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Review

Craniosynostosis: current conceptions and misconceptions

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Abstract: Cranial bones articulate in areas called sutures that must remain patent until skull growth is complete. Craniosynostosis is the condition that results from premature closure of one or more of the cranial vault sutures, generating facial deformities and more importantly, skull growth restrictions with the ability to severely affect brain growth. Typically, craniosynostosis can be expressed as an isolated event, or as part of syndromic phenotypes. Multiple signaling mechanisms interact during developmental stages to ensure proper and timely suture fusion. Clinical outcome is often a product of craniosynostosis subtypes, number of affected sutures and timing of premature suture fusion. The present work aimed to review the different aspects involved in the establishment of craniosynostosis, providing a close view of the cellular, molecular and genetic background of these malformations.

1. Introduction

During early childhood, malformations that impact on growth and development must be given special consideration. Sutures function as key growth centers of the skull during the early years of life [1]. Hence, as the embryonic brain grows, a proportional amount of skull growth is needed at the sutures in order to create the necessary space for the expanding brain. The main role of sutures is to ultimately permit brain growth by coordinating skull expansion in the presence of a developing brain [2]. Suture patency is therefore critical at this stage of life. Craniosynostosis, defined as the premature closure of one or more of the cranial vault sutures, results in a variety of associated skull and subsequent facial deformities secondary to skull growth restrictions that may severely impact on child wellbeing, potentially increasing intracranial pressure, commonly resulting in visual disturbances, frequent headaches and learning/developmental delays [3]. In addition, the inability of the skull to create the adequate space for brain growth by expanding perpendicularly to the fused sutures results in a compensatory expansion of the cranial vault in a direction parallel to these sutures [4], with a compensatory overgrowth at other suture sites, progressively rendering an abnormal head shape [5]. Hence, head shape will be a product of the direction and number of the affected sutures, coupled with the order and timing in which these sutures synostose. The earlier the onset of craniosynostosis, the more dramatic the effect of these malformations is on subsequent cranial growth and development, whereas late synostosed cranial sutures may render a nearly normal-shaped skull [6].

Craniosynostosis can be expressed as an isolated clinical feature, or in association with other clinical symptoms, as part of different types of syndromes. Isolated craniosynostosis is the most commonly observed expression of these malformations, whereas syndromic craniosynostosis is a less frequent finding, usually associated with the fusion of multiple sutures and distinct manifestations in the face, trunk and extremities [5]. The prevalence of isolated craniosynostosis ranges from 0.7 to 6.4 per 10,000 live births [7,8]. Premature closure of the sagittal suture is the most common type of craniosynostosis, followed by metopic, unilateral coronal, bilateral coronal, and lambdoid, in order of decreasing prevalence [8]. There is a higher frequency of sagittal craniosynostosis among males than females (4:1 ratio), whereas unilateral coronal craniosynostosis is either slightly more frequent among females or expresses no sex predilection (3:2 ratio). However, metopic, lambdoid and bilateral coronal synostosis demonstrate no sex predominance [5]. In the absence of craniosynostosis, sutures normally fuse with advancing age from front to back and lateral to medial.

Multiple signaling pathways regulate premature fusion of cranial vault sutures, and the fibroblast growth factor receptors (FGFRs) appear to play an important role in bone growth regulation and subsequent establishment of these malformations [9]. The full genetic/epigenetic signaling pathways of this unique and refined developmental process generating craniosynostosis is not yet fully understood; however, different pieces of this intricate puzzle have been slowly elucidated over the past few decades. In addition, the magnitude of advances in genetics has permitted a better understanding of the interplay between genes, regulatory molecules and cellular biology. The present review was designed to (1) establish a better understanding on the process of craniofacial growth and development that when altered, may lead to craniosynostosis, and (2) a focus on the pathogenesis and genetics of these malformations.

2. Clinical aspects of craniosynostosis

Craniosynostosis can be classified based on the number and type of affected sutures, but classification can also be approached by phenotype variations, if part of a broader range of malformations and/or functional impairments (syndromic craniosynostosis) or not; in which case, the manifestation of craniosynostosis is understood as an isolated finding (non-syndromic craniosynostosis). A population-based study within the United States showed a prevalence of 9% of syndromic craniosynostosis among affected patients [7], contrasting with other authors who reported a 20% prevalence of this type of synostosis [10]. Though over 150 craniosynostosis-associated syndromes have been described, the slow unraveling of the genetics and mechanisms behind these syndromes have permitted the construction of pathways that may relay future ideas for treatment and/or prevention of this condition. However little is known of the etiology of non-syndromic craniosynostosis. For the purpose of this review our discussion of the clinical aspects of craniosynostosis will center on the affected sutures and consequences of such malformations.

3. Single-suture synostosis

3.1. Sagittal suture

Early closure of the sagittal suture is by far the most prevalent form of isolated craniosynostosis, accounting for approximately half of all nonsyndromic forms of these malformations (40–47%), with a reported birth prevalence of 1.9 per 10,000 live births, affecting 3.5 males for every 1 affected female [7,8,11,12]. Hence, gender is a very important risk factor in nonsyndromic sagittal synostosis, along with macrosomia and maternal age [7,8]. Early sagittal suture fusion can be observed in craniofacial syndromes, but syndromes that manifest sagittal craniosynostosis either as an isolated form or in association with other subtypes is usually of rare occurrence (Table 1). Some of these syndromes include cranioectodermal dysplasia 1 [13], hypophosphatemic rickets [14], Loeys-Dietz syndrome 1 [15] and Frank-ter Haar syndrome [16]. In the presence of isolated sagittal craniosynostosis, the cranial vault portrays a scaphocephalic (dolichocephaly) appearance (Figure 1), which can be described as a long and narrow head, with wide or narrow temporal regions, narrowing toward the top of the head, associated with ridging on the path that follows the fused suture, generating the appearance of an inverted boat keel [17]. This head shape is the result of compensatory growth that takes place posteriorly at the lambdoid suture and anteriorly at the metopic and coronal suture sites [18].

3.2. Metopic suture

The birth prevalence of metopic craniosynostosis has been described as being 0.8 in 10,000 live births. Previous epidemiological work from Australia verified a higher risk of metopic craniosynostosis among males [8]; however, a recent population-based study in the United States failed to show any significant association between metopic synostosis and male gender [7]. In addition, high maternal age and multiple birth (e.g., twins and triplets) have been previously associated with an increased risk of isolated metopic craniosynostosis [7,8]. Since the metopic suture tends to close at a very young age (Table 2), differential diagnosis must be made to assure the presence of craniosynostosis. In this case, true premature fusion may be clinically ascertained by the presence of an obvious bony ridge in the

Table 1. Genetic and phenotypic profiles in common syndromic craniosynostosis.

	Genotype					Phenotype			
Syndrome	OMIM #	Gene(s)	Cytogenetic location	Common Mutations[*]	Inheritance	Frequency of Occurrence[**]	Craniosynostosis	Syndrome	Ref
Apert	101200	FGFR2	10q26.13	c.755C>G (p.Ser252Trp), c.758C>G (p.Pro253Arg)	Sporadic, with AD reported	9.9-15.5/1,000,000 BP	Coronal, multisuture	Normal intelligence or various degrees of mental deficiency, ventriculomegaly midface hypoplasia, cutaneous and bony syndactyly of hands (central 3 digits always involved) and feet	[145,169]
Crouzon with acanthosis nigricans	612247	FGFR3	4p16.3	c.1172C>A (p.Ala391Glu)	AD	0.1/100,000 BP	Multisuture	Female preponderance, crouzonoid characteristics, atypical widespread acanthosis nigricans, choanal stenosis, hydrocephalus, hyperplasia, thickening of the axilla, hypopigmentation of surgical scars, melanocytic nevi, oral abnormalities, including cementomas of the jaw and cleft palate	[170-172]
Crouzon	123500	FGFR2	10q26.13	c.1025G>A (p.Cys342Tyr), c.833G>T (p.Cys278Phe), c.938A>G (p.Tyr328Cys), c.1021A>C (p.Thr341Pro), c.1026C>G (p.Cys342Trp), c.1032G>A (p.Ala344Ala), c.1061C>G (p.Ser354Cys)	AD	0.9/100,000 BP	Multisuture, coronal, sagittal, lambdoid	Hypertelorism, exophthalmos and external strabismus, parrot-beaked nose, short upper lip, hypoplastic maxilla (" crouzonoid" facies), and relative mandibular prognathism	[134,136, 173,174]

Pfeiffer	101600	FGFR1, FGFR2	8p11.23-p11.22, 10q25, 10q26.13	FGFR1: c.755C>G (p.Pro252Arg). FGFR2: c.833G>T (p.Cys278Phe), c.1024T>C (p.Cys342Arg)	AD	1/100,000 *I*	Coronal and lambdoid, sagittal (occasional)		[135,152, 175,176]
Carpenter syndrome I	201000	RAB23	6p11.2	RAB23: c.434T>A (p.Leu145*)	AR	< 1/1,000,000 *P*	Multisuture	Brachydactyly, syndactyly, preaxial polydactyly, midface hypoplasia, hypertelorism, broad flat nasal bridge, epicanthus, upslanted palpebral fissures, highly arched eyebrows, low-set ears, obesity, cryptorchidism, congenital heart disease	[27,177]
Carpenter syndrome II	614976	MEGF8	19q13.2	MEGF8: c.7099A>G (p.Ser2367Gly) ; c.1342C>T (p.Arg448*); c.595G>C (p.Gly199Arg)	AR		Metopic		
Muenke	602849	FGFR3	4p16.3	c.749 C>G (p.Pro250Arg)	AD	1.8/100,000 *BP*	Coronal (often bilateral with or without associated sagittal or metopic), sagittal.	Female preponderance, brachycephaly/plagiocephaly/ macrocephaly, temporal bossing, hypertelorism, ptosis or mild proptosis; strabismus, hearing loss, midfacial hypoplasia; and highly arched palate, cleft lip, brachydactyly, clinodactyly, broad thumbs and toes, developmental delay	[178-180]
Saethre-Chotzen	101400	TWIST1	7p21.1	TWIST: Ex1-2 del***; c.394C>T (p.Arg132Trp)	AD	3/100,000 *BP*	Coronal	Short stature, brachycephaly, acrocephaly, low-set frontal hairline, gross ptosis of eyelids, small and low-set ears, beaked nose, dilated parietal foramina, interdigital webbing, and hallux valgus or broad great toe with bifid distal phalanx, intracranial hypertension	[166]

* [182]; ** Estimated prevalence (*P*), birth prevalence (*BP*) or incidence (*I*) [173,181-183]; *** Deletion of exons 1 and 2.
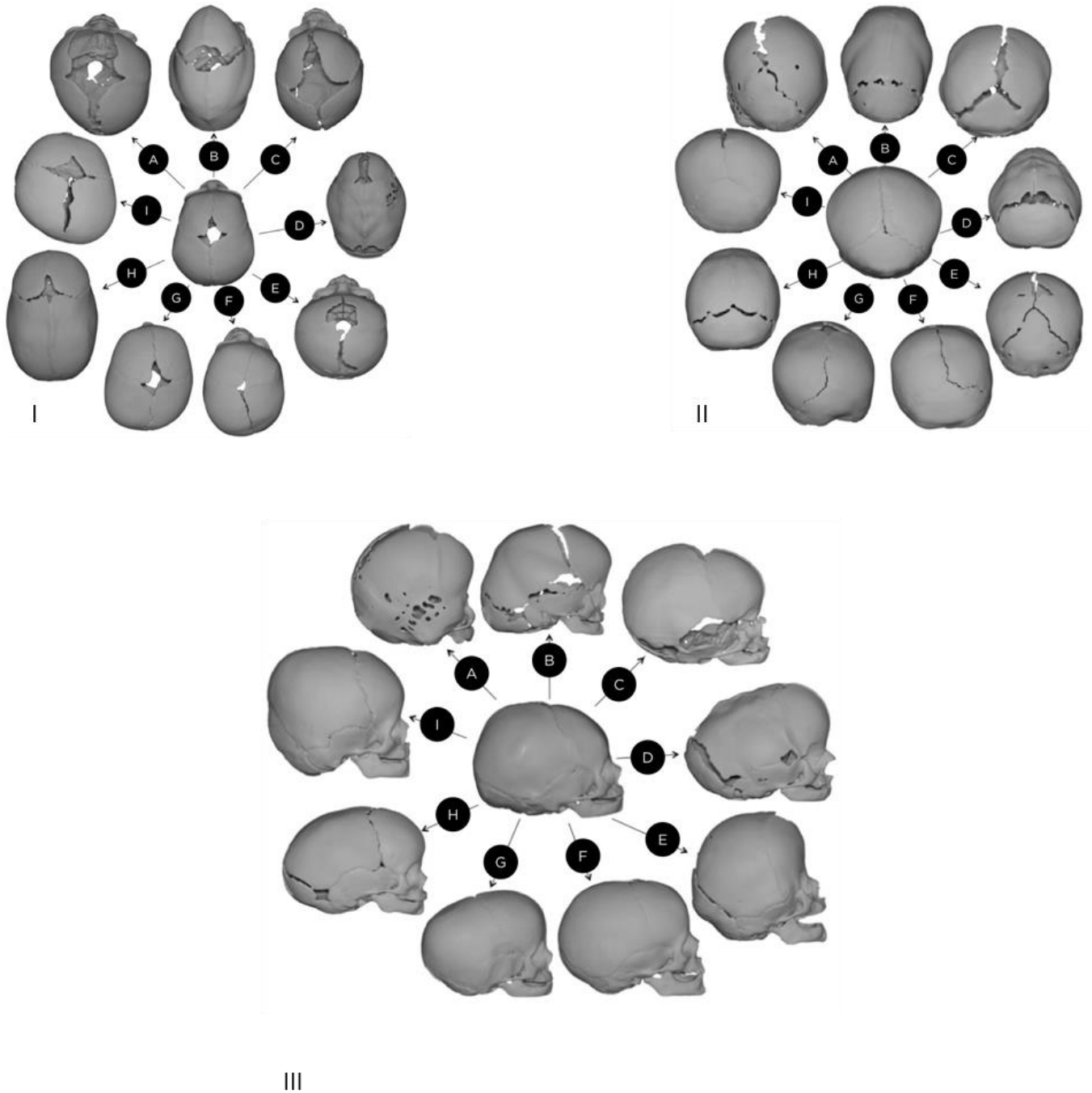
Figure 1. Superior (I), posterior (II) and lateral (III) views of infant skull models in: A) Apert syndrome; B) Carpenter syndrome; C) Crouzon syndrome; D) Pfeiffer syndrome; E) Bicoronal synostosis; F) Unicoronal synostosis; G) Lambdoid synostosis; H) Sagittal synostosis; I) Plagiocephaly. Figure midpoint shows model of a normal infant skull. Models by 3D Medical Systems, Golden, CO (USA).

Table 2. Embryological characteristics of major cranial vault sutures.

Sutures	Origin[a]	Period of intrauterine development[b]	Normal age of closure[c*]
Metopic	Neural crest	13–18 weeks	9–11 months
Sagittal	Mesoderm-neural crest boundary	13–18 weeks	30–40 years
Paired coronals	Mesoderm-neural crest boundary	13–18 weeks	30–40 years
Paired lambdoids	Mesoderm-neural crest boundary	13–18 weeks	30–40 years
Paired squamosals	Mesoderm-neural crest boundary	13–18 weeks	30–40 years

[a][49] [b][75] [c][6,76]

*Sagittal, paired coronals and paired lambdoids may initiate closure prior to 30 years-age. Metopic suture may initiate fusion at 3 months-age.

metopic suture area associated with lateral growth restriction of the frontal bones, often resulting in a triangular keel-shaped head with a horizontally narrowed forehead and temporal areas, known as trigonocephaly. Hypotelorism may also be observed. The mere presence of a metopic ridge, without other related clinical features does not necessarily indicate a diagnosis of craniosynostosis [19] Diagnostic conundrums may be settled by the use of CT scans and other imaging techniques [20]. Compensatory growth will occur at the expense of the coronals and sagittal sutures [21]. Metopic craniosynostosis may be preferentially expressed in an array of syndromes, including chromosomal abnormalities, such as disorders expressing del(9)(p22p24) or monosomy 9p syndrome (MIM 158170) and del(11)(q23q24) or Jacobsen syndrome (MIM 147791) [22], as well as other genetic disorders including Bohring-Opitz syndrome (MIM 605039) [23], 3MC syndrome 2 (MIM 265050) [24], trigonocephaly (MIM 614485) [25], Greig cephalopolysyndactyly syndrome (MIM 175700) [26], Carpenter syndrome (MIM 604267) [27], among others.

3.3. Coronal suture

Given that coronal sutures exist in pairs, this type of craniosynostosis can be expressed on either a unilateral or bilateral fashion. A previous cohort study showed that non-syndromic coronal suture synostosis are more frequently associated with genetic causes than other non-syndromic craniosynostosis subtypes [28]. In addition, the non-syndromic bilateral coronal synostosis was found to be more often familial than its unilateral form [29]. Children with unilateral non-syndromic coronal synostosis expressing single gene mutations were more prone to require squint correction and hearing aids than children with similar phenotype and no identified mutations [28]. Clinically, head shape in its unilateral form exhibits an ipsilateral flattening of the frontoparietal bone with a compensatory contralateral frontal bossing (plagiocephaly) (Figure 1). In addition, the greater wing of the sphenoid fails to descend, while the orbit elongates superior and laterally, resulting in a characteristic "harlequin" deformity that can be observed radiographically. However, bilateral coronal craniosynostosis accounts for an anterior-posterior shortening of the head, with lateral and vertical compensatory growth and an established brachycephalic shape [21]. Unicoronal craniosynostosis has been described as expressing a lower risk among males [8]; thus expressing a higher prevalence among girls. The birth prevalence of coronal craniosynostosis is of 0.7 in 10,000 live births, representing approximately 17% of all nonsyndromic craniosynostosis cases [7].

3.4. Lambdoid suture

Lambdoid craniosynostosis is one of the least prevalent isolated types of nonsyndromic craniosynostosis, and this form of synostosis is only rarely genetically determined. Some of the syndromes associated with this condition are Hurler syndrome (MIM 607714) [30], Simpson-Golabi-Behmel syndrome type 1 (MIM 312870), Pfeiffer syndrome (MIM 101600) and Crouzon syndrome (MIM 123500) [31] (Table 1). Male gender has been recently identified as a significant risk factor for the development of lambdoid craniosynostosis. The birth prevalence of lambdoid craniosynostosis has been reported as 0.7 per 10,000 live births [7]. Clinically, the distinction between lambdoid craniosynostosis and positional plagiocephaly represents a challenge, specially considering the low frequency of these malformations. These infants present ipsilateral flattening of the occiput with a compensatory bulging of the mastoid, and the presence of contralateral growth in the parietal area [21]. 3D computed tomography often provides greater diagnostic accuracy, allowing evaluation of analogous and/or related conditions such as synostosis of the asterion region or mendosal suture [11]. Lambdoid synostosis has also been significantly associated with increased duration of the first stage of labor [32].

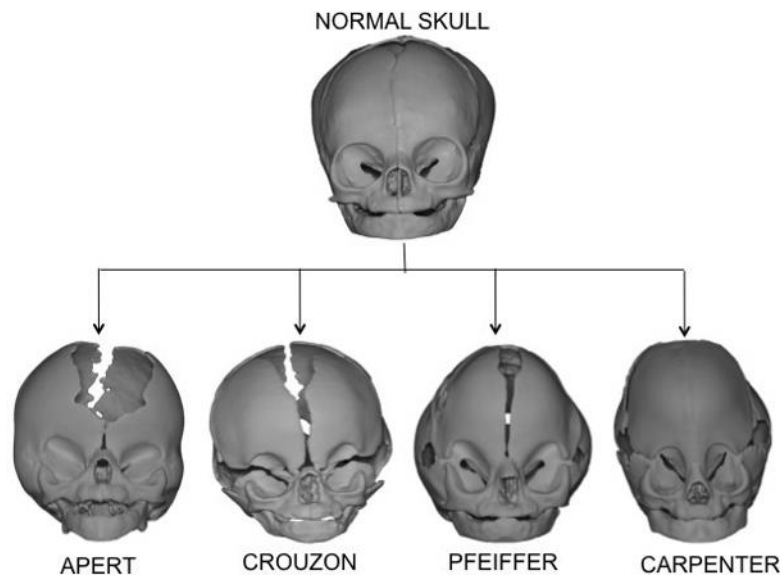## 4. Multiple suture synostosis

Figure 2. Frontal view of skull models with different suture patterns based on specific types of syndromic craniosynostosis in comparison with a normal infant skull. Models by 3D Medical Systems, Golden, CO (USA).

The involvement of multiple suture phenotypes is generally associated with syndromic craniosynostosis (Figure 2). Nevertheless, multiple suture synostosis does not seem to significantly relate to gender, and its risk has been shown to moderately increase in cases of forceps deliveries [8]. In addition, multisuture craniosynostosis has also been associated with a higher incidence of acquired Chiari deformations (especially with lambdoid involvement), the need for multiple operative procedures, and a greater incidence of developmental delays than isolated craniosynostosis [33]. Nonsyndromic

multisuture phenotypes though rare were reported among nearly 8% of infants with nonsyndromic craniosynostosis, and the most frequently observed suture combination was sagittal and lambdoid sutures [7].

5. Cranial development

During human prenatal development, gastrulation is considered to be complete with the formation of the three primary tissue layers of the embryo—ectoderm, mesoderm, and endoderm, marking the beginning of tissue differentiation. Cells that give rise to the tissues of the head originate from these three germ layers. Often recognized as a fourth tissue layer, the neural crest arises from the ectoderm at the most dorsal aspect of the neural tube. Migration of neural crest cells away from the closing and/or closed neural folds allows for the identification of the cranial neural crest [34]. Cranial neural crest consists of pluripotent stem-like cells that migrate dorsolaterally in response to specific signal transduction instructions, producing the craniofacial mesenchyme which forms most of the hard tissues of the head such as bone, cartilage, and teeth, representing one of the key features of craniofacial development. Hence, depending on its location within the embryo, neural crest cells express the ability to differentiate into any of several different cell types, to the extent of being able to produce nerves under certain conditions when transplanted into regions that wouldn't otherwise produce this type of tissue [35]. In addition, the specification, migration, proliferation, survival, and ultimate fate determination of the cranial neural crest plays an important role in regulating craniofacial development. Alterations in the fate determination of these cells influenced by insults of genetic and/or environmental origin may result in craniofacial malformations [34]. Candidate intercellular regulatory molecules of this process [36] include members of the TGF-β family [37], fibroblast growth factors (FGFs) [38], platelet-derived growth factor (PDGF) [39], and Wnt gene products [40]. At this stage, the head has mesenchyme originating from paraxial mesoderm and the cranial neural crest [41], and tissue margins should remain intact. Previous data confirms a generalized segregation of neural crest and cephalic paraxial mesoderm cells in the ectocranial mesenchyme, and demonstrates the complexity and unidirectional nature of the mammalian calvarial mesoderm-neural crest boundary; the paraxial mesoderm compartment (coronal suture mesenchyme and parietal bone) remains impermeable to neural crest cells, whereas the postulated neural crest-derived compartment (frontal bone) permits lineage intermixing [42]. Tissue-tissue interaction at this area controls development of the cranial vault. Hence, gene mutations compromising tissue boundary, mixing cranial neural crest and mesodermal tissues, may result in the premature fusion of cranial sutures [43,44].

The skull (cranium) consists of a complex arrangement of bones (Figure 3), categorically divided during its developmental phase into neurocranium and viscerocranium, originating from mesenchyme, which encircles the developing brain. The viscerocranium is derived from the neural crest, and is composed of 14 bones (the jaws and other pharyngeal arch derivatives): the paired nasal bones, maxillae, palatine bones, lacrimal bones, zygoma and inferior nasal conchae, along with the singular vomer and mandible [34]. In humans, the neurocranium (cranial vault and base) functions as a protective case that surrounds the brain, comprising the frontal, sphenoid, ethmoid, occipital and paired temporal and parietal bones [34]. The superior portion of the neurocranium or cranial vault is constituted by part of the frontal and occipital, as well as the paired parietal bones [6], whereas the lower part of the neurocranium or skull base forms the floor of the cranial vault and separates the brain from other facial
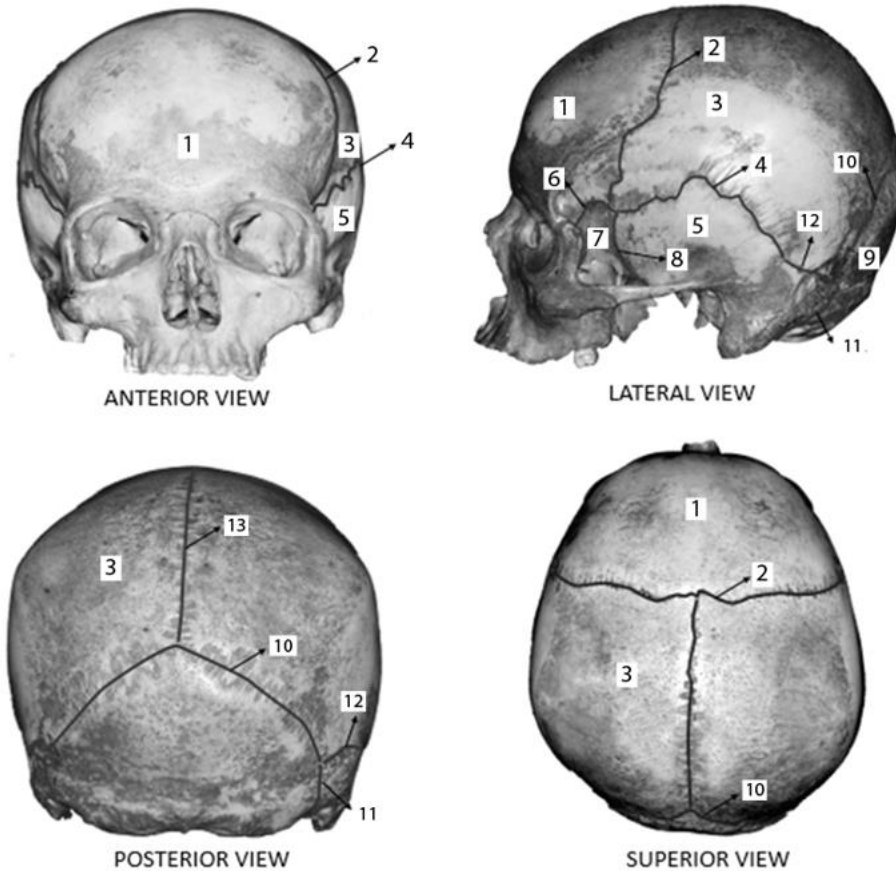
Figure 3. Anterior, posterior, lateral and superior views of an adult skull, showing different cranial vault bones and sutures: 1) frontal bone; 2) coronal suture; 3) parietal bone; 4) squamous suture; 5) temporal bone; 6) frontosphenoid suture; 7) sphenoid bone; 8) sphenosquamous suture; 9) occipital bone; 10) lambdoid suture; 11) occipitomastoid suture; 12) parietomastoid suture; 13) sagital suture.

structures, and is constituted by the ethmoid, sphenoid, paired temporal bones and part of the occipital and frontal bones. Previous work [45-48] led to conflicting results as to the embryological origin of the cranial vault. The use of quail-chick a chimera model allowed Noden (1978) to demonstrate the neural crest origin of the prefrontal, maxilla, premaxilla, nasal bones, and the mixed origin of the frontal, sphenoid and temporal bones [45,46]. Noden (1988) confirmed these previous findings and showed by extrapolation of avian and amphibian models the mesoderm origin of the parietal and occipital bones. These findings agreed with earlier work from Le Lièvre (1978) showing the neural crest origin of the facial part of the skull, the mesoderm and neural crest origin of the cranial vault structures [47]. Couly and coworkers subsequently used the quail-chick chimera model but operated on embryos at an earlier stage and allowed chimeras to develop until skull formation was complete and all bones could be identified. The authors concluded that the cranial vault bones originated exclusively from the neural crest lineage, contradicting the previous concept of the neural crest-mesoderm origin of the cranial vault bones. At the time, the ability to discern the geneses of embryonic cells represented a challenge that was difficult to overcome due to limitations on the available avian model (avian-mammalian cranial vault

dissimilarities, e.g., small avian parietal bone), embryo culture, and cell labeling techniques. This impasse ended in 2002, when Jiang and coworkers demonstrated through a sophisticated transgenic mouse model that the frontal bone originates from neural crest lineage and parietal bones are mesodermally derived [49]. The neural crest also gives rise to the anterior cranial base [50], as well as to the dura mater that lie beneath the frontal and parietal bones [49,51]. The occipital bone has a dual origin (endochondral and intramembranous) and consists anatomically of 4 parts surrounding the foramen magnum—the basilar, squamous (supraoccipital), and 2 condylar (exoccipital) parts; its development precedes that of most skull bones and originates from six ossification centers [52]. The supra and exoccipital parts of the occipital bone originate from the occipital somites, which consist on cellular masses derived from paraxial mesoderm [34].

The calvaria and cranial base bones differ not only in embryonic origin; their dissimilarity extends to the type of ossification that prevails within these bones. The calvaria is intramembranous in origin, while endochondral ossification predominates in the cranial base bones. The body and ramus of the mandible are formed by intramembranous ossification [53]. During intramembranous ossification, bone formation occurs via direct differentiation of the undifferentiated mesenchymal cells into osteoblasts, bypassing the development of a cartilaginous prototype, whereas endochondral ossification requires the differentiation of neural crest and mesodermal cells into chondroblasts, with subsequent syntheses of a cartilaginous template [18]. The cranial vault is a dynamic structure formed from multiple membranous flat bones that are sheathed by periosteum and endosteum or dura mater, contain limited bone marrow space, and are connected at the edges by fibrous sutures. This complex structure has the dual function of protecting the encased central nervous system, simultaneously enabling brain growth and expansion throughout its developmental stage, made possible by a coordinated integration of bony and connective tissue structures [18].

6. Understanding sutures

Craniofacial bones are united at junctions called sutures, composed of two osteogenic fronts interposed by suture mesenchyme [54]. Cranial sutures are highly specialized tissues, essential for skull growth, surpassing the concept of these tissues being mere articulation of two bones [55,56]. Cartilaginous tissue has been previously observed within human metopic suture, in addition to a mix of chondroid tissue and bone matrix, bridging the gap between the two frontal bones [57] and suggesting that suture fusion is a product of chondroid or endochondral ossification, in spite of the intramembranous origin of the cranial vault bones [53]. These findings have been confirmed in a mouse model based on expression of Sox9 in the suture [58]. However, sutures are considered to be "intramembranous growth sites" [59]. Without cranial sutures, craniofacial bones may increase in thickness, but not in length or width. A more in-depth definition establishes that sutures are sites that contain osteogenic progenitors confined between flat calvarial bones that developed from embryonic mesenchyme, which may proliferate or differentiate in response to signaling pathways [21]. According to current research, dynamic exchanges within the triad of dura mater, brain and skull tightly regulate growth through an intricate maze of mechanical interactions and inter-tissue crosstalk [60,61]. This highly regulated mechanism may fail, compromising the preservation of unmineralized suture mesenchyme during the developmental period, essential for the continuous growth of the adjacent bones [62]. In humans, growth and development of the bones that compose the cranial vault initiate at approximately 23–26

days of gestation, and cranial sutures maintain growing capacity until the third decade of life [6,63], when fusion of these bones mark the completion of cranial vault growth. However, since brain growth is the main driving force for skull expansion, by 30 months of life, when approximately 80–85% of brain volume growth is complete, skull enlargement is almost finalized [5]. Thus, suture patency is maintained for the duration of brain growth, after which suture fusion occurs [55,56].

Genesis of the cranial suture system occurs subsequent to condensation, and as a continuum of the ossification of the cranial facial bones, becoming at this instance key growth sites in neonatal and postnatal cranial expansion. Growth occurs at the expense of osteogenic fronts present at the sutural edges of the opposed bones [59,64]. In addition to mesenchyme, unmineralized type I collagen can be found within this area [65,66], signifying that suture mesenchyme could be a product of fibrogenic and mesenchymal cells, with the ability to differentiate into different cell lineages, such as fibroblasts, chondroblasts, and osteoblasts [62,65]. A balanced interrelation between fibroblastic and osteoblastic activity is essential for suture patency to be maintained. It has been previously demonstrated that osteoprogenitor cells of frontal (neural crest derived) and parietal (mesoderm derived) bones express distinct embryonic and postnatal bone forming and regenerative capabilities. Neural crest derived frontal bone holds superior osteogenic potential [67]. Embryonic origin of tissues renders variations in the expression pattern of different signaling pathways [68].

Sutures have several functions, including the abilities to: (a) enable the tight passage of the newborn through birth canal by a combination of suture overlap and parietal bone deformation, (b) serve as shock absorbers, (c) allow for the occurrence of brain growth, and (d) prevent the separation of bones [6,69]. Developmentally, facial and cranial sutures express important differences [70]. Facial bones are surrounded by fibrous periosteal capsules that are fully established by the seventeenth week of gestation, whereas cranial bones develop in a preformed continuous fibrous membrane and only develop fibrous capsules after birth [71]. Unlike facial sutures, which close around the 7th–8th decades, cranial sutures close earlier in life [72]. Hence, for the purpose of this review we will focus exclusively on sutures of the cranial vault.

In humans, the cranial vault area of the neurocranium contains 5 major sutures [6], namely the paired coronal sutures found bilaterally at the junction of the parietal and frontal bones; paired lambdoid, bilaterally at the junction of the parietal and supraoccipital bones; paired squamosal, bilaterally running across the junctions of the temporal, and parietal bones; the singular sagittal, which separates the paired parietal bones; and a singular metopic (interfrontal), which separates the paired frontal bones. Other minor cranial sutures include: the sphenofrontal, sphenoparietal, sphenotemporal and masto-occipital sutures [6]. The coronal and sagittal sutures provide the greatest contributions to skull growth [73]. Any defect in the ossification process of the occipital bone will give rise to a mendosal suture (accessory suture), and conditions like posterior plagiocephaly [74]. Direction of suture closure normally goes from posterior to anterior and lateral to mid suture areas [75]. However, the metopic suture normally closes in an opposite direction, and fuses first, as early as 3 months, with complete fusion occurring by 9 months of age [76] (Table 2). This is followed by the sagittal, coronal, lambdoid and squamosal sutures, which may initiate fusion at approximately 22, 24, 26 and 35 years-age respectively [69]. Timing of suture closure may vary with gender [77]. The mesoderm-neural crest boundary initiates formation of all calvarial sutures, with the exception of the metopic suture, which is entirely neural crest derived [50,73].

The fibroblast growth factors/fibroblast growth factor receptors (FGF/FGFR), bone morphogenetic proteins (BMP), transforming growth factors (TGF), runt-related transcription factor 2 (RUNX2),

transcription factor twist (TWIST1), msh homeobox 2 (MSX2) and neural EGFL like 1 (NELL-1) constitute important regulatory genes and participating pathways involved in suture growth [36]. FGFR mutations leading to craniosynostosis appear to alter cell differentiation within sutures sites rather than their proliferative capabilities [59], but evidence has shown a connection between suture synostosis and higher proliferative ability of cellular elements within these areas [78]. These findings do not outcast the knowledge that craniosynostosis can be the outcome of either a numerical increment in cell division or an increased ability to acquire specialized bone forming features. Currently, what we do know about the mechanisms and mechanics underlying craniosynostosis malformations is that in most cases they appear to connect either upstream or downstream to the FGF/FGFR signaling pathways, which are liable to exert dichotomous effects over osteoblast differentiation, at times stimulating but occasionally suppressing their actions to the point of leading to cell death [9]. Balance between these functions is essential to maintain suture patency. In vitro addition of FGF to osteoblasts downregulates several Wnt target genes suppressing osteoblast differentiation, and concomitantly upregulates the transcription factor SOX2 which carries the inherent ability to suppress Wnt signaling pathways [79]. The osteoblast inhibiting properties of Wnt signals may occur independently, or alongside with bone morphogenetic proteins (BMPs) [80]. Interestingly, exposure of osteogenic fronts and mid-sutural mesenchyme to BMP4 did not generate synostosis, but did render increased tissue volume within both areas. Hence, presence of BMP4 triggered the expression of both Msx1 and Msx2 genes in sutural tissue [79]. Downstream from the FGF/FGFR signaling system other important genes and pathways arise that may potentially interfere in early suture closure. Noggin, a gene that encodes a BMP-antagonist, is expressed in the node, notochord, and dorsal somite, and though not essential, it is required for normal growth and patterning of the neural tube [81]. BMPs are considered to carry bone-inducing properties, but have been shown to affect morphogenesis and function of many organ systems [82], and are expressed in the mesenchyme of patent sutures. Previous research has shown suppression of Noggin by FGFR2 as well as by mutated FGFR signaling, potentially hindering BMP antagonism, and allowing bone formation to be kept intact with resultant synostosis [83].

7. Understanding mechanisms of suture growth

As previously stated, the presence of unmineralized suture mesenchyme is a *sine qua non* for continuous growth of the calvarial bones. Consecutive and frequently overlapping cellular events render recruitment of mesenchymal cells, followed by their proliferation and differentiation into osteogenic cell lineage, peripherally and closest to the bony plates of the respective suture bones. These advancing osteogenic fronts produce and gradually deposit collagen fibers and minerals (bone matrix) to bony plates, generating growth at the so-called osteogenic fronts. At this instance, preservation of suture growth relies on a tightly controlled mechanism to avoid perpetuation of osteogenesis, allowing preservation of sutural existence. Programed cell death (apoptosis) may be critical in this self-preservation process [84,85]. In addition, suture preservation depends on a balanced association between fibroblasts and osteoblasts [62].

Previous studies demonstrated the secretory influence of this tissue by carrying out *in vivo* [86] and *in vitro* [87] experiments in the presence and absence of dura mater from the coronal sutures of rats. These studies showed that: (1) the presence of dura mater conferred resistance to ossification, (2) soluble factors play an important role in suture ossification, (3) biochemical interactions between the dura mater

and the calvaria seem to outweigh the importance of mechano-sensory stimulation generated by the cranial base. However, the dura mater has been demonstrated to serve a dual role in the process of suture growth, intermediating biomechanical stimulation and secretory functions. Furthermore, these roles appear to interact [88] allowing the dura mater to regulate bone growth, contributing to suture patency, as well as signaling, differentiation and proliferation of suture cells [60]. Osteogenic growth factors, cytokines, and shed growth factor receptors are largely secreted by the dura, triggering changes to bone structure and to the underlying nervous tissues [55]. Thus, mechanical stimuli upregulate genes and transcription factors, subsequently increasing protein synthesis, sutural cell proliferation, ultimately generating skull growth.

In brief, activation of sutural cells is probably a result of strain originating from bone or from bone mechanical derivatives, such as fluid flow through an extracellular matrix of tissues [89]. Cells have an inherent capacity to communicate and respond to different signaling mechanisms, but they do not have a previous knowledge of need for differentiation. This understanding comes from the acknowledgement and response to molecular cues conveyed via genes that communicate specific coordinates and needs for phenotype change or relocation. Endogenous (rather than exogenous) forces great enough to deform cell membranes and cytoskeleton (strain) can trigger cell communication and sutural growth [90,91]. The cytoskeleton consists of an integrated system of tension and compression-resistant forces properly elicited by a complex and sophisticated structure of microtubules and microfilaments that maintains cell shape [92]. Disruption of this equilibrium through the exertion of quasi-static tensile forces generated by a growing brain will establish the need for re-accommodation of the cranial bones through growth, in order to reduce strain and re-establish balance. Hence, skull shape is "forced" to mimic brain growth [61]. This network is translated into biochemical signaling; hence, cross-talk between brain, dura mater and calvarial bones.

8. Extracellular matrix and craniosynostosis mechanisms

Another important piece of this puzzle rests on the interaction between suture cells with their non-cellular environment, or extra cellular matrix (ECM). Proteoglycans, glycosaminoglycans, hyaluronic acid, fibronectin, collagens, and other glicoproteins compose the ECM complex. These molecules are unevenly distributed within tissues, as well as during different developmental stages, providing adhesion, cell-to-cell communication and cellular differentiation, playing an essential role during craniofacial development [93] A better understanding of the role of cell-ECM interactions on suture growth are made possible by understanding receptors and their role in craniofacial development, because intracellular signaling mechanisms frequently initiate at cell surface and are a result of protein-receptor interactions. Whether cells are interacting with each other or with their surrounding ECM, it must be established that different families of receptors carry the ability to trigger different responses, generating signals for specific cellular activities. Studies have demonstrated a possible interaction between ECM molecules and the cytoskeleton [61,92]. Cells also respond to their ECM surroundings through integrin receptors or proteoglycan receptors such as the syndecan family, known to function as heparin sulphate co-receptors in fibroblast growth factor signaling, linking cells directly to the extracellular matrix [36,94]. Integrin receptors, growth factor receptors and cytoskeleton-coupling proteins are a part of protein complexes called focal adhesion. Growth factor receptors express intrinsic kinase activity. Though integrins are devoid of kinase activity, upon binding to ECM molecules integrins trigger

autophosphorylation of other proteins (focal adhesion kinase, paxillin, Src and ILK). These proteins will cluster creating focal adhesions [36]. Under stress, the ECM can be affected by deformation. These zones of cell-matrix adhesion will convey stress into the cytoskeleton, generating cellular deformation. Hence, ECM-cell receptor connections transfer signals across the cell membrane into the cytoplasm and ultimately the nucleus, influencing expression of target genes [95].

Previous *in vitro* studies in fibroblast and osteoblast cultures obtained from patients diagnosed with Apert's and Crouzon syndrome has helped elucidate some of these interactions. Findings from these studies suggest that changes in ECM composition and distribution could explain the alterations in osteogenic processes, and account for pathological variations in cranial development, such as craniosynostosis, since the balanced interaction among ECM, cytokines (e.g., interleukins) and growth factors (e.g., TGF-β and FGF2) modulate cellular response and osteogenic events [96] Experimental evidence shows that fibroblasts from Apert patients synthesize and secrete greater amounts of glucosaminoglycans than normal cells [97], and an imbalanced production of cytokines (IL-1 and IL-6) is observed in these cells, and thought to be of pathogenic significance [98]. The authors believe these cytokines may act as autocrine regulators of ECM production in Apert fibroblasts. In addition, osteoblasts from Apert patients express and secrete more TGF-β1 than normal osteoblasts *in vitro*, and addition of FGF2 to Apert osteoblasts lead to a decline in the levels of TGF-β1 [99], suggesting the existence of different patterns in the TGF-β1 cascade secondary to changes in the balance between TGF-β1 and FGF2 associated with these cells [96]. Osteoblasts from Crouzon patients have shown altered ECM production and changes in the gene expression of specific ECM proteins triggered by FGF2, demonstrating for the first time the ability of Crouzon osteoblasts to still respond to exogenous FGF2, in spite of the presence of mutations affecting FGF2 receptors [100].

9. What we know about genes frequently associated with craniosynostosis

Intricate interactions of signaling pathways regulate skull growth. This network includes, but is not restricted to, the FGF-FGFR system, MSX1–2, SHH, BMPs, and TGF β1–3 [79,101-103]. Frequent similarities in the signaling expression within calvarial sutures seem to occur; however, unique expression of specific mechanisms may prevail within specific sutures, which is confirmed by the fact that different mutations are linked to the expression of distinct craniosynostosis phenotypes [2], and differences in the incidence of craniosynostosis subtypes can also be observed [8]. In addition, previous work has demonstrated that the coronal suture is the one most likely to demonstrate premature fusion in genetic forms of craniosynostosis, and 21% of all craniosynostosis cases are of genetic origin [28]. Recently, Twigg and Wilkie [2] published an updated list of various different craniosynostosis mutations expressed among 57 different genes that have been recurrently associated with premature suture fusion, although the frequency of the association of these mutations with craniosynostosis may vary. Inspection of these human mutations allows us to draw a few important conclusions: (1) most of the identified mutations lead to different syndromic types of craniosynostosis; (2) at present, FGFRs 2–3 are the genes most frequently linked to early suture fusion; (3) the most common craniosynostosis phenotype generated by these mutations is multi-suture synostosis, followed by coronal, sagittal, metopic and lambdoid craniosynostosis; (4) association of these mutations with craniosynostosis can vary in frequency; (5) finally, genes that cause craniosynostosis tend to express constitutive activation mutations.

The unraveling of craniosynostosis genetics started in 1993 when Jabs and colleagues [104] demonstrated the existence of a heterozygous point mutation encoding a specific missense amino acid substitution among all affected members of a family expressing the Boston type craniosynostosis, a previously identified autosomal dominant condition characterized by high penetrance, variable craniosynostosis and skull morphology phenotypes [105]. The gene in question was Msx2, which is part of the Msx homeobox gene family. Almost contemporaneously, it was noted that Msx2 expression occurred within the calvarial sutures of mice, demonstrating a role of this homeobox gene in skull morphogenesis, and providing twofold evidence linking Msx2 to this craniosynostotic syndrome [28]. Subsequently, Ma et al. [106] demonstrated that the mutated (P148H) form of Msx2 expressed enhanced DNA binding affinity of the Msx2 protein, due to a lower dissociation rate of the Msx2–DNA complex. Wilkie et al. [107] showed the importance of Msx2 levels for proper human skull development by revealing the presence of heterozygous loss-of-function mutations (deletion of the entire gene and intragenic mutations, e.g. RK159-160del and R172H) of the homeobox gene Msx2 in three unrelated families, contrasting with the previously identified Msx2 homeodomain mutation (P148H), and resulting in delayed suture formation and enlarged parietal foramina. Hence, gain or loss of function in an Msx2-mediated pathway of calvarial osteogenic differentiation may either render premature closure or wide-open suture phenotypes. In addition to Msx2 in mammals, this family of genes incorporates two other physically unlinked members, namely Msx1 and Msx3 [108]. In addition, Msx1 and Msx2 genes are targets of the BMP and FGF signaling systems [79].

Msx1 and Msx2 are transcription factors typically expressed in overlapping patterns at multiple sites of tissue-tissue interaction, especially epithelial-mesenchymal interactions during organogenesis [109-111]. Various mechanisms control Msx gene expression, including retinoids, antisense "quenching", growth factor regulation, as well as regulatory interactions with other transcription factors [108]. As regulatory proteins, Msx modulate craniofacial, limb, and nervous system development by functioning as transcriptional repressors [112,113]. Despite similarities on preference of DNA-binding sites and transcription repressor capabilities, Msx1 and Msx2 display important molecular distinctions [111] that allow for specific unique properties, with Msx2 expressing a greater DNA binding-affinity, while Msx1 acts as a more potent repressor [108,114]. While Msx3 expression appears to be limited to the dorsal neural tube [115], Msx1 and Msx2 are observed in multiple organs, but are strongly expressed in developing craniofacial sites [116]. Msx genes play an important role in the transduction of growth factor signals responsible for cranial suture development. Studies of murine craniofacial development showed (1) presence of Msx1 and Msx2 in the suture mesenchyme and dura mater, and (2) important dissimilarities between Msx1 and Msx2 with overlapping but non-identical expression patterns [112]. Hence, the expression of Msx1 enters the postnatal stages of cranial development, whereas Msx2 shows significant postnatal decline [79]. Evidence suggests that while dura mater signaling regulates suture patency during the prenatal period, the osteogenic fronts are the main signaling source, postnatally [79]. There is an apparent correlation between spatial and temporal expression of Msx1 and Msx2 genes and stages of craniofacial morphogenesis. In addition, mutations in Msx1 have been demonstrated to cause dental developmental anomalies and orofacial clefting [117], but thus far have not been associated with craniosynostosis.

The mammalian fibroblast growth factor receptors (FGFRs) comprise a group of 4 distinct but related proteins (FGFR1, FGFR2, FGFR3, FGFR4) that integrate a family of transmembrane single-pass tyrosine kinase receptors [9]. Based on phylogeny, FGFRs relate closely to the vascular endothelial

growth factor receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFRs) [118]. Four FGFR genes have been identified in humans [119-122]. Recently, a fifth FGFR subtype was identified in human fetal cartilaginous tissue [123], also known as FGFR5 [124], but currently designated as FGFRL1 (fibroblast growth factor receptor like 1) [125]. This novel receptor is encoded by a gene (FGFRL1) that was mapped to chromosomal band 4p16, in close proximity to the FGFR3 gene [123]. FGFRs 1–4 interact at various levels to at least 18 different FGF ligands (FGF1-FGF10 and FGF16-FGF23) [126]. These ligand-receptor connections usually occur either at epithelial or mesenchymal tissues. However, FGF1 activates both splice isoforms [127]. In addition to bonding FGFRs, FGFs express a high binding affinity to heparin. Nevertheless, FGFRs are not activated by FGF11–14. These molecules are known as fibroblast homologous factors, being highly homologous in sequence and structure to FGFs, in addition to expressing high heparin-binding affinity. Interestingly, splicing can generate various isoforms of these 4 FGFR genes, and although their primary role centers on signal transduction secondary to binding of the different FGF molecules, these receptors may also trigger biological responses by interacting with other classes of ligands [128].

During development, receptor function appears to be dictated by ligand expression. It has been previously demonstrated that addition of FGF2 to fetal coronal sutures generated intrasutural expression of osteopontin, with FGFR2 expression in the surrounding tissue; hence, favoring osteogenic differentiation [129]. Although FGF2 is the most abundant factor in the cranial vault area [130] and is found in high levels in fusing sutures [131], FGFs seem to act in concert throughout the developmental period and other less abundant FGFs such as FGF8 have demonstrated important ligand-receptor interactions [132]. Additionally, *in vitro* experiments showed a dose-dependent response of mesencephalic neural crest cells to FGF2, suggesting that FGFs may contribute to skeletogenic differentiation of the cranial neural crest [133]. The presence of FGF4 on osteogenic fronts has been shown to stimulate suture synostosis, and induce expression of Msx1 [79]. The FGF family is a structurally related group of polypeptide growth factors, known as mitogenic cytokines and classically considered to be paracrine factors that mediate a broad range of biological activities, including cellular proliferation, migration, and differentiation, mitogenesis, angiogenesis, embryogenesis, and wound healing [126,127]. Binding of FGF to FGFRs 1–4 requires the presence of heparin sulfate proteoglycan as a cofactor; once FGF binds to FGFR, a signaling cascade is initiated, receptor dimerization occurs at the cell surface level by forming a complex which consists of a double set of FGFs, heparin sulfate chains and FGFRs. Each FGF bind to the two FGFRs, and in sequence, inter-receptor contact occurs, enabling transphosphorylation of specific tyrosine residues in the cytoplasmic domain of the receptors [127]. In spite of its structural similarity to other FGFRs, FGFRL1 characteristically lacks the intracellular tyrosine kinase domain [124], harboring a C-terminal domain unable to signal by transautophosphorylation. This receptor may function as a decoy receptor, since it binds to FGF ligands and heparin with high affinity [125].

The identification of craniosynostosis-associated mutations in FGFR genes [134-137] provided a glimpse into the critical role played by the FGF-FGFR complex in skull bone embryogenesis and ossification. Human embryos show early and intense expression of FGFR1 and FGFR2 in the mesenchyme of the unossified skull vault, whereas low levels of FGFR3 are detected during this stage [130]. Once ossification has been established, FGFRs 1–3 are co-expressed in proliferating pre-osteoblasts and osteoblasts in the cranial vault and facial regions [130]. FGFR2 is solely expressed in proliferating osteogenic stem cells. In addition, FGFR1 and FGFR2 signaling pathways regulate osteogenic function,

with FGFR3 likely playing an auxiliary role in this process [138]. Hence, FGFR2 controls proliferation and is more extensively expressed within mid-suture, whilst FGFR1 regulates differentiation of these cells. Signaling balance between FGFR1 and FGFR2 is of the utmost importance in maintaining suture patency [60]. FGFR3 appears to play a role in tissue interaction, and is expressed in the osteogenic membrane. Evidence suggests that FGFR2 displays a more important role than FGFR3 during embryogenesis [139]. The expression of Fgfr4 is only observed in the cranial musculature [138], and FGFRL1 may impact negatively on cell proliferation, conversely stimulating cell differentiation [125].

Relevant craniosynostosis mutations, predominantly missense, have been identified involving FGFRs1–3 genes. These mutations bestow functional changes on the mutated receptor in a ligand-independent or ligand-dependent manner via different mechanisms that generate distinct, though at times similar, craniosynostosis syndromic phenotypes [2]. As transmembrane highly conserved receptors, the FGFR complex (FGFRs 1–4) hold important structure resemblance consisting of three extracellular immunoglobulin-like domains (Ig), a single transmembrane helix, and a cytoplasmic split tyrosine kinase (TK) domain [118]. The extracellular realm of these receptors include a single extracellular ligand binding domain encompassing three extracellular immunoglobulin-like (Ig) domains (Ig I–III), a continuous stretch of 7–8 acidic amino acids comprising the linker that connects Ig I and Ig II ("acid box"), and a positively charged Ig II region. The Ig domains II and III have specific sites that bind FGFs and heparan sulphate proteoglycans (ligand-binding pocket), whereas alternative splicing of the Ig III domain establishes ligand-binding specificity [118]. Autosomal dominant FGFR mutations found within the extracellular, transmembrane, or tyrosine kinase domains, by enhancement of receptor-ligand binding affinity [140], illegitimate ligand-receptor binding specificity [141], ligand independent dimerization [142,143], as well as ectopic splice form expression [144], have been identified in different syndromes [139].

FGFR2 maps to human chromosome 10q25.3–26. A wide spectrum of FGFR2 mutations has been previously reported, and the IgIIIa/IIIc region was identified as a genuine mutation hotspot [139]. Specific FGFR2 missense substitutions (Ser252Trp and Pro253Arg) in the linker between IgII and IgIII were found to associate with Apert syndrome (MIM 101200) in 40 unrelated cases, allelic with Crouzon syndrome, wherein clinical findings include presence of craniosynostosis, but absence of limb abnormalities [145]. Mutations affecting the transmembrane domain (Ty375Cys) and the linker region interposing IgIII and the transmembrane domains (Ser372Cys) generate Beare-Stevenson cutis gyrata syndrome (MIM 123790), an autosomal dominant condition characterized by cutis gyrata, acanthosis nigricans, craniosynostosis, craniofacial dysmorphism, digital anomalies, umbilical and anogenital abnormalities and early death [146]. Mutations within the FGFR2 B exon were previously associated with Crouzon syndrome (MIM 123500), and sequence variations were identified in 9 out of 20 unrelated Crouzon syndrome patients. Different mutations were identified, among which a cysteine replacement in IgIII, disrupting the structural integrity of the affected Ig domain [136]. Another FGFR2 mutation was also demonstrated in the IgIIIc domain in association with Jackson-Weiss syndrome (MIM 123150), an autosomal dominant condition that expresses craniosynostosis, foot anomalies, and great phenotypic variability, that has been mapped to the same chromosomal region (10q25–q26) as Crouzon syndrome and FGFR2 [147,148]. Thus, scientific evidence demonstrates that FGFR2 mutations may have a wide range of different phenotypic outcomes [134]. In 2005, McGillivray and coworkers [149] described a 3 generation pedigree of a family with 11 members affected by a cranyosinostosis syndrome (MIM 609579) that characterized by scaphocephaly, macrocephaly, hypertelorism, severe maxillary retrusion,

and mild intellectual disability. Absence of shallow orbits and ocular proptosis allowed the authors to exclude the diagnosis of Crouzon syndrome. Molecular analysis revealed a novel FGFR2 mutation which unlike most of the previously characterized mutations was located on exon 14, and consisted of a heterozygous base change c.1576A→G, resulting in a non-conservative amino acid substitution (K526E) within the intracellular TK1 domain. This mutation appears to have a very important role in the function of FGFR tyrosine kinase domain. Structural models of the wild-type (K526) and mutant E526 FGFR2 tyrosine kinase domain were produced to understand the pathogenic significance of this mutation. The mutant model predicted that the E526 mutation renders stabilization of the active conformation of the A loop of the FGFR2 tyrosine kinase domain, which probably occurrs in the absence of activating ligand (e.g. ligand independent activation).

Early evidence showed the presence of a mutation (Pro252Arg) in exon 5 of the FGFR1 gene in seven out of seven affected individuals with Pfeiffer syndrome (MIM 101600) originating from unrelated families [135], yet genetic heterogeneity of this syndrome seems to be an important genomic characteristic [150]. Studies in mice carrying a mutation orthologous to the Pfeiffer syndrome mutation in humans provided supporting evidence that the FGF/FGFR1 pathway interacts with the core-binding transcription factor α subunit type 1 (CBFA1), which then regulates alkaline phosphatase, osteocalcin and bone sialoprotein, influencing intramembranous ossification by favoring early suture fusion [151]. Further studies showed different mutations in FGFR2 gene could also cause Pfeiffer syndrome [152,153], while other mutations that were identified on the B exon of FGFR2 were associated with both Jackson-Weiss (MIM 123150) and Pfeiffer syndromes (MIM 101600) [120]. In addition, FGFR2 missense mutations that may result in Pfeiffer syndrome (T1036 to C and G1037 to A) can also cause classical Crouzon syndrome [154]. It is clear that variability exists within the clinical phenotypes of many craniosynostosis syndromes. The gene variants in the FGFR genes are a perfect example of how the genetic background on which the variant operates influences the ultimate phenotype. Most mutations related to Crouzon, Pfeiffer, and Jackson-Weiss syndrome represent missense mutations and are located within the IgIII domain of FGFR2c. A large number of these mutations result in a gain or loss of cysteine residue. From a molecular point of view, the presence of abnormal intermolecular disulfide bonds between unpaired cysteine residues may explain the phenotypic results generated by the FGFR2 mutations that cause these syndromes [155].

Recently, a distinct perinatal lethal skeletal dysplasia (bent bone dysplasia syndrome, MIM 614592) expressing poorly mineralized calvarium, craniosynostosis, dysmorphic facial features, prenatal teeth, hypoplastic pubis and clavicles, osteopenia, and bent long bones was identified and associated with *de novo* missense mutations within the transmembrane domain (p.M391R and p.Y381D) of FGFR2, leading to deficient plasma-membrane targeting (loss of function), but possibly preserving intracellular activity (nuclear gain of function) [156]. A genetic paradigm has been that dwarfing syndromes associate with FGFR3 mutations, while mutations affecting FGFRs 1–2 generate the majority of the craniosynostosis malformations. Nevertheless, FGFR3 mutations have been associated with craniosynostosis syndromes such as Muenke syndrome (P250R mutation) (MIM 602849), thanatophoric dysplasia type II (MIM 187601) and a form of Crouzon syndrome with skin characteristically affected by acanthosis nigricans (MIM 612247). Osteoglophonic dysplasia (MIM 166250), a disorder which combines skeletal and craniosynostosis phenotypes has also been previously associated with FGFR1 mutations (Y372C, mapped to the extracellular juxtamembrane region), and other FGFR1 missense mutations for this syndrome have been described as well [157,158]. Ibrahimi and coworkers [159]

explored the mechanisms by which Pro252Arg and Pro250Arg mutations activate FGFR1c and FGFR3c, respectively, using surface plasmon resonance and X-ray crystallography. The authors measured the binding of Pro252Arg FGFR1c, Pro250Arg FGFR3c and their respective wild-type receptors to FGF1-10, aiming to quantify mutant FGFR–FGF interactions. Both Pro252Arg FGFR1c (type I Pfeiffer syndrome) and Pro250Arg FGFR3c (Muenke syndrome) showed enhanced ligand binding capabilities in comparison with their respective wild-type receptors. The FGFR2c linker domain mutations that were associated with Apert syndrome demonstrated increased binding of the mesodermally expressed FGF7 and FGF10, resulting in autocrine signaling [155], while FGFR1c and FGFR3c mutations enhanced the binding of FGF9, abundant in the calvaria. These findings implicate FGF9 as a potential pathophysiological ligand for mutant FGFRs in mediating craniosynostosis, and explain the more severe limb abnormalities observed in Apert syndrome in comparison with type I Pfeiffer and Muenke phenotypes. The presence of abnormal intermolecular disulfide bonds between unpaired cysteine residues may be the molecular explanation behind FGFR2 mutations that cause Crouzon, Jackson-Weiss and Pfeiffer syndromes [160].

Another important part of this network lies in the transcription factor TWIST1, which has been previously associated with an autosomal dominant condition in humans, known as Saethre-Chotzen syndrome (MIM 101400) [161]. TWIST is found in developing suture mesenchyme, and when upregulated reduces osteoblast differentiation, diminishing chances of craniosynostosis. The mutations affecting TWIST generate haploinsufficiency and result in unilateral and bilateral coronal craniosynostosis. Over 50 mutations affecting the TWIST1 gene were previously identified in patients with Saethre-Chotzen syndrome [162]; however, similar overlapping phenotypes have been found in association with mutations affecting FGFR3 (P250R) and FGFR2 (VV269-270 deletion) emphasizing the genetic heterogeneity of this syndrome. Since findings in the same gene give rise to different conditions, e.g. Crouzon, Pfeiffer, Jackson Weiss, Apert and Saethre-Chotzen syndromes, and mutations affecting different genes might lead to the same clinical appearance, the authors suggest that TWIST and FGFRs may interact within the same molecular pathway, modulating suture development in humans [163]. It appears as if loss-of-function mutations affecting TWIST may increase FGFR expression leading to suture fusion. Conversely, an increase in TWIST expression may translate into a lower FGFR expression, preserving suture patency. The effect of TWIST on osteoblast differentiation has been previously demonstrated in the coronal suture of developing mice, demonstrating its ability to decrease osteoblast differentiation and suppress FGFR2 expression in sutures [164,165]. Although, essentially based on functional differences, recent work has suggested a separation between classic Saethre-Chotzen syndrome and Muenke' s syndrome [166]. In addition to other important contrasting features of these two conditions, Muenke syndrome more often includes developmental delay, whereas Saethre-Chotzen syndrome is at a higher risk of increased intracranial pressure. However, these clinical issues do not negate the otherwise highly similar phenotypic characteristics, and the possibility of interrelated signaling systems, as previously suggested.

In 2013, Sharma et al. [167] reported 38 heterozygous mutations associated with the gene encoding transcription factor 12 (TCF12), a member of the basic helix-loop-helix (bHLH) E-protein family. These mutations imparted loss-of-function, and consisted of 14 nonsense, 15 frameshift, 7 splicing and 2 missense changes, being predominantly expressed in patients with bilateral and unilateral coronal synostosis. Some of these patients presented with Saethre-Chotzen syndrome but did not show TWIST1 mutations, confirming phenotypic variability and overlap of features between TCF12 mutation-

associated syndrome and Saethre-Chotzen syndrome. Incomplete penetrance of the TCF12 mutation-associated syndrome was also suggested [168]. Features of the TCF12 mutation-associated syndrome include isolated coronal synostosis at birth (brachycephaly or plagiocephaly), prominent ear crus, ptosis and brachydactyly. TCF12 appears to act in concert with TWIST1, possibly by heterodimerisation, rendering normal development of the coronal sutures ultimately by regulating preservation of tissue boundary, and/or inhibition of osteogenegic differentiation via actions on RUNX2, BMP or FGFR signaling pathways [167].

10. Conclusions

Cranial bones articulate through areas called sutures. These fibrous joints are constituted by mesenchyme that must remain unmineralized until physiological skull growth is finalized. Sutures present specificities that should be considered, given that time of fusion and incidence of premature fusion differ among them. A tightly regulated mechanism prevents premature and persistent osteogenesis, preserving suture patency. This interplay involves cross-talk between brain, dura mater and calvarial bones. Mechanisms of suture growth must modulate osteogenesis at the right site and timing, until growth is complete. Different genes may influence this process, and the FGFR genes have been associated with the most common craniosynostotic syndromes. Failure or disturbances within this network of signals may arise secondary to different genetic mutations, frequently upstream or downstream of the FGFR signaling pathway. Different and often overlapping phenotypes have been associated with syndromic and non-syndromic craniosynostosis. Further studies are warranted to fully elucidate the underlying intricacies that may lead to craniosynostosis. Most studies within this realm have concentrated on craniosynostosis related syndromes, but limited information is currently available on the genetics and etiologic factors causing isolated craniosynostosis.

Conflict of interest

All authors declare no conflict of interest in this paper.

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