint. Originally developed by Koskinen-Moffett, Jacob et al. employed this system to show decreased expression of Indian hedgehog and *noggin* to be associated with constraint-induced suture fusion.[60,61]

Similar to mice and rats, a rabbit model of familial non-syndromic craniosynostosis has also been developed to study pathologic suture fusion.[62-64] This model has demonstrated many similarities with the human condition: autosomal dominant transmission and variable phenotypic expression including unilaterally affected animals, delayed-onset suture synostosis and animals with complete bilateral fusion.[65] Moreover the size of the rabbit, in contrast to mice and rats, has facilitated studies looking at post-surgical re-stenosis. In rabbits with coronal synostosis, investigators have transplanted "wild-type" dura mater allografts into suturectomy sites following removal of pathologically fused sutures.[66] This resulted in a reduction of post-operative refusion rates, highlighting the important role dura mater plays in dictating overlying bone formation.

While significant advances in our understanding of cranial suture biology have been achieved using mammalian models, non-mammalian models possess some unique advantages which have rendered them invaluable. Our laboratory has used the zebrafish which possess a short reproductive cycle, large number of progeny, and a high degree of genetic and developmental conservation with humans.[67] Furthermore, they have transparent embryos which permits direct visualization of morphogenesis, including that of the craniofacial elements, without the need for much processing.[68] Genetic manipulation and screening

on a whole genome basis can also be performed given that the entire genome has been sequenced. Interestingly, however, the zebrafish differs from humans and murine models in that the PF suture remains patent. Therefore, ongoing studies into the molecular mechanisms which confer this difference are being performed.

Over the past few years, our laboratory has also introduced *Xenopus laevis* as another potential model for studying suture biology.[69] In *Xenopus*, both the frontal and parietal bones are fused, leaving only two sutures, a midline suture that fuses during metamorphosis and a posterior suture that remains patent.[69] *Xenopus laevis* is also unique in that a dramatic transformation takes place from a cartilaginous to bony skull. A better understanding of the molecular program underpinning this process may provide novel insights into development of the calvarium.

The Role of Dura Mater

While investigations continue on defining the aberrant biomolecular events within the suture mesenchyme and osteogenic fronts, a paracrine effect of neural crest derived dura mater on overlying suture fate has also been purported from both in vivo and in vitro animal studies. Interruption of this interaction in the PF suture by interposition of a silicon sheet has been shown to delay suture fusion in rats.[70] Surgical relocation of the SAG suture over PF dura mater and PF suture over SAG dura mater resulted in abnormal SAG suture fusion and PF suture patency.[69,71] Finally, transplantation of COR suture complexes without the underlying dura mater into parietal defects in rats revealed that in the absence of dura mater, the coronal suture fused. In contrast, when COR sutures were transplanted with the underlying associated dura mater, patency was maintained.[72]

The importance of dura mater interaction with the overlying suture has also been highlighted by Mooney et al. using the rabbit model. Following coronal suturectomy, re-fusion could be prevented by dura mater allografts from WT rabbits allowing for normal unrestricted craniofacial growth.[66] More recently, in vitro co-culture experiments by Cooper and colleagues showed that associated dura mater from fused coronal sutures induced increased alkaline phosphatase activity in osteoblasts harvested from the fused coronal suture.[73] This effect was not observed, however, in osteoblasts derived from wild-type rabbits. Lastly, in vivo experiments similar to those performed in the rat have demonstrated that interposition of a physical barrier between the dura mater and coronal suture complex could successfully inhibit dura-mater-derived osteogenesis in rabbits.[74] Such invaluable studies have thus deepened our comprehension into the myriad of influences guiding suture fate and provide alternative avenues for subsequent development of therapeutics aimed at preventing post-operative resynostosis.

Future Directions: Approaching a New Era of Targeted Therapeutics

While the mainstay of treatment for craniosynostosis remains surgical, accumulating data from insightful clinical and animal studies have rendered the tantalising prospect of an entirely non-surgical approach to craniosynostosis possible. Furthermore, advances in our understanding of biomolecular mechanisms involved in suture fusion along with an evolution of surgical approaches may permit the use of combination therapies which would employ improved surgical technique in concert with pharmacological or genetic adjuncts to minimize morbidities of total cranial vault remodelling.

The first reported surgical intervention for sagittal synostosis was made by Lannelongue in 1890 who described a strip craniectomy to remove the prematurely fused suture. Early attempts at surgical correction, however, were plagued by post-operative refusion at craniectomy sites. Alternative techniques have subsequently been described including the pioneering work of Paul Tessier in the 1970s who developed an approach for total calvarial vault remodeling.[7] This allowed for immediate volumetric expansion and correction of the misshapen skull but has also been associated with increased operative times, hospital stays, and blood loss requiring transfusion.[8-12] In 1998, Jimenez and Barone described the first minimally invasive endoscopic repair for sagittal synostosis, demonstrating shorter operative times, reduced blood loss, and earlier hospital discharge.[75] Importantly, however, minimally invasive approaches achieve less immediate remodeling and instead rely on the adjunctive post-operative use of skull-molding helmets which can range from several months to a year. Nevertheless, endoscopic cranial suture release is frequently performed and is the favored technique at many centers for certain types of craniosynostosis.[11]

Although the evolution of surgical technique is a vital aspect of improving clinical care and outcome for patients with craniosynostosis, the significant advances in our understanding of the biomolecular underpinnings guiding cranial suture development hold promise for a potential paradigm shift in treatment and/or prevention of craniosynostosis. Given the well-established role of FGF signaling in the etiopathogenesis of craniosynostosis, FGF receptor downregulation or interference with the signaling cascade presents an avenue for development of pharmacological therapy. Ueno et al.[76] first described use of a truncated FGFR-1, which lacks its cytoplasmic domain, to inhibit signal transduction by FGF ligands.[76] Greenwald et al. subsequently employed the same truncated FGFR-1 in rat calvarial osteoblasts, demonstrating impaired MAP kinase activation in response to FGF-2.[53] Furthermore, when this dominant-negative FGF receptor was introduced in utero into the PF sutures of fetal rats, postnatal fusion of the PF suture was prevented. This elegant study demonstrated the possibility that by reducing the physiological level of FGF signaling through use of a truncated receptor, abnormal suture fusion may be prevented. Alternatively, RNA interference could be used to target suppression of specific FGF receptors in patients with gain-of-function mutations. Promising results have been demonstrated in mice using a shRNA targeting a mutant form of FGFR-2.[77] This was shown to prevent an Apert-like syndrome in mice. Importantly, while this technique could provide a highly specific and efficient reduction in gene expression, clinical translation would demand the need for development of a means for safe, targeted delivery and durable suppression throughout calvarial development.

Modulation of downstream FGF signal transduction is another appealing target for directed pharmacological therapy. Treatment of Apert syndrome osteoblasts with the MAP kinase inhibitor PD98059 has been shown to reduce IL-1α expression which is pathologically upregulated in these cells.[78] Similar inhibitors of the MAPK pathway may also impede pathological suture fusion resulting from increased FGF signaling activity.[79] In a mouse model of Apert syndrome, treatment of mutant mice with U0126 significantly inhibited craniosynsotosis.[77] These findings highlight the potential for small-molecule inhibitors to be used in the treatment of specific gain-of-function mutations observed in syndromic craniosynostosis.

The TGFβ signaling pathway has also attracted significant attention as a potential target for pharmacological therapy. Given elevated isoforms of TGF-β in prematurely fused sutures, Opperman et al. demonstrated that delivery of TGF-β antibodies could alter suture fate in an ex vivo murine organ culture system.[50,80,81] Delivery of neutralizing antibodies to TGF-β3 resulted in aberrant suture fusion while TGF-β2 antibodies was found to rescue sutures from osseous obliteration.[81] More recently, suppression of TGF-β1 through RNA interference in mouse primary dura cell culture has been shown to downregulate expression of *FGF-2* and *FGFR-1*.[82,83] This again raises the exciting prospect of using siRNAs to suppress genes involved in promoting suture fusion. But as mentioned above, an efficient and safe means to deliver siRNA constructs must be first developed to achieve clinical efficacy and ongoing studies are evaluating a variety of synthetic vehicles including nanoparticles, liposomes, and other lipid-like materials.[84]

While investigations continue to develop novel pharmacological approaches targeting the biomolecular pathways involved in pathologic suture fusion, studies have also identified potential candidates that may serve as useful adjuncts to contemporary surgical treatment. As previously described, Warren et al. demonstrated that overexpression of the BMP antagonist Noggin could prevent physiologic suture fusion in mice.[58] In contrast, studies have also shown downregulation of Noggin to enhance signaling by endogenous BMPs resulting in increased osteogenesis. Based on these findings, Cooper and colleagues have evaluated the ability for exogenous delivery of Noggin to inhibit postoperative resynostosis in both mice and rabbits.[85,86] Introduction of Noggin in a slow-resorbing collagen vehicle was found to significantly improve maintenance of post-suturectomy defects compared with control animals which demonstrated complete re-ossification. These data thus suggest that Noggin may have a potential therapeutic role as an adjunct to surgery, preventing postoperative re-fusion in children with craniosynostosis.

Conclusion

As our understanding of the molecular and genetic underpinnings of craniosynostosis improves, the prospect of pharmacological or genetic therapy for the treatment or prevention of premature suture fusion becomes increasingly tangible. Such an approach, however, has yet to overcome significant obstacles in order to translate into the clinical setting. The demonstration of multiple signaling pathways involved in guiding suture fate and the complexity of cross talk between these pathways may render a one pathway pharmacological approach insufficient. Moreover, the highly conserved pathways which have been associated with craniosynostosis also play a variety of key physiological roles during development. Therefore, any potential therapy must be focused in a spatiotemporal manner to avoid unwanted deleterious consequences. Contemporary approaches to this heterogeneous and potentially debilitating condition remain surgical, and evolution of current techniques continues with the goal of minimizing morbidities associated with such surgical interventions. Toward this end, exploitation of targeted pharmacological therapy as an adjunct to prevent post-operative suture re-fusion may be readily incorporated. Nevertheless, the promise for novel pharmacological/genetic-based therapies has become increasingly real, and the possibility to obviate need for surgery remains an enticing prospect. With continued studies in clinical genetics and biomolecular mechanisms involved in premature suture fusion, new insights will undoubtedly be made which could, the in the future, usher in a new era of non-surgical therapy for craniosynostosis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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