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CLINICAL GENETICS (J STOLER, SECTION EDITOR)

Closing the Gap: Genetic and Genomic Continuum from Syndromic to Nonsyndromic Craniosynostoses

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Abstract Craniosynostosis, a condition that includes the premature fusion of one or multiple cranial sutures, is a relatively common birth defect in humans and the second most common craniofacial anomaly after orofacial clefts. There is a significant clinical variation among different sutural synostoses as well as significant variation within any given single-suture synostosis. Craniosynostosis can be isolated (i.e., nonsyndromic) or occurs as part of a genetic syndrome (e.g., Crouzon, Pfeiffer, Apert, Muenke, and Saethre–Chotzen syndromes). Approximately 85 % of all cases of craniosynostosis are nonsyndromic. Several recent genomic discoveries are elucidating the genetic basis for nonsyndromic cases and implicate the newly identified genes in signaling pathways previously found in syndromic craniosynostosis. Published epidemiologic and phenotypic studies clearly demonstrate that nonsyndromic craniosynostosis is a complex and heterogeneous condition supporting a strong genetic component accompanied by environmental factors that contribute to the pathogenetic network of this birth defect. Large population, rather than single-clinic or hospital-based studies is required with phenotypically homogeneous subsets of patients to further understand the complex genetic, maternal, environmental, and stochastic factors contributing to nonsyndromic craniosynostosis. Learning about these variables is a key in formulating the basis of multidisciplinary and lifelong care for patients with these conditions.

Keywords Craniosynostosis · Suture · Sagittal synostosis · Coronal synostosis · Genome wide association study · Whole exome sequencing

Abbreviations

NSC	Nonsyndromic craniosynostosis
3D-CT	Three-dimensional computed tomography
FGF/FGFR	Fibroblast growth factor/fibroblast growth factor receptor
BMP	Bone morphogenetic protein
BBS	Bardet–Biedl syndrome
ERF	ETS2 repressor factor
TCF12	Transcription factor 12

Introduction

Craniosynostosis, which always involves the premature fusion of one or more of the neurocranial sutures and can include many associated dysmorphologies of the craniofacial complex, is a relatively common congenital malformation [1]. The incidence of craniosynostosis is estimated to be in the range of 1 in 2,000–2,500 live births and occurs in all ethnic groups [2–5].

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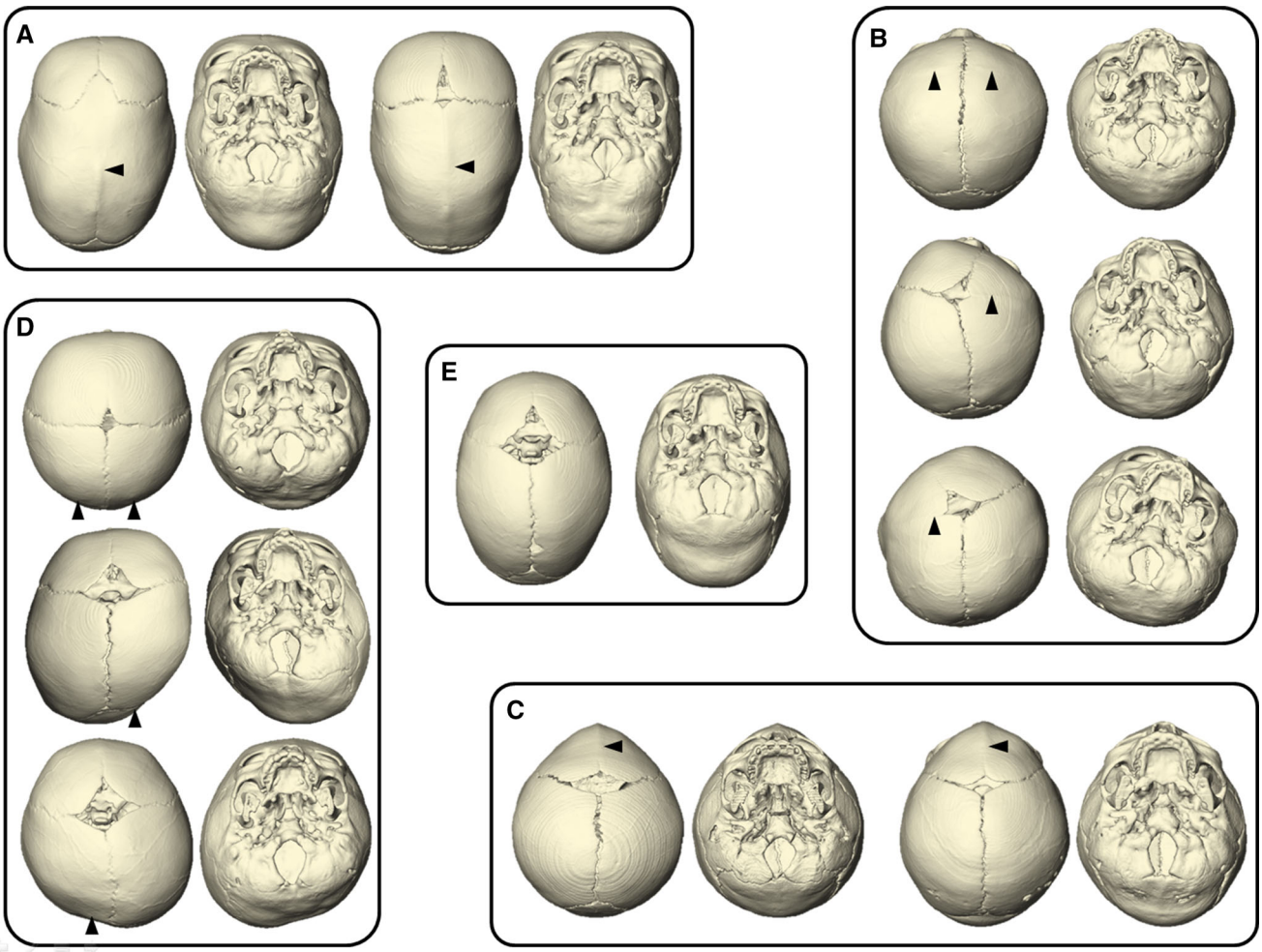

Fig. 1 Variation in cranial vault shape depicted by three-dimensional computed tomography (CT) reconstructions of infants with various types of single-suture craniosynostosis (superior and inferior (i.e., ectocranial) views; anterior aspect of skull at *top*, posterior aspect of skull at *bottom*). Those pictured are cases of single-suture craniosynostosis from our archive of 3D CT images and include skulls of infants with **a** sagittal craniosynostosis; **b** bicoronal, right unicoronal, and left unicoronal craniosynostoses (from *top* to *bottom*); **c** metopic craniosynostosis; **d** bilateral, right unilateral, and left unilateral lambdoidal craniosynostoses (from *top* to *bottom*); **e** unaffected individual. The *black arrow* heads indicate the sutures that are prematurely closed. Though most attention has been focused on cranial vault shape, the cranial base and facial skeleton are also dysmorphic in craniosynostosis conditions. Not to scale

Approximately 85 % of cases are nonsyndromic, and 92 % are non-familial. The other 15 % of cases are defined as having one of the more than 180 known craniosynostosis syndromes, at least 50 % of which follow a Mendelian pattern of inheritance [6, 7].

The frequency of fusion of each of the cranial vault sutures varies. Sagittal synostosis, the most common of the isolated craniosynostoses, occurs in 45–58 % of all craniosynostoses with males more often affected than females (M:F ratio of 3.5:1) [3, 8]. The fusion of the midline sagittal suture results in scaphocephaly, a skull shape that is relatively longer than normal along the anterior-posterior axis and narrowed mediolaterally (Fig. 1a). However, there is a great variability in the scaphocephalic morphology of sagittal synostosis that involves not only the cranial vault but also the facial skeleton and cranial base [9]. Little is known about the sources of this heterogeneity.

Coronal synostosis occurs in 20–30 % of all cases of craniosynostosis with females more often affected than males (M:F 1:2) [10, 11]. The overt cranial dysmorphology of coronal craniosynostosis varies depending upon whether premature closure of the coronal suture occurs bilaterally involving both the right and the left side of the skull (resulting in brachycephaly) or unilaterally (resulting in anterior plagiocephaly or asymmetry) (Fig. 1b). Unilateral coronal synostosis occurs twice as often as bilateral coronal synostosis. Progressive frontal plagiocephaly or flattening sometimes results from the fusion of the fronto-sphenoidal or fronto-zygomatic sutures and is detected by the detailed three-dimensional computed tomography (3D-CT) imaging

of the basilar coronal ring sutures involving the ethmoid-sphenoidal sutures [12, 13]. Metopic synostosis, resulting in trigonocephaly (Fig. 1c), had an estimated prevalence of 6–7 in 100,000 live births prior to 2000, but in the past decade its presentation in some medical centers in Europe and the US has increased as much as fourfold for unknown reasons [14, 15]. Approximately 67 % of all metopic cases are nonsyndromic, and 92 % are non-familial [16]. Male to female ratio has been estimated to be about 3:1. Lambdoid synostosis, resulting in posterior plagiocephaly (Fig. 1d), is estimated to represent about 1 % of all craniosynostosis [1].

Candidate Gene Mutations Found in Nonsyndromic Craniosynostosis

Causative mutations for craniosynostosis have been primarily identified in coronal craniosynostosis syndromes most often within the *FGFR1*, *FGFR2*, *FGFR3*, *TWIST1*, and *EFNB1* genes, but the list of new genes involved in less common syndromes is growing (Table 1), reviewed by Passos-Bueno et al. [17], Wilkie et al. [18], and Jabs and Lewanda [19], and there is an association between the pattern of facial dysmorphogenesis and causative mutation for some of these syndromes [20•]. However, the genetic etiology of nonsyndromic craniosynostosis (NSC) remained poorly understood until very recently. Over the last two decades, the search for genetic mutations underlying NSC has focused on "hotspots" of genes that are known to cause syndromic craniosynostosis [21]. Rare mutations in *FGFRs, TWIST1, LRIT3, ALX4*, *IGFR1, EFNA4, RUNX2*, and *FREM1* have been reported in a minor fraction of NSC cases (Table 1).

An example of a successful identification of a single point mutation in a candidate gene is the fibroblast growth factor receptor 3 (FGFR3) Pro250Arg mutation associated with individuals initially diagnosed with isolated coronal craniosynostosis [22, 23]. The identification of the FGFR3 Pro250Arg mutation resulted in the definition of Muenke syndrome [23] characterized by a highly variable phenotype with some individuals appearing phenotypically normal [24] demonstrating a reduced penetrance of the mutation at about 80 % [18]. It has been estimated that the FGFR3 Pro250Arg mutation may account for 4–12 % of isolated unilateral and 30–40 % of isolated bilateral coronal synostosis cases [25, 26] with a population prevalence of about 1 case per 30,000 [18].

Other rare gene mutations have been identified for isolated synostosis [27]. However, some of these mutations were present not only in the affected probands, but also in other members of the family, who had craniofacial dysmorphisms (but not craniosynostosis) or were unaffected suggesting incomplete penetrance [28–32]. In one case of sagittal NSC, the FGFR2 Ala315Thr mutation was reported [33]. Two cases with sagittal NSC were found to carry Ser494Thr and Cys592Tyr mutations in LRIT3, a protein believed to regulate maturation and signaling of FGFR1 [28]; another three sagittal NSC cases had Val7-Phe, Lys211Glu, and Pro306Leu mutations in ALX4, a homeobox containing transcription factor regulating calvarial development through interactions with Wnt and bone morphogenetic proteins (BMPs) [31]. TWIST1 mutations, Ala186Thr, Ser201Tyr, and Ser188Leu in the TWIST Box domain, were found in two cases of the isolated sagittal synostosis and in one case of isolated left coronal synostosis, respectively [29, 30]. Insulin-like growth factor 1 receptor (IGF1R) mutations, R406H, N857S, and R595H, were found in two cases of isolated sagittal and one with coronal synostosis, and rare variants P190S and M446V were also detected [34]. For coronal NSC, an FGFR2 Ala315Ser mutation was reported in a patient with unicoronal synostosis and a birth history of breech presentation and skull compression [35]. EFNA4 His60Tyr, Pro117Thr, and Asn157LysfsX45 mutations have been reported in three patients with coronal NSC [32]. An FGFR1 Ile1300Trp mutation was found in one case of metopic NSC with facial skin tags [36]. Recently, a 1.1 Mb duplication encompassing *RUNX2* and mutations in *FREM1* has been associated with metopic NSC [37, 38]. Apart from *IGFR1* and *FREM1*, the above genes can be linked directly to *TWIST1* and the FGF signaling pathway, which ultimately interact to control the entry of mesenchymal cells into osteoblastic differentiation in the developing suture; it is speculated that FREM1 may also bind FGFs to modulate the FGF pathway [38].

Novel Genes Identified in Craniosynostosis Using Genomic Technologies

In the past 2 years, important breakthroughs have been achieved with the identification of new genes associated with sagittal and coronal NSC. Justice et al. [39••] reported susceptibility loci for sagittal NSC near *BMP2* and *BBS9*. Twigg et al. [40••] showed that the reduced dosage of *ERF* causes sagittal, lambdoid, and multisuture craniosynostoses in cases diagnosed as isolated or syndromic craniosynostosis. Finally, Sharma et al. [41••] identified mutations within *TCF12* associated with unilateral and bilateral coronal craniosynostosis in patients with isolated or syndromic craniosynostosis. Here, we review these mutations in genes that may account for a significant number of patients diagnosed with NSC.

Table 1 Genes and pathways in syndromic and nonsyndromic craniosynostosis

Gene	Phenotype/condition[a]	Calvarial sutures[b]	Mutation type	Pathways/function	Reference
ABCC9	Cantu syndrome	Coronal, sagittal	Probable loss of function	K^+ Channel	[79]
ALPL	Hypophosphatasia infantile type	Coronal, sagittal, lambdoid	Loss of function	Mineralization	[80, 81]
ALX4	Nonsyndromic craniosynostosis	Sagittal	Gain of function	BMP	[31]
CD96	C syndrome, Opitz trigonocephaly	Metopic	Loss of function	Adhesion	[82]
CHST3	Autosomal recessive Larsen syndrome	Sagittal	Loss of function	GAG sulfation	[83]
CYP26B1	Craniosynostosis with radiohumeral fusions and other skeletal and craniofacial anomalies	Coronal, lambdoid	Loss of function	Retinoic acid metabolism	[84]
EFNA4	Nonsyndromic coronal synostosis	Coronal	Loss of function	EPH/EPHRIN	[32]
EFNB1	Craniofrontonasal syndrome	Coronal	Haploinsufficiency	EPH/EPHRIN	[85]
ERF	Complex craniosynostosis, nonsyndromic sagittal or lambdoid synostosis	Multiple	Loss of function	ERK1/2	[40]
FAM20C	Non-lethal osteosclerotic bone dysplasia	Multiple	Loss of function	Mineralization	[86]
FBN1	Shprintzen–Goldberg syndrome	Coronal, sagittal, metopic	Loss of function	TGF-β	[87]
FGF3, FGF4	Syndromic multiple craniosynostosis	Multiple	Duplication	FGF	[88]
FGFR1	Osteoglophonic dysplasia	Multiple	Constitutive activation	FGF	[89]
FGFR1	Pfeiffer syndrome	Coronal	Enhanced ligand affinity	FGF	[90]
FGFR1	Trigonocephaly, metopic synostosis with facial skin tags	Metopic	Gain of function	FGF	[36]
FGFR2	Antley–Bixler syndrome without genital anomalies or disordered steroidogenesis	Coronal, lambdoid	Enhanced ligand affinity	FGF	[91]
FGFR2	Apert syndrome	Coronal	Enhanced ligand affinity	FGF	[92, 93]
FGFR2	Beare–Stevenson syndrome	Coronal	Constitutive activation	FGF	[94]
FGFR2	Bent-bone dysplasia-FGFR2 type	Coronal	Loss of function	FGF	[95]
FGFR2	Crouzon syndrome	Coronal	Constitutive activation	FGF	[96, 97]
FGFR2	Jackson–Weiss syndrome	Coronal	Constitutive activation	FGF	[97]
FGFR2	Pfeiffer syndrome	Coronal	Constitutive activation	FGF	[98–100]
FGFR2	Nonsyndromic coronal synostosis	Coronal	Enhanced ligand affinity	FGF	[35, 101]
FGFR2	Nonsyndromic sagittal synostosis	Sagittal	Gain of function	FGF	[33]
FGFR3	Crouzonodermoskeletal syndrome	Coronal	Constitutive activation	FGF	[102]
FGFR3	Muenke syndrome	Coronal	Enhanced ligand affinity	FGF	[23]
FGFR3	Nonsyndromic coronal synostosis	Coronal	Enhanced ligand affinity	FGF	[103]
FGFR3	Thanatophoric dysplasia Type II	Coronal, sagittal, lambdoid	Constitutive activation	FGF	[104]
FREM1	Nonsyndromic trigonocephaly	Metopic	Loss of function	Extracellular signaling	[38]
GLI3	Greig cephalopolysyndactyly syndrome	Sagittal, metopic	Loss of function	SHH	[105, 106]
GPC3	Simpson–Golabi–Behmel syndrome	Coronal	Loss of function	Extracellular signaling	[107]
IFT122	Cranioectodermal dysplasia 1 (Sensenbrenner syndrome)	Sagittal	Loss of function	Cilia	[50]

Table 1 continued

Gene	Phenotype/condition[a]	Calvarial sutures[b]	Mutation type	Pathways/function	Reference
IFT43	Cranioectodermal dysplasia 3 (Sensenbrenner syndrome)	Sagittal	Loss of function	Cilia	[51]
IGF1R	Isolated single-suture craniosynostosis	Coronal, sagittal	Loss of function	IGF	[34]
IHH	Craniosynostosis and syndactyly	Multiple	IHH duplication	IHH	[108]
IL11RA	Craniosynostosis and dental anomalies syndrome	Multiple	Loss of function	JAK/STAT	[109]
JAGGED1	Alagille syndrome	Multiple	Loss of function	Notch	[110, 111]
LMX1B	Nail–patella syndrome	Coronal	Loss of function	Transcription	[18]
LRIT3	Nonsyndromic sagittal synostosis	Sagittal	Loss of function	FGF	[28]
MEGF8	Carpenter syndrome	Coronal, sagittal, metopic	Loss of function	Signaling	[112]
MSX2	Boston-type craniosynostosis	Coronal, sagittal, metopic	Enhanced DNA binding affinity	FGF/BMP	[113–115]
MSX2	Craniosynostosis	Sagittal, lambdoid	Trisomy	FGF/BMP	[116, 117]
OSTM1	Infant osteopetrosis, craniosynostosis, Chiari malformation	Coronal, sagittal	Loss of function	Osteoclast function	[118]
POR	POR syndrome with genital anomalies and disordered steroidogenesis	Coronal, lambdoid	Loss of function	Retinoic acid metabolism	[119]
RAB23	Carpenter syndrome	Coronal, sagittal, metopic, lambdoid	Loss of function	SHH	[120]
RECQL4	Baller–Gerold syndrome	Coronal, lambdoid	Loss of function	DNA repair	[121]
RUNX2	Nonsyndromic synostosis	Metopic	Duplication	Transcription	[37]
SH3PXD2B	Borrone dermato-cardio-skeletal	Sagittal, lambdoid	Loss of function	Adhesion	[122]
SH3PXD2B	Frank–ter Haar syndrome	Sagittal	Loss of function	Adhesion	[123]
SKI	Shprintzen–Goldberg syndrome	Coronal, sagittal	Loss of function	TGF-β	[124]
SOX6	Craniofacial dysostosis	Sagittal, lambdoid	Loss of function	Transcription	[125]
TCF12	Bicoronal synostosis	Coronal	Loss of function	TWIST	[41]
TGFBR1	Loeys–Dietz type 1	Sagittal (multiple)	Loss of function	TGF-β	[126, 127]
TGFBR2	Loeys–Dietz type 2	Sagittal (multiple)	Loss of function	TGF-β	[126]
TWIST1	Saethre–Chotzen syndrome	Coronal	Loss of function	TWIST	[57, 58]
TWIST1	Nonsyndromic coronal synostosis	Coronal	Loss of function	TWIST	[29, 30]
TWIST1	Nonsyndromic sagittal synostosis	Sagittal	Loss of function	TWIST	[30]
WDR19	Cranioectodermal dysplasia 4 (Sensenbrenner syndrome)	Sagittal	Loss of function	Cilia	[52]
WDR35	Cranioectodermal dysplasia 2 (Sensenbrenner syndrome)	Sagittal	Loss of function	Cilia	[53]
ZEB2	Mowat–Wilson syndrome with craniosynostosis	Coronal, metopic	Loss of function	Transcription	[128]

[a] For nonsyndromic craniosynostosis phenotypes, the gene change may be non-penetrant rather than a disease-causing mutation in some individuals

[b] Principle sutures involved are listed

Susceptibility Loci for Sagittal NSC Near BMP2 and BBS9

Though sagittal NSC is the most frequent form of craniosynostosis, the genetic basis for most cases is unknown, and only rare gene mutations have been identified until recently [27]. Justice et al. [39••] conducted a genome wide association study (GWAS) of 130 non-Hispanic case-parent trios of European ancestry followed by the replication analysis of 172 unrelated non-Hispanic Caucasian cases and 548 controls to identify susceptibility loci for sagittal NSC near *BMP2* and within *BBS9*. The discovery/replication meta-analysis demonstrated the combined odds ratios of 4.38 (95 % CI 3.51–5.45; $P = 1.1 \times 10^{-39}$) and 0.24 (95 % CI 0.17–0.32; $P = 5.6 \times 10^{-20}$), respectively. BMP2 is a member of the TGF-β superfamily and a key growth factor regulating osteoblast development [42]. The BMP and FGF pathways interact and are important in skull growth [43–45]. *BBS9* is a member of the BBSome, a multiprotein complex localized in the primary cilium that is involved in coordinating many developmentally important signaling pathways including platelet-derived growth factor receptor α, sonic hedgehog, and Wnt [46, 47]. The BBSome is also implicated in intraflagellar transport [48]. BBS9 loss-of-function mutations have been found in Bardet–Biedl syndrome (BBS) patients [49]. Although BBS-affected individuals do not present with suture phenotypes, there are ciliopathy conditions that have craniosynostosis as a feature such as cranioectodermal dysplasia (Sensenbrenner syndrome) [50–53].

As of this writing, no study phenotypically characterizing sagittal NSC cases with or without *BMP2* or *BBS9* variations has been published. Although the morphology of the cranial vault has been observed as a defining characteristic in craniosynostosis, qualitative assessments of calvarial dysmorphology have shown consistent variability in NSC (Fig. 1) [54], and the exact source of this variation remains unknown. A recent quantitative study of craniofacial shape in 43 infants with nonsyndromic sagittal synostosis using 3D-CT reconstruction and morphometric methods confirmed variation in cranial vault morphology [9]. In all cases studied, the central portion of the sagittal suture was the first to fuse (probably prenatally), and at least two different developmental paths toward complete fusion of the sagittal suture exist either in the anterior section or in the posterior section of the sagittal suture being the second to fuse. The analyses showed association between the variation in craniofacial shape and the exact path of fusion of the sagittal suture. Comparable morphometric studies should be completed using cases carrying either *BMP2* or *BBS9* NSC-associated variants to determine whether or not these genetic influences correspond with specific phenotypes.

ERF and TCF12 Mutations in Patients with Craniosynostosis

Using whole exome sequencing of seven unrelated individuals with bilateral coronal synostosis and negative for previously described mutations [55], two additional genes, *ERF* and *TCF12*, were found to have mutations in two or more patients with craniosynostosis [40••, 41••]. Heterozygous mutations in ERF, an inhibitory ETS family transcription factor that is negatively regulated by the extracellular signal-related kinases 1 and 2 (ERK1/2) of the mitogen-activated protein kinase (MAPK) signaling pathway, were found [40••]. Some of the patients with the ERF mutations were syndromic with sagittal or multisuture synostosis, craniofacial dysmorphology, Chiari malformation, and language delay, and others were nonsyndromic with sagittal, unilateral or bilateral lambdoid, and multisuture synostosis. ERF was found to bind close to regulatory sites recognized by RUNX2, an essential regulator of osteoblast differentiation, and could interfere with transcriptional activation by RUNX2. Thus, loss-of-function mutations of ERF in these patients have a similar effect to FGFR-phosphorylated ERK activation observed in FGFR-related craniosynostoses. Reduced ERF function in these conditions can result in the upregulation of RUNX2 activity, leading to changes in osteoblast differentiation and potential premature ossification of cranial sutures.

Heterozygous mutations in TCF12, transcription factor 12, were also identified in syndromic and nonsyndromic patients with unilateral and bilateral coronal and multisuture synostoses [41••, 56]. Syndromic patients had additional features of craniofacial dysmorphism and external ear and minor limb anomalies. *TCF12* mutations were found in 32 % of subjects with bilateral and 10 % with unilateral coronal synostosis. TCF12 heterodimerizes with class II basic helix-loop-helix transcription factors including TWIST1. Loss-of-function mutations in *TWIST1* have previously been shown to cause the Saethre–Chotzen syndrome, a craniosynostosis condition with coronal fusion and minor limb anomalies [57, 58]. The TCF12-TWIST1 heterodimer is likely to regulate the specification of the coronal suture between the neural crest-derived frontal bones and mesoderm-derived parietal bones [32, 59]. As in the case of ERF, these dimers may inhibit osteogenic differentiation via actions on RUNX2 and FGFR signaling pathways [60].

Genetic Risk

To estimate the proportion of craniosynostosis patients that screen positive for a gene mutation, a study was conducted on 326 children, who were born from 1993 to 2002 and required surgical treatment in a craniofacial unit in Oxford, England

[18]. Genetic diagnoses were made for 21 % of all craniosynostosis cases, and the FGFR3 P250R mutation was the single most common mutation, accounting for 24 % of cases with genetic diagnoses (5 % of all cases). Those with genetic diagnoses were associated with increased rates of many complications. Children with the clinical diagnosis of nonsyndromic unicoronal or bicoronal synostosis were more likely to have an identified causative mutation than those with other sutural involvement. In the extended Oxford birth cohort (cases born from 1998 to 2006), *TCF12* mutations were identified in approximately 1.0 % of craniosynostosis cases [41]. While the patients with *TCF12* mutations had a more benign course than patients with FGFR3 P250R or TWIST1 mutations, 14 % had developmental delay or learning disabilities and two were diagnosed with autism. *ERF* mutations explain an additional 1.2 % of etiology for the cohort [40].

Cellular and Animal Model Investigations of Craniosynostosis

Both the genome wide expression analysis of primary osteoblasts derived from craniosynostosis patients and the creation of mutant mouse models allow experimental analysis of craniosynostosis phenotypes and the roles of newly-discovered genes in craniosynostosis. Gene expression profiling of human craniosynostosis samples has been recently reviewed [61]. As an example, a recent survey of 199 NSC patient-derived osteoblasts, including sagittal, metopic, and coronal cases, suggested the common involvement of *FGF7*, *SFRP4*, and *VCAM1* and the role of extracellular matrix interactions in the craniosynostosis phenotypes [62].

Mouse models of activating *Fgfr* mutations and of *Twist1* loss-of-function have been invaluable in understanding the coronal synostosis and the role of the neural crest/mesoderm boundary forming this suture [63•, 64]. *Erf* was shown to be expressed within calvarial sutures in the mouse, and the conditional deletion of *Erf* demonstrated that loss of *Erf* was causative for craniosynostosis [40]. While heterozygous null *Tcf12* mice alone did not show craniosynostosis, reduction of *Tcf12* significantly increased the incidence and severity of craniosynostosis in *Twist1* heterozygous null mice, supporting the model of Tcf12 interaction with Twist1 [41]. Gene expression within sutures can be readily determined in mice, and this knowledge is crucial in understanding the connection between gene mutations and specific patterns of suture fusion or other resulting craniofacial dysmorphologies, exemplified by *Fgfrs1–3* and *Twist1,* which have distinct expression patterns within sutures [65]. The restriction of craniosynostosis to specific sutures may reflect the suture-specific expression of some genes. For example, in the mouse model of Greig cephalopolysyndactyly syndrome, the *Gli3* (Xt-J/Xt-J) mouse, the lambdoid sutures fuse and interfrontal suture development is anomalous, and these are the sites of strong embryonic calvarial *Gli3* expression [66]. A comprehensive knowledge of murine sutural gene expression, particularly at embryonic stages, would facilitate the identification of human craniosynostosis candidate genes. Furthermore, the mouse provides a model for the potential therapeutic amelioration of craniosynostosis. Chemical inhibition of Fgfr tyrosine kinase activity, or of the activity of effector kinases downstream of Fgfrs, results in reduced craniosynostosis in mouse models of Crouzon, Apert, and Beare-Stevenson syndromes [67–70].

Conclusions

The etiology of approximately three quarters of patients diagnosed with craniosynostosis is not known. Thus, much work is needed in the elucidation of causal mutations proximate to the *BMP2* and *BBS9* gene loci and in identifying new genes involved in craniosynostosis. Since craniosynostosis is a complex heterogeneous condition, the interplay between genetic variants and environmental exposures may explain the low heritability for NSC, and their combined action may elucidate processes underlying the variable dysmorphogenesis of the facial skeleton and cranial base, portions of the skull that do not encompass the cranial vault sutures. These factors may also contribute to the lack of distinct disease phenotypes across diagnostic groups and the current lack of identified molecular causes in some cases. Mutations in genes initially identified in syndromic cases may contribute to causation in milder phenotypes including nonsyndromic or non-penetrant cases. The large population of patients with nonsyndromic craniosynostosis embodies a fundamental need for more work, as well as a fertile research area for the discovery of novel genetic, maternal, and environmental factors. Harnessing next generation sequencing technology and bioinformatics analysis with our understanding of the genome, transcriptome, and epigenome will help to elucidate the etiology of craniosynostosis, both syndromic and nonsyndromic.

As noted previously, although sutural fusion is the most frequent feature studied and treated, craniosynostosis also refers to the abnormal development of the bones of the skull associated with dysmorphic skull shape. In animal models for human craniosynostosis syndromes, the abnormal skull shape can be detected before the premature closure of cranial vault sutures 71•, 72•]. The development of animal models for craniosynostosis [70, 73, 74] has already revealed many molecularly driven three-dimensional morphological changes in soft tissues of the head and skull that were not apparent in humans [71•, 75, 76•, 77]. These changes are more difficult to evaluate quantitatively in humans where observations are routinely made postnatally and there is a lack of appropriate morphological control data

sets to make meaningful comparisons to abnormal phenotypes. Human cases provide access to population-based molecular screens, and more recently the chance to link genotype with phenotype [20], but do not provide easy access to the molecularly-based processes that result in the highly variable, abnormal cranial morphology of syndromic and nonsyndromic cases of craniosynostosis. This access will be necessary in order to understand what unites the various molecular causes of craniosynostosis (Table 1) at the genomic and phenotypic levels. Mouse models provide access to both the processes that underlie these integrated sets of anomalies and the networks that produce them. Emerging technologies (e.g., optical projection tomography [78]) allow direct study of the correspondence between the spatiotemporal dynamics of gene expression patterns, morphogenesis, and morphological diversity.

The coordinated assimilation of results from human- and animal model-based research that build on respective discoveries is crucial to understand the variation in the integrated anomalies that together define craniosynostosis conditions. These associated anomalies contribute to additional health issues critical to effective clinical care of people with craniosynostosis conditions including type and timing of surgery, treatment of comorbidities, and long-term effects on neuropsychological aspects and quality of life. Future research applied to large molecular datasets, analysis of pathways and networks, and the complexity of the craniosynostosis phenotype will require integrative analyses by multidisciplinary teams of physicians and scientists including system and developmental biologists, quantitative anatomists, epidemiologists, geneticists, medical specialists, and surgeons.

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