BBA Clinical 6 (2016) 165–176

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: www.elsevier.com/locate/bbaclin

Review article

Signaling mechanisms implicated in cranial sutures pathophysiology: Craniosynostosis

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ARTICLE INFO

Article history:
Received 25 February 2016
Received in revised form 19 April 2016
Accepted 27 April 2016
Available online 29 April 2016

Keywords:
Sutures
Craniosynostosis
Molecular signaling
Polycystins
Mechanical stimuli
Craniosynostosis therapies

ABSTRACT

Normal extension and skull expansion is a synchronized process that prevails along the osteogenic intersections of the cranial sutures. Cranial sutures operate as bone growth sites allowing swift bone generation at the edges of the bone fronts while they remain patent. Premature fusion of one or more cranial sutures can trigger craniosynostosis, a birth defect characterized by dramatic manifestations in appearance and functional impairment. Up until today, surgical correction is the only restorative measure for craniosynostosis associated with considerable mortality. Clinical studies have identified several genes implicated in the pathogenesis of craniosynostosis syndromes with useful insights into the underlying molecular signaling events that determine suture fate. In this review, we exploit the intracellular signal transduction pathways implicated in suture pathobiology, in an attempt to identify key signaling molecules for therapeutic targeting.

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1. Introduction

The human skull is a dynamic structure formed from multiple bones connected at the osteogenic edges by fibrous joints, the cranial sutures.

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http://dx.doi.org/10.1016/j.bbacli.2016.04.006
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Cranial expansion occurs at the sutures. Premature fusion of sutures leads to craniosynostosis, a congenital disorder which causes cranial deformations and potential cognitive impairment. While the full etiology remains clouded in uncertainty, several genetic mutations have been elucidated and implicated in craniosynostosis. Several transgenic models have been used for identifying the signaling mechanisms involved in premature fusion. Recent findings have correlated fibroblast growth (FGF), bone morphogenetic protein (BMP), and wingless-type integration site (Wnt) signaling in craniosynostosis and potential therapeutic strategies have been established. This review largely focuses on these signaling pathways but also includes an extensive analysis of the role of the mechanical stimuli and the mechanosensory proteins Polycystins in suture formation. We hypothesize that Polycystins play a pivotal role in mechanosensation and mechanotransduction and function at an epistatic level along with the aforementioned pathways. Polycystins could be of paramount importance as tools for diagnosis and treatment either at an embryonic or postnatal level.

2. Cranial vault development

2.1. Human skull

The vertebrate skull is an exquisitely complex structure and is formed from two main parts—the neurocranium and the viscerocranium. The neurocranium surrounds and protects the brain and the sensory organs (optic, olfactory, and otic). The viscerocranium includes the bones of the face and the palatal, pharyngeal, temporal, and auditory bones [1]. This review will focus on the neurocranium. The neurocranium is principally formed from five bones, the paired frontal and parietal bones and the unpaired occipital bone [2]. Initially, the development of the skull starts with the superficial migration of mesenchymal stem cell populations (MSCs) from the embryonic epithelium to brain and surface ectoderm destinations. MSCs are pluripotent cells that can differentiate into chondrocytes, osteoblasts, myoblasts, and adipocytes [3].

2.2. Origin of craniofacial mesenchymal tissues

Craniofacial mesenchymal tissues have three origins: neural crest, paraxial mesoderm, and lateral mesoderm [4]. Accumulating evidence indicates that the bones are of mixed embryonic origin, arising from neural or mesoderm crest. Neural crest cells (NCC) are pluripotent cells that migrate from the embryonic epithelium and, once they reach their final destination, condensate into blastemas and differentiate into several cell types thus forming craniofacial structures. Dysregulation of their levels of proliferation or differentiation respectively leads to congenital craniofacial disorders as reviewed in Mishina and Sneider [3].

Initially, the craniofacial neural crest cells (CNCC) contribution was investigated by performing chick–quail transplantation experiments. These studies showed that the anterior calvarial bone is derived from neural-crest whereas the posterior bone from paraxial mesoderm. Further studies in a transgenic mouse that expresses a marker for neural crest cells, using the Cre-lox system, have enabled researchers to label genetically cell populations and trace their origin. More specifically, murine transgenic reporter gene models that labeled cell types with galactosidase under the Wnt1 promoter demonstrated that the skull is formed from mesenchyme of two different origins, the mesoderm and neural crest [5,6]. Therefore, the migration of neural crest cells was further elucidated and demonstrated that parietal bones are of mesodermal origin, whereas the frontal bone is of neural crest in origin [7].

At the time of NCC migration, growth factor signaling via bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and Wnts, as well as other signaling pathways, including Delta/Notch, retinoic acid, Hedgehog (Hh), and their downstream targets, are involved in cell fate determination, growth, differentiation, and survival [1]. In particular, NCCs, which comprise the cranial region, play a distinct role as they differentiate into osteoblasts and chondrocytes in addition to other types of cells, and an imbalance in their portion can lead to severe craniofacial anomalies, an example of such, craniosynostosis, which will be further analyzed below.

2.3. Cranial sutures

Cranial sutures emerge as soon as the developing bones approximate [8]. Skull flexibility is enabled by the presence of cranial sutures, fibrous structures separating the bone plates and human skull, essential for the expansion and subsequent growth of the skull. The skull expansion is perpendicular to the orientation of sutures during expansion. Sutures constitute major bone growth sites and are regarded as complexes, composed of the two osteogenic bone fronts, the mesenchymal/fibrous tissue of the suture, the underlying dura mater, and the overlying pericranium [2].

Four cranial sutures separate the five cranial bones; the sagittal suture exists between the two paired parietal bones, the coronal suture between the frontal and parietal bones, the metopic between the two frontal bones, and the lambdoid between the occipital and parietal bones. This arrangement is similar to other species. With the exception of the posterior frontal suture which closes at humans around 18 months of age, and similarly in mice in the first 45 days of life, all other sutures remain patent and unossified [2].

Two ossification processes take place during cranial development, intramembranous and endochondral ossification. The distinct difference between these two processes is that during endochondral ossification a cartilaginous intermediate arises, whereas in intramembranous ossification, condensed mesenchyme cells directly differentiate into osteoblasts and form bone tissue without any cartilaginous precursor [9]. During intramembranous ossification, osteoblasts secrete an extracellular matrix, consisting of type I and other forms of collagens and proteoglycans. Mineralization proceeds and the flat bones of the skull expand, from the skull base toward the apex of the cranium [2].

Ossification of the skull is mainly via intramembranous ossification and is a procedure orchestrated by the suture mesenchyme and the dura mater, the rigid membrane that adheres to the inner surface of the vault and separates it from the brain. However, although skull bones are ossified through intramembranous ossification, suture fusion can undergo both types of mineralization. The mesenchymal cells of the suture mesenchyme proliferate and differentiate into osteoblasts during cranial expansion that deposit collagen fibers and minerals to the bony osteogenic ends to increase their size. Ossification can also be accomplished under mechanical stimuli, when the cells lying on osteogenic fonts become bone through intramembranous ossification [10] (Fig. 1).

Sutures being obviously less stiff than the bones they join may play mechanical roles. Their fibrous identity plays a pivotal role in various procedures, such as resistance to tensile or compressive forces, during the osteogenic expansion of the skull. In the skull, sutures are subjected to three different types of strain. The strain is sensed by cells and in comparison to stress it can be measured [8]. Thus, sutures are bone growth sites which respond to biomechanical signals which are induced by the underlying brain. These signals are translated into cell signaling at sutures, resulting in the transcription of osteoblast differentiation, thus bone ossification [11].

Forces that are sensed and absorbed by sutures include sudden forces such as sudden impacts, cyclic loading which involves blood vessels pulsation [12], feeding, locomotion and quasi-static loading due to tensile strains caused by internal organ pressure, intracranial pressure or forces implemented by the dura mater [8]. Dura mater has been found to play a vital role in suture patency and fusion. Studies have found that sutures devoid of dura mater, respond with fusion [13]. Thus, dura mater and its underlying growth and paracrine factors may regulate suture patency and fusion.

Several studies have imposed extrinsic forces such as an orthodontic spring on sutures of the palate. Tensile loads were mainly used, owing to

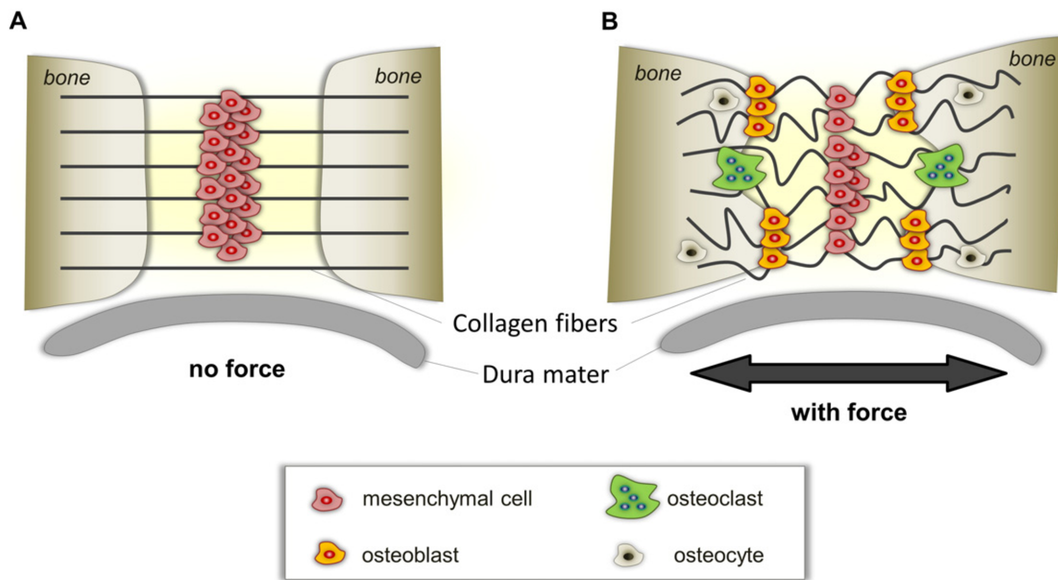

Fig. 1. *The cranial suture complex*. The cranial suture complex is composed of the two osteogenic bone edges, the suture mesenchymal tissue and the underlying dura mater. *A*: The mesenchyme at rest consists of undifferentiated cells and collagen fibers which are at a parallel state. *B*: When force is implemented, the collagen fibers adopt different orientations and the mesenchymal cells differentiate into various cell types (osteoblasts, osteoclasts, osteocytes); bone homeostasis is regulated by both osteoblasts and osteoclasts. Osteoblasts through intramembranous or endochondral ossification form bone at the suture osteogenic fonts and the human vault expands; osteoclasts mediate bone resorption; osteocytes remain buried in the bone matrix of the cranial vault.

their therapeutic potential of bone growth, but compression forces were also applied. Because of force imposition, cell and molecular reactions were observed. In rats and mice, static tensile loads have been applied in vivo to the sagittal suture resulting in differential growth during fetal and infant life. An optimal energy absorption and stress transfer are correlated to well-oriented collagen fiber sutures and a higher hierarchical suture cell morphology [14].

In terms of morphology, this strain causes an elevation in suture width and cell changes such as changes in size, number, and vascularity. In addition, an increased osteoid production leads to mineralization at the osteoid fronts and an increased matrix production suggesting that 2 weeks stretching of mouse sagittal sutures causes up-regulation of the osteogenesis factors alkaline phosphatase and osteopontin [15]. In addition, another in vitro study detected that force led to the premature fusion of sagittal suture, further connecting craniosynostosis to epigenetic factors [16].

Sutures also maturate and form fibered structures that resist static tensile strains due to intracranial pressure, absorb cyclic loading during mastication and locomotion, and act as shock absorbers during external effects such as brain traumas. The study of their complex nature, formed from a range of extracellular matrix zones, different cell populations, and a distinct vasculature, is complicated, as all these parts respond differently to mechanical stimuli. The same applies for the periodontal ligament (hPDL), the connective tissue between the tooth and alveolar bone, which is, in fact, a suture.

Due to expansion, a decrease in suture stiffness has been observed further highlighting the interaction between increased loading and osteogenic response. Taking the aforementioned data together, it is suggested that cyclic forces, either in tension or compression, induce sutural changes and skull modeling [17].

Although cranial sutures initially remain thin and straight between bones, they become interdigitated with age. As a result of the interdigitation of sutures, the application of force to one area of a suture can cause stresses of different origin and at various areas of the same suture. This complex and different forces can lead to different movements in the sutures that can result in a complete displacement of the suture [18]. Mathematical models have demonstrated this behavior by employing different models of mechanical stimulus and time-dependent processes. Skull expansion and suture growth involve cell signaling pathways, which are likely controlled by secreted paracrine factors and responsive to differently implemented mechanical stimuli [19].

2.4. Craniosynostosis

During development, cranial sutures need to remain patent, while allowing rapid bone formation at the edges of the bone fronts [20]. If ossification is not synchronized, and there is failure of the signaling pathways to maintain suture patency, then functional impairment can be caused, a prime example of which is craniosynostosis.

Craniosynostosis, with a birth prevalence of 1:2000–2.500 live births, is heterogeneous in its presentation and can be classified into two categories, syndromic and non-syndromic [1]. The premature closure of sutures is associated with a distorted skull shape, with elevated intracranial pressure, visual and respiratory impairment, and neurological dysfunctions such as inhibited intellectual ability. Epidemiological data have correlated craniosynostosis with multiple pregnancies, prematurity, high birth weight, and elevated paternal and maternal age [21].

Non-syndromic craniosynostosis accounts for 70% of the cases and occurs when the main defect in the individual is fused sutures. On the contrary, syndromic craniosynostosis is associated with further morphological manifestations. Frequencies of different types of craniosynostosis vary, but in general sagittal synostosis is the most common (40–55%), coronal (20–25%), metopic (5–15%), multiple suture synostosis (5–15%), and lambdoid (0–5%) [22].

Despite the genetic etiology of several forms of craniosynostosis, there are several epidemiological factors, either extrinsic or intrinsic, that lead to the premature fusion of the suture. The effect of the mechanical strain transmitted by the growing brain to maintain suture patency, chromosomal abnormalities of genes related to osteogenesis, as well as extrinsic forces applied to the skull, may contribute to the manifestation of craniosynostosis. Craniosynostosis has been reported as a clinical feature in many syndromes, the most common of which include Apert, Saethre–Chotzen (SC), Muenke, Crouzon syndromes [1].

At a genetic basis, craniosynostosis has been linked to mutations of several osteoblastogenic molecules including the fibroblast growth factor receptors (FGFRs), homeobox protein MSX-2 (MSX2), Ephrin-B

(EFNB), Twist-related protein 1 (TWIST), and Runt-related transcription factor 2 (RUNX2). Some of these genes have been elucidated from rare craniosynostosis syndromes such as Boston-type craniosynostosis (MSX2), Saethre–Chotzen syndrome (haploinsufficiency of the Twist nuclear transcription factor gene) as reviewed in Twigg and Wilkie [1].

Currently, craniosynostosis can be treated surgically, an intervention that remodels the skull and creates extra space for the brain to develop. As a number of signaling pathways, cytokines and growth factors have been correlated to suture patency, a further understanding of their function could lead to the development of innovative translational treatment methods [23].

2.5. Mechanical forces and skull expansion

Mechanical forces can be from an external (stretch, sound, breathing, mastication, swallowing) or an internal source (fluid flow, blood pressure, osmotic pressure, heart pulsation) and according to the cell type that are applied may vary in intensity [24]. The fact that the mechanical stress is translated into a biochemical response by the cell implies that there is an interaction between the extra cellular matrix (ECM) and the internal cytoskeleton. The cytoskeleton is resistant to tensile and compressive forces due to the existence of microtubules and microfilaments. Stress-based deformation of the ECM alters the cytoskeleton and deforms the cell through stress applied at focal adhesion sites [25].

Growth and development are the net result of environmental adjustment of genetic inheritance. Sutures are composed of mesenchymal cells which ultimately differentiate into chondrogenic, osteogenic, and fibrogenic cells. The fate of these cells is influenced by genes and environmental factors, including mechanical forces. Mechanical forces form bone and regulate cartilage formation by regulating gene expression, differentiation, maturation, and other signaling pathways [26].

Mechanical forces can cause deformation of the cell membrane or the cytoskeleton by evoking changes in cell signaling pathways implicated in cell differentiation, proliferation, and the production of extracellular matrix molecules. Notably, an ideal mechanical force is considered to be the minimum force that causes the maximum mineralization in the shortest time period [27]. In addition, according to Wolff's' law, bone remodeling is accomplished because of the mechanical demands it has to withstand [18].

During skull expansion, cranial sutures play a fundamental role in its proper expansion and development, as its cells of mesenchyme origin sense forces, and according to its tension, induce mineralization of the cranial bones. Mechanosensing involves interaction of various protein compounds including integrins, actin microtubules, and adherent junctions. Many of these proteins are connected to signaling pathways, which induce calcium signaling or other pathways [28]. Interestingly, the term "Suture mechanobiology" is used to support the importance of the mechanical stimuli that are capable of regulating sutural growth and their translation into signals which contribute to biological growth [29].

2.6. Polycystins and mechanical stimuli

The protein family of Polycystins (PCs) is implicated in mechanosensing, as it has been shown that polycystins interact with many mechanosensing compounds. Polycystins 1 and 2 (PC1, PC2) have been reported as key mechanosensor molecules implicated in severe structural abnormalities (cysts formation, aneurysms) as well as colorectal cancer and atherosclerosis [30–32]. In addition, they have been implicated in various processes, including renal flow sensing, vascular pressure and flow mechanosensation, brain injury, skeletal development, and osteoblast differentiation. PCs are integral proteins expressed in human tissues, including kidneys, blood vessels, pancreas, liver, and the skull. They are localized in the primary cilium, at the plasma membrane, and at the endoplasmic reticulum (ER), where they interact with various partners and trigger signaling transduction [30].

PC1 was first identified by positional cloning as a gene mutated in 85% of patients with autosomal dominant polycystic kidney disease (ADPKD) and its role in renal pathology and kidney development has been intensively studied. PC1 (460 kDa) is an 11-segment integral membrane protein, which constitutes a long N-terminal extracellular region and a short intracellular C-terminal region of 200 amino acids. PC1 interacts with its partners through protein motifs localized on its N-terminus, including flanked leucine-rich repeats (LRRs), Ig-like domains (ILDs or PKD repeats), a G-protein-coupled receptor proteolytic site (GPS), and a C-lectin-like domain (CLD). Interestingly, a study which analyzed the mechanical identity of the PC1 N-terminus, using single molecule force spectroscopy, showed that PC1 is characterized by extensibility due to the unfolding/refolding of its Ig domains. Thus, this trait may be essential for cell elasticity and cell signaling [33,34].

In addition, such binding sites, involve PC1 in protein–protein interactions, including ligand-binding sites, implicating that it participates in interactions that take place in the extracellular environment. PC1 is a promising candidate in mechanotransduction as it spans the cellular membrane and connects the extracellular matrix with the cytoskeleton and intracellular signaling pathways [30].

More interestingly, PC1 is found localized at the primary cilium and at the plasma membrane being involved in interactions between proteins and between protein-carbohydrates. PC1 has been reported to interact with proteins localized at adhesion points, adherent junctions, and desmosomes. PC1 mediates cell-to-cell adhesion and intercellular interactions and a small intercellular domain which is implicated in signal transduction by activating cytoplasmic effectors. The intracellular C-terminal has been reported to be implicated in signal transduction in several signaling pathways including JAK–STAT, the mammalian target of rapamycin (mToR), the Wnt, the activator protein 1 (AP-1), and the calcineurin–Nuclear factor of activated T-cells (NFAT) pathway [30,35,36].

Polycystin 2 (PC2, TRPP2, 110 kDa) is also a six-segment transmembrane spanning integral membrane. Both the N- and C-termini are intracellular. The C-terminus contains a calcium-binding EF-hand domain (EF), an endoplasmic reticulum (ER) retention domain, and a coiled-coil domain (CC) and the N-terminus contains a ciliary sorting motif (CSM). PC2 is located in the ER [37]. PC1 is required for the translocation of PC2 from the ER to the nucleus [38]. PC2 is a member of transient receptor (TRP) channel family proteins. It has been shown that PC2 interacts with cytoskeletal proteins as well as other mechanosensitive ion channels in different cells, including potassium-selective stretch-activated potassium channels and non-selective cationic SAC channels. In a model where PC2 was knocked down with the use of siRNAs, it was reported that PC2 mediates cell-to-cell adhesions partially through E-cadherin [38].

Recent studies have correlated mechanical forces to biological responses. In a recent study, using an optimal tissue culture system for applying short-term mechanical stretching on pre-osteoblastic cells derived from human periodontal ligament tissue (hPDL), was demonstrated that PC1 modulates osteoblastic gene transcription and bone cell differentiation through the calcineurin/NFAT signaling pathway [35]. Another study has shown that PC1/PC2 is a flow-tensing protein complex in endothelial cells, responds to shear stress, and induces cell proliferation changes leading to atherosclerosis [32].

2.7. Molecular mechanisms and craniosynostosis

2.7.1. FGF signaling

A signal transduction cascade is based on a ligand-dependent dimerization of growth factor receptors. Dimerization brings the intracellular tyrosine kinase domains into proximity, leading to autophosphorylation on tyrosine residues, initiating phosphorylation events. The subsequent activation of downstream targets results in the transmission of signals

to the nucleus which may lead to cell differentiation or proliferation. FGFRs are tyrosine kinase receptors that contain three extracellular immunoglobin-like domains (D1–D3), one hydrophobic transmembrane domain and one cytoplasmic tyrosine kinase domain. In general, mammals comprise 18 FGF types, which have been grouped into six distinct subfamilies, based on sequence homology and phylogenetic analysis reviewed in [39].

FGFs are growth factor receptors that, once activated, mediate various signal pathways implicated in multiple developmental processes. FGFs play a fundamental role in cellular proliferation, migration and differentiation, mitogenesis, angiogenesis, embryogenesis, and wound healing. FGFs signal through cell surface FGF receptor (FGFR) Tyr kinases, encoded by four distinct genes in mammals (FGFR 1–4). FGFs bind to FGFRs, which brings the intracellular receptor kinase domains into close proximity, such that phosphorylation, and hence activation of the kinases can occur. The activated receptor kinases, thereafter, phosphorylate and activate intracellular substrates, such as FGFR substrate 2a (FRS2a) and phospholipase Cγ1 (PLCγ1). The activated FRS2a substrate initiates the downstream signaling pathways, the RAS–MAPK pathway or the PI3K–AKT pathway, whereas the activation of PLCγ1 leads to calcium release and activation of protein kinase C (PKC).

The RAS/MAPK kinase pathway affects cellular proliferation and differentiation. Examples of MAP kinase effectors include c-Jun.-N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 mitogen-activated kinase. FGF signaling pathway also interacts with the Wnt canonical pathway and is crucial for the differentiation process of mesenchymal cells either to osteoblasts or chondroblasts [39,40].

Mutations in FGF ligands are autosomal dominant forms and have been associated with different craniosynostosis syndromes. FGFR mutations affect the ligand-binding domain affinity, resulting in decreasing receptor specificity or increasing receptor activity [41–45]. Several point mutations in FGFRs have been identified and related to craniofacial malformations.

Mutations in FGFR2 (S252T and P253R), account for the majority of Apert, Crouzon, and Pfeiffer syndrome cases. All exhibit a characteristic Crouzon-like facial appearance, with protruded eyes, shortened face, and premature fusion of the coronal sutures [22]. Analysis of bone marrow samples indicated proliferation of osteoprogenitor cells in these tissues, implicating FGF in the early stages of embryogenesis.

Apert syndrome patients share characteristics, such as biocoronal synostosis and syndactyly of the hands and feet. Other cases have been reported with cleft palate, and learning disability. Nearly all Apert syndrome mutations arise de novo and have been shown to originate exclusively from the father [46].

Mutations in FGFR1 (P252R) are associated with Pfeiffer syndrome [47], and changes in FGFR3 (P250R) have been linked to Muenke syndrome [48]. Pfeiffer syndrome is characterized by broad, deviated thumbs. Crouzon syndrome is usually the mildest of the FGFR2-associated disorders and the clinical diagnosis is suggested by the combination of crouzonoid faces and absence of major abnormalities of the hands and feet. The distribution of mutations causing Pfeiffer and Crouzon syndromes in FGFR2 overlaps considerably [46].

Several translational animal models have been established using the aforementioned mutations. Deletion of both FGFR1 and FGFR2 genes leads to lethal phenotypes. Mice with FGFR2 (Cys342Tyr) missense mutation generated by Eswarakumar and colleagues demonstrated a Crouzon phenotype [49]. A mouse model carrying an FGFR1 gain of function mutation P252R, related to Pfeiffer syndrome has also been studied, with a premature fusion noted in multiple sutures. This phenotype exhibited premature and coronal suture fusion, midface hypoplasia, and facial asymmetry [50]. Increased bone ossification further supported that FGFR1 is important in osteoblast differentiation. Of all the FGF receptors, FGFR2 seems to be most implicated in suture fusion [51]. FGF2 has been identified in dura mater, by immunohistochemistry, during suture fusion. In further studies, an increase in FGF2 mRNA in translational models, by using a viral vector, causes premature suture fusion [52].

2.7.2. TGF-β/BMP signaling

Organ murine model systems of cranial sutures have allowed for the definition of TGF-β (transforming growth factor beta) as a potential regulator of suture fusion or patency. TGF-β is not associated with a known form of craniosynostosis, but a series of studies by Opperman et al. demonstrated that TGF-β1, β2, β3 are expressed in sutures and the dura mater [53–55]. More specifically, TGF-β2 seems to promote suture fusion and TGF-β3 patency.

In a tissue analysis from sutures of human infants, either synostotic or controls, elevated TGF-β immunoreactivity was found in fused sutures. Also, TGF-β has been correlated to its downstream ERK1/2 pathway as application of an ERK1/2 inhibitor disrupts the expression and phosphorylation of TGF-β in a fused suture [56].

BMPs (bone morphogenetic proteins), another member of TGF-β superfamily, have been reported to induce osteogenesis and regulate a variety of clinical disorders, such as cancer, skeletal and vascular diseases. BMPs have also been implicated in suture fate, as studies have localized BMP2, BMP4 in both fused and patent sutures. A recent study of patients with non-syndromic sagittal synostosis highlights the importance of BMPs in craniosynostosis. Genome-Wide Association analysis identified single nucleotide polymorphisms (SNPs) in a region downstream of BMP2 and BBS9 [57]. BMP2 plays a vital role in mesoderm formation during embryogenesis and BBS9 is a component of protein aggregation that has been correlated to craniosynostosis. BBS9 is part of a protein complex which induces the moving of cargo molecules in and out of cilia. Cilia act as a platform on which signaling pathways take place [57].

BMPs bind to type I and II transmembrane serine/threonine receptors. When both receptors are present, the binding affinity increases dramatically. Activated BMP receptors, initiate signaling by phosphorylating downstream targets, Smad1, Smad5, and Smad8 proteins, which then interact with Smad4 and translocate into the nucleus. BMP signaling is regulated by extracellular and intracellular modifiers. Extracellularly, BMP signaling is regulated by the cell surface in compliance with secreted molecules. These include noggin and heparin sulfate proteoglycans, such as glypicans [58]. Noggin is expressed in non-fused sutures and over-expression of Noggin prevents fusion of the posterofrontal suture that normally fuses by postnatal day 45. Noggin is a BMP antagonist, known to be important for suture patency.

Warren et al. found that the differential expression of the BMP antagonist Noggin was responsible for the sutural fate. In sagittal and coronal sutures that remained patent Noggin inhibited the signaling capacity of BMPs. In contrast, Noggin when absent in fused sutures, allowed BMP activity [59].

MSX1 and MSX2 are transcription factors which belong to genes encoding homeodomain proteins, a class of transcriptional regulators that play important role in development. MSX1/MSX2 were originally isolated by homology to the Drosophila msh (muscle segment homeobox) gene. MSX2 is located on a locus on chromosome 5 and is regulated by BMP-SMAD signaling. A single amino substitution in the homeodomain of the human MSX2 gene is associated with the autosomal dominant disorder Boston-type craniosynostosis [60]. Enhanced expression of MSX2 in mice increases bone growth of parietal bones into the sagittal suture and a subsequent increase in osteoblastic cells is observed [61]. Additionally, $MSX1^{-/-}$ homozygotes manifest a cleft palate model and abnormalities of the frontal and parietal bones [62]. Recent studies have further elucidated their function. MSX1/MSX2 mutant mice exhibited a distinct retardation in the migration of neural crest-derived cells and a subsequent disorganization of neural patterning [63]. Recent findings have observed in mutant MSX1/MSX2 mice ectopic bone in the frontal foramen. This formation of ectopic bone is associated with elevated BMP signaling [64].

2.7.3. Wnt signaling

The Wnts (wingless-type integration sites) are a large family of secreted glycosylated proteins that consists of 19 members present in mammals. Wnt signaling pathway is one of the most conserved molecular pathways, which controls several cellular processes, cell proliferation, differentiation, migration, as well as the patterning of cranial neural crest cells, as reviewed in Logan and Nusse [65]. It has been reported that Wnt signaling is important for the proliferation of the neural crest-derived mesenchyme [66]. Deficiency of Wnt signaling, either by low-density lipoprotein receptor-related protein 6 (Lrp6) deletion or with mutated Wnt3/Wnt9 antagonists, resulted in facial clefts and abnormalities in midfacial development [67].

Wnts signal through the canonical β-catenin pathway and also through two non-canonical pathways, the Wnt/Ca^{2+} and the planar cell polarity pathway. It is believed that all pathways are involved in craniofacial development. The canonical pathway is regulated by β-catenin. When Wnt ligands bind to Frizzled (FZD) receptors, a cytoplasmic accumulation of β-catenin is triggered, which leads to its translocation into the nucleus. In the nucleus, β-catenin binds to lymphoid enhancer factor/T cell transcription factor (LEF/TCF). This binding activates downstream transcription. The Wnt/Ca^{2+} pathway is characterized by an intracellular calcium release, possibly via G-proteins. This pathway is also composed of activated phospholipase C and protein kinase C (PKC). The planar cell polarity pathway is implicated in the migration of cells within the palatal shelves. Thus, a crosstalk between these canonical and non-canonical pathways may regulate the Wnt signaling as reviewed in Baron and Kneissel [68].

β-catenin is a central signaling component of the canonical Wnt signaling. Disruptions of β-catenin in neural crest cells result in lack of skeletal structures and elevated neural crest cell differentiation. β-catenin is essential in determining whether cells of mesenchymal origin will differentiate into osteoblasts or chondrocytes regardless of regional destinations [69]. On the other hand, activation of β-catenin causes an increase in mesenchymal cells and an elevated presence of immature, undifferentiated osteoblasts [70].

Axin2, however, acts as a negative regulator of the canonical Wnt pathway, by promoting degradation of β-catenin [71]. Axin2, in Axin1$^{-/-}$ mice, is expressed in the osteogenic fronts and periosteum of developing sutures. Targeted disruption of Axin2 in mice induces premature fusion of cranial sutures, through an enhanced differentiation of osteoprogenitors, thus an accelerated osteoblast proliferation and an ultimate mineralization [72]. It was mentioned earlier that the PF suture in the mouse fuses through endochondral ossification by postnatal day 45 and is ightly regulated by canonical Wnt pathway process. Recent studies used an Axin2$^{-/-}$ mouse model and investigated PF sutures. It was shown that PF sutures lack psychological endochondral ossification and are characterized by patency. These findings were further correlated to BMP signaling. In Axin2 mutants, BMP is up-regulated, functioning as a positive feedback mechanism [73]. This mechanism results in a change of the cellular localization of β-catenin, which localizes towards a membrane fraction, which enables cell–cell interactions during skull formation. An ectopic cartilage was also found, which probably led to the delayed suture fusion.

TWIST is a basic helix–loop–helix transcription factor and is located on chromosome [74]. Mutations in TWIST cause Saethre–Chotzen syndrome [74]. Patients with Saethre–Chotzen syndrome, demonstrate coronal craniosynostosis, facial asymmetry, limb abnormalities, and ear crura [75]. The TWIST protein is thought to participate in osteoblast differentiation and proliferation, as it is expressed in the osteoprogenitor cells within the sagittal and coronal sutures [46]. In homozygous mice, deletion of TWIST is lethal as they died at embryonic day 11.5. Their prominent phenotype was due to failure of cranial sutures to fuse [76]. This study implied that TWIST regulated the patterning and fate of neural crest-derived cells. Thus, a following study produced a heterozygous loss of function mutation in the TWIST gene, in a murine model. TWIST heterozygous mice showed coronal fusion, resembling the Saethre-Chotzen Syndrome in humans [77]. It should be pointed out that a variety of mutations, nonsense, missense, insertions, and deletions in Saethre–Chotzen syndrome patients, have been found in the coding region of the TWIST gene. Also, the Notch ligand Jagged1 is thought to be a downstream target of TWIST, as TWIST mutants demonstrate a decreased Jagged1 expression. Conditional knockout of Jagged1 leads to coronal craniosynostosis [78].

3. Signaling interactions in cranial sutures

The craniofacial skeleton includes the neurocranium and facial bones, which undergo intramembranous and endochondral ossification, respectively. The aforementioned signaling pathways are all involved in the regulation of its development and growth. A delicate balance exists between proliferation and differentiation in maintaining the anatomy of the cranial sutures. FGF, BMP, and Wnt signaling, along with their downstream or upstream targets, are critical players. Signals from the dura mater may also regulate the maintenance of sutural patency prenatally, whereas signals in the osteogenic fronts dominate after birth, suggesting that polycystins may act as mechanosensors and trigger the signaling cascade at a postnatal level. Indeed, the understanding of the signaling networks that control the commitment and differentiation of suture mesenchyme cells will not only expand our basic knowledge of the molecular mechanism of cranial suture development but will also aid our ability to develop therapeutic means of intervention in craniosynostosis.

FGF signaling is present both in endochondral and intramembranous bones and regulates their development reviewed in Marie [79]. FGF2, FGFR1, and FGFR2 have been found in the cranial vault during embryogenesis [46,80]. The morphology and patency of sutures are regulated by the presence of FGFR1 and FGFR2, which act in parallel with other osteogenic-related genes. It was shown that if FGF2 is applied to the osteogenic fonts via beads, ossification is accelerated. A subsequent study which blocked FGF2 with neutralized beads revealed that osteogenesis was induced, implicating an FGF2 role in bone formation. FGF signaling has been integrated with activation of the transcription factor TWIST, which causes craniosynostosis via haploinsufficiency [81]. On the contrary, FGFs cause craniosynostosis due to gain-function mutations [82].

Haploinsufficiency of the transcription factor TWIST1 is associated with SC syndrome and is manifested by craniosynostosis. It was shown that the TWIST forms heterodimers with ubiquitously expressed inhibitor of DNA-binding/differentiation proteins (bHLH) (T/E) and homodimers (T/T), can have different functions. More specifically, this study supported the hypothesis that T/T reside at the osteogenic fonts and T/E in between sutures. The formation of dimers throughout the sutures interferes in other pathways related to suture patency. For example, T/T dimers up-regulated FGFR2 expression, resulting in increased FGF signaling, and a subsequent increase in BMP signaling through binding to SMAD proteins. T/E dimers, on the other hand, up-regulate thrombospondin 1 (TSP1) expression, which activates TGF-β [83]. Depending on the TGF-β isoform, this can lead to suture patency or fusion. Further studies, proved that mice TWIST homodimers resulted in elevated FGFR2 levels, which ultimately resulted in craniosynostosis. In the same study, the authors demonstrated that in heterodimer Twist mice, suture fusion could be inhibited by limiting FGF signaling [84]. Obviously, it can be inferred that if craniosynostosis is due to an initiating mutation in a relevant gene, then inhibition of a relative signaling pathway could hinder suture fusion.

Wnt signaling plays a pivotal role during osteogenesis, as it both promotes the differentiation process of the precursor cell population and the commitment of these cells to the osteoblast lineage. Conditional β-catenin deletion in the head results in replacement of the cranial vault by cartilage as well as in reductions in osteogenic factors, such as RUNX2 mRNA levels [85]. It is apparent that β-catenin regulates target gene expression.

One of its targets is the TWIST family, and it has been reported that β-catenin activates both TWIST1 in vitro and TWIST2 in vivo, identifying them as nuclear targets [86,87]. Furthermore, TWIST1 inhibits chondrogenesis in vitro, suggesting that TWIST1 could repress chondrogenesis in vivo [87].

TWIST1 is a requisite in early migration and survival of cranial mesenchyme populations, which formulate the skull bones. A study by Yoshida and colleagues showed that during the establishment of the coronal (COR) suture area, TWIST is responsible for the regulation of the sutural mesenchyme and its subsequent osteoblast differentiation. These authors showed in calvarial organ culture, where they inhibited TWIST using morpholino-antisense oligonucleotides that COR sutural capacity was narrower, and its fusion was enhanced due to TWIST inhibition [88]. Therefore, its presence along with the presence of signaling pathways regulating skull formation at early migration stages plays a pivotal role.

In further studies, Behr and colleagues, building on the previous observations, demonstrated that active canonical Wnt signaling is responsible for cranial suture patency and that low levels of Wnt signaling allow craniosynostosis to appear [89]. More specifically, they further elucidated that the cranial fusion is attributed to chondrogenesis, a procedure normally inhibited by TWIST [89].

Additionally, another component of Wnt-β-catenin signaling pathway Axin2 is implicated in craniosynostosis. In a deficient Axin2 murine model, mice exhibited skeletal abnormalities resembling craniosynostosis in humans. A genetic disruption of Axin2 alters β-catenin [90] and this, in turn, results in the induction of synostosis-osteoblast-related genes, including the FGF–FGFR family, suggesting a potential link between FGF and Wnt signaling. Also, it is reported that Wnt orchestrates suture closure and skull development by maintaining a balance between FGF and BMP signaling.

MSX2 is a member of the homeobox gene family, whose transcription is regulated by BMP and TGF-β. Mice over-expressing MSX2 develop premature fusion of coronal and sagittal sutures. MSX2 and TWIST function cooperatively in the processes of osteoblast differentiation and proliferation. MSX2 is also related to BMP signaling, as it is regulated by BMP4 which in turn is regulated by the canonical Wnt pathway in other tissues. The fact that BMP antagonist Noggin is expressed in patent sutures further enforces the observation that BMP signaling is critical for regulating suture patency, and inhibited BMP signaling may contribute to craniosynostosis (Fig. 2).

Cranial sutures in rats and mice have been used for biomechanical studies, but limited information is available on the cellular and molecular events induced when such sutures are exposed to mechanical stress.

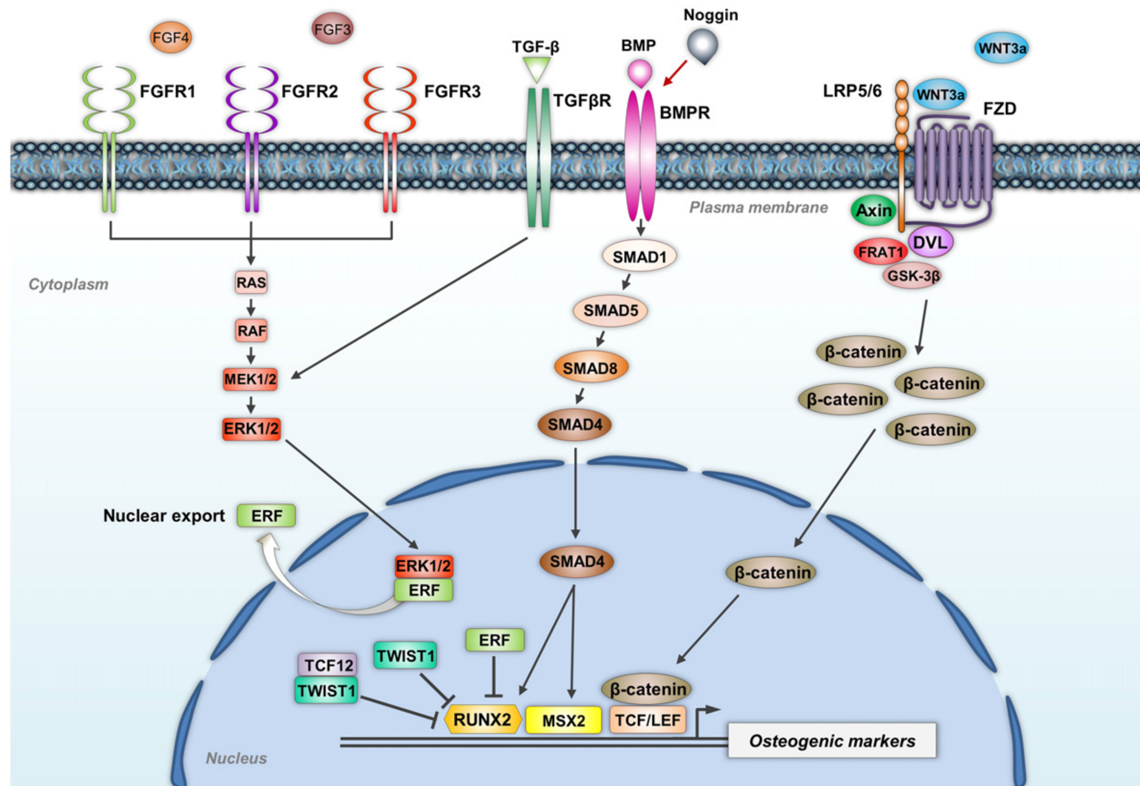

Fig. 2. *Signal transduction pathways in cranial development*. The figure schematically illustrates the central signal transduction cascades implicated in cranial suture development. Possible impairment of these pathways can lead to premature cranial fusion thus craniosynostosis. *FGF signaling*: FGFs (FGF4, FGF3) bind to FGF receptor Tyr kinases; FGFRs are phosphorylated hence activated and further activate intracellular substrates, which initiate downstream signaling pathways such as the RAS–MAPK pathway. The phosphorylated ERK1/2 translocates to the nucleus where it interacts with the transcription factor ERF, which is then exported from the nucleus. Within the nucleus ERF can bind to RUNX2 and repress RUNX2 expression. TWIST also represses RUNX2 by forming a heterodimer with TCF12. Mutations in FGFRs are associated with craniosynostosis syndromes, as over-expression of FGFRs triggers the constant expression of ERK1/2, thus RUNX2 expression is not abrogated and osteogenesis occurs. *TGF-β signaling*: TGF-β signaling is correlated with downstream ERK1/2. *BMP signaling*: BMPs bind to type I/II transmembrane receptors. Activated receptors initiate signaling by phosphorylating downstream targets Smad1, Smad5, Smad8 proteins, which interact with Smad4 which translocates into the nucleus. BMP over-expression leads to the expression of RUNX2, thus to cranial fusion. BMP signaling is regulated by the antagonist Noggin. *Wnt signaling*: The Wnts signal through the canonical β-catenin pathway. Wnts bind to FZD receptors and an accumulation of β-catenin is triggered. β-catenin translocates to the nucleus and binds to TCF/LEF transcription factors. Subsequent transcription of osteogenic markers follows. BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; CK1, casein kinase 1; DVL, the scaffolding protein Disheveled; ERF, ETS domain-containing transcription factor; ERK1/2, extracellular signal-regulated kinase 1/2; FGF3 and FGF4, fibroblast growth factors 3,4; FGFR1/2/3, fibroblast growth factor receptors 1/2/3; FRAT1 proto-oncogene; FZD, Frizzled receptor; GSK3-β, glycogen synthase kinase 3; LRP5/6, low-density lipoprotein receptor-related protein 5/6; MEK, mitogen-activated protein kinase kinase; MSX2, homeobox protein MSX-2; RUNX2, runt-related transcription factor 2; TCF12, transcription factor 12; TGF-β, transforming growth factor beta; TGFβR, transforming growth factor beta receptor; TCF/LEF, T cell factor/lymphoid enhancer factor; (TWIST), twist-related protein 1.

Studies where mice were subjected to midpalatal suture expansion in vivo demonstrated that midpalatal force promoted cartilage formation [91]. PC1 and PC2 are promising mechanosensory molecules potentially involved in this process since previous studies have shown their implication in skeletogenesis [91,92]. It has been reported that PC1-deficient mice present restricted growth effects at the skull base and in craniofacial sutures, without however knowledge of the underlying molecular mechanisms [92]. Mice with floxed alleles of PC1 were crossed with Dermo-1-Cre, Wnt1-Cre, and Osx-Cre delete strains, to conditionally remove PC1. Mutant animals showed a premature closure of the presphenoid and sphenooccipital synchondroses at the cranial base [91].

Furthermore, conditional deletion of PC2 in neural crest-derived cells provoked mice mutants which showed craniofacial deformities, including mechanical trauma, fractured molar roots, distorted incisors, alveolar bone loss, and compressed temporomandibular joints, in addition to abnormal skull shapes. It is worth mentioning that mutants showed no indication of these phenotypes at embryonic stages, suggesting that in utero heads do not receive significant mechanical stress [93].

Recent findings establish correlation between PC1 and β-catenin in autosomal dominant polycystic kidney disease, where PC1 C-terminus tail (CTT) undergoes cleavage and is released intracellularly. It has been demonstrated that the CTT binds to β-catenin and co-localizes in the nucleus. This has led to the view that CTT inhibits the ability of β-catenin and Wnt ligands to activate the TCF gene. Loss of PC1, after being silenced in MC3T3-E1 osteoblastic cell line by using lentivirus-mediated shRNA technology, impaired mechanical strain, as PC1 deficiency resulted in the loss of ability to sense external mechanical stimuli, thus promoting osteoblastic proliferation and differentiation [94].

The importance of this effect could be clearly demonstrated in cranial suture development, as cranial sutures constantly respond to mechanical stimuli. It has been shown that osteoblasts require the mechanosensory molecule PC1 to respond to mechanical forces, by enhancing osteoblastic mechanoresponse, through a potential PC1/β-catenin action [94].

4. Polycystins and cranial sutures

Skeletal abnormalities had firstly been reported in PC1 deficient mice which had lethal phenotypes [95]. Then mice with floxed alleles of PC1 were crossed with Dermo-1-Cre, Wnt1-Cre, and Osx-Cre delete strains, so as to remove conditionally PC1. Mutant animals showed a premature closure of the presphenoid and sphenooccipital synchondroses at the cranial base [91] correlating the central role of polycystins in cellular mechanosensation and mechanotransduction processes.

PCs act as mechanosensory proteins in cranial development. Skull expansion depends on tensile forces, thus, PC1 is a mediator as it controls the proliferation of the sutural mesenchymal cells. In a Wnt-Cre, Pkd1 mouse model, PC1-deficient mice displayed an impaired response to tensile force. In this model, wild-type mice under induced midpalatal suture expansion revealed ossification at the bone joints, which was a result of an intense proliferation, within the sutural mesenchyme.

Therefore, PC1 is related to osteoblast differentiation and bone formation at sutural edges. Taking into account that recent studies have reported that proliferation and differentiation of chondrocytes and osteoprogenitor cells are modulated by mechanical stress [96], we can speculate that PC1 can be implicated in this procedure.

For example, tensile forces applied across the frontonasal suture in rabbits, led to the expansion of the craniofacial system [97]. Furthermore, in vivo cyclic and static loading of the cranial base of growing rabbits, enhanced chondrocyte proliferation at synchondroses at the cranial base [98]. However, the molecular mechanism underlying the response of suture cells to mechanical strain remains poorly understood. A hypothesis which we support is that PC1 is involved in the process of mechanotransduction and mechanoresponse during skull growth.

According to studies by Hou B, it has been suggested that PC1 is displayed on the surface of sutural osteoprogenitor cells and interacts with the collagen fibers, which are the internal part of the suture [91]. Collagen fibers absorb tensile and compressive forces that are present within a suture. Then, osteoprogenitor cells respond by proliferation, osteoblast differentiation and subsequent bone deposition. This model suggested by Hou B, in suture development, aligns with previous studies which implicate PC1 in osteoblast differentiation. Most notably, a study by Xiao et al. further demonstrated that PC1 serves as a connection between cells and induces cell-to-cell adhesions, acting as a mechanosensor protein, whose absence in vitro led to skeletal abnormalities and decrease in RUNX2 levels [99]. Therefore, it is suggested that under the presence of PC1, expression of the osteoblastic indicator RUNX2 is enhanced, an enhancement which results in bone formation. Also, in the same studies, PC1 deficiency resulted in increased levels of phosphorylated ERK1/2. Hyperactivation of ERK1/2 signaling pathway has been linked to premature closure of the coronal suture and led to craniofacial malformations, as elevated ERK1/2 led to a continuously active FGFR2 [100]. In addition, in an in vivo model where ERK1/2 was inhibited, it prevented craniosynostosis [101]. Further studies have correlated PC1 deficiency with increased proliferation and dedifferentiation of tubular epithelial cells [102].

5. Therapies

Currently, the main treatment of craniosynostosis is surgery. An aim would be to design therapies in craniosynostosis to prevent the rapid refusion of the skull that occurs after surgery to separate the fused bones, reducing the need for repeated surgery. Thus, some of the aforementioned signaling pathways pose promising candidates for medical treatment/diagnosis of craniosynostosis.

FGFR signaling pathway remains the most important molecule in the pathological development of craniosynostosis, as mutations were initially found in FGFRs. Moreover, FGFR signaling is a critical player in osteogenesis. Treatments with FGFR inhibitors on osteoblast-like cells or osteoblast cultures from coronal sutures of Apert syndrome have correlated a non-properly working FGFR pathway to abnormal osteoblast proliferation, differentiation, and apoptosis [103–106].

The first study that characterized FGFR2 as a possible candidate was by Greenwald et al. [106], who injected adenoviral vectors in rat sutures, which either inhibited or enhanced FGF expression. In all calvarial models, the posterior frontal suture fuses postnatally. In rats, to prevent fusion of the posterior frontal suture, infection with a dominant-negative FGFR1 construct, abrogated overactive FGFR signaling in vivo [107]. Meaning that, when FGF was inhibited then suture patency was maintained whereas, when enhanced, bone formation was triggered.

Also, mouse calvaria isolated from the Crouzon-like FGFR2C342Y mouse model cultured in the presence of FGFR tyrosine kinase inhibitor PD173074 exhibited coronal suture patency. On the contrary, premature suture fusion was observed in untreated mutants [108]. Eswarakumar and colleagues reported in a Crouzon-like craniosynostosis murine model system that substitution of two amino acids, L424A and R426A, in the extracellular membrane domain of an activated Fgfr2c, prevent the recruitment and tyrosine phosphorylation of docking protein Frs2a, resulting in normal skull development [109].

In addition, a most promising study was conducted by Shukla et al., in which they cured mice with the Apert syndrome FGFR2 mutation by intraperitoneally injecting an inhibitor of MEK1/2, the U0126, into the pregnant mother. A subsequent postnatal fusion of the cranial sutures was prevented. They proposed that the mutant pups had a normal phenotype after birth, but their phenotypic response was unstable at a postnatal level. It is highlighted that probably the dosage of the inhibitor or its delivery may have led to this variability of response [101]. At this

point, it could be mentioned that forces due to skull expansion at postnatal level may have altered the stability of the sutures. Therefore, PCs can be implicated in the process. Further studies, in a similar model to the Apert syndrome, showed that treatment with MEK1 inhibitor PD98059 partially alleviated coronal suture fusion [100].

These data also suggested that the application of small molecule inhibitors may decrease FGFR tyrosine kinase activity and activate the downstream target ERK1/2. These data may contribute to the development of novel, non-invasive treatment options at a pre-birth level to treat craniosynostosis. It was also proven that FGFR inhibitor can be injected ectopically to prevent premature suture fusion and also refusion of surgically corrected previously fused sutures, a recurrent problem in treating craniosynostosis patients.

Glycosaminoglycans (GAG) regulate FGF/FGFR signaling. Thus, it has been proposed that inhibitory GAGs that down-regulate FGF/FGFR signaling could be potentially useful drugs for treating Apert syndrome. More specifically, proteins like heparin sulfate and chondroitin sulfate (CS) are involved in facilitation of FGF–FGFR ligand binding and osteoblastic differentiation. In this study, researchers manipulated levels of GAGs and FGF ligands, and it has been illustrated that due to variable co-operative binding activity, an inhibition of mutant FGFR signaling in Apert syndrome was observed [110].

Other studies have used recombinant technology, using human antibodies and small interfering RNAs (siRNAs). Noggin, an antagonist of the BMP proteins, is expressed postnatally in the patent cranial sutures, and the expression is suppressed by overactive FGF/FGFR signaling [59]. In a rat model transplanted with mutant FGFR2 osteoblasts, craniosynostosis was evident. In the abnormal fusing sutures, researchers found a downregulation of Noggin. By application of recombinant human Noggin, craniosynostosis was deterred [111]. In a following study, the application of cells expressing exogenous Noggin was proven to be an inhibitor of cranial synostosis in mice after the abrogation of the cranial suture. Due to the fact that Noggin treatment inhibited bone formation, Noggin therapy may be beneficial to traditional surgical repair of craniosynostosis [112,113].

Several studies using human tissues or organ systems have shown that fusion of cranial sutures may be related to an over-expression of TGF-β2 [114,115]. In an in vivo study of a New Zealand white rabbit model, with a bilateral coronal suture synostosis phenotype, the over-expression of TGF-β2 was obliterated with antibodies sharing neutralizing characteristics, in the synostotic sutures after surgery. In addition, in further studies in which inhibitory TGF-β2 antibody was infused in a collagen matrix, inhibited postsurgical synostosis and improved volume and craniofacial growth [116,117].

RNA interference has been used by recent studies, both in vitro [118] and in vivo [101]. Gosain and colleagues used endogenous anti-TGF-β1 small interfering RNA to target and knockdown TGF-β1 mRNA transcripts, which are expressed during cranial suture formation and may affect FGFR signaling. Ultimately, a decrease in mRNA levels of FGF2 and FGFR1, as well as a successful knockdown of TGF-β1, was observed.

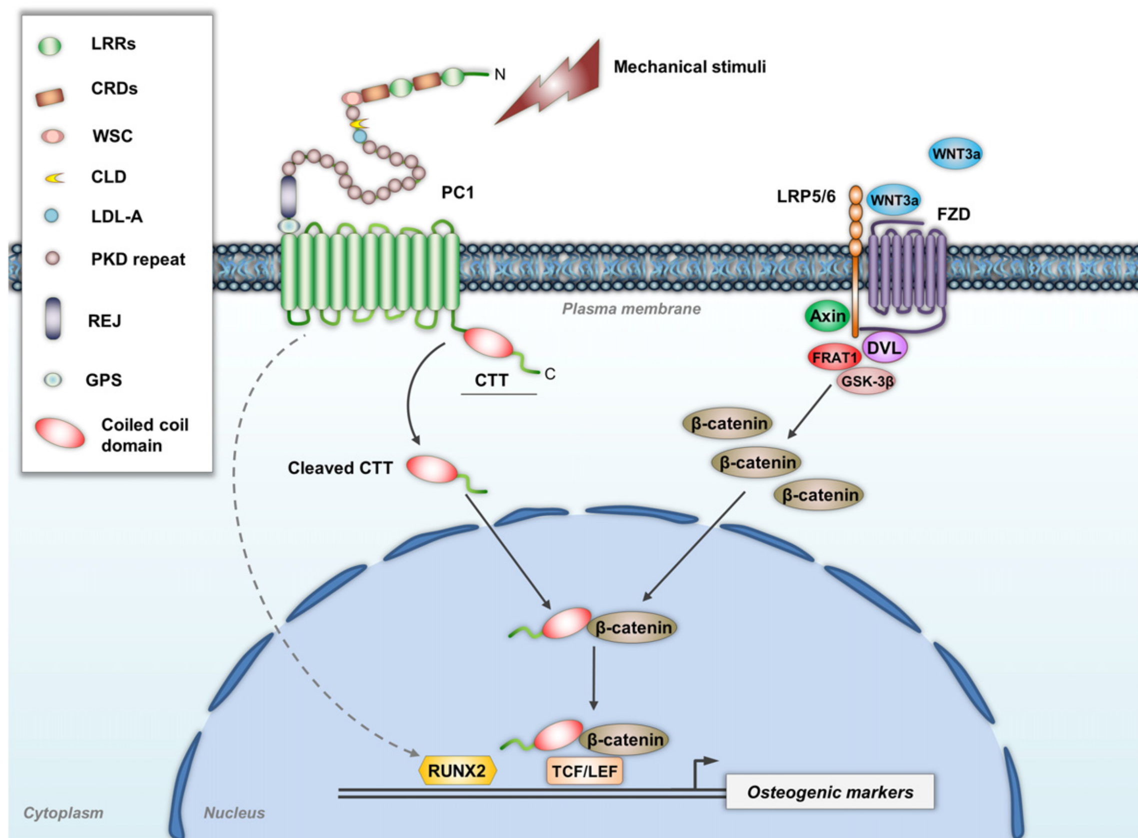

Fig. 3. *Schematic model of Polycystin 1 and Wnt signaling interaction.* PC1 is an 11-segment integral membrane protein, which consists of an N-terminal extracellular region and a short intracellular C-terminal part. Mechanical stimuli activate PC1, its C-terminal tail is cleaved and translocates to the nucleus where it binds to β-catenin. The complex of PC1/β-catenin then interacts with TCF/LEF transcription factor complex and initiates osteogenic gene expression therefore suture fusion. In the absence of mechanical cues, mechanosensory protein PC1 is inactive, Wnt remains silent, and FGF/BMP signaling pathways are induced and cause acceleration of the premature suture fusion. CTT, C-terminal tail; CK1, casein kinase 1; CRDs, cysteine-rich domains; DVL, the scaffolding protein Disheveled; CLD, C-lectin like domain; FZD, Frizzled receptor; GPS, G-protein-coupled receptor proteolytic site; GSK3-β, glycogen synthase kinase 3; LDL-A, low-density lipoprotein receptor class A; LRP5/6, low-density lipoprotein receptor-related protein 5/6; LRRs, leucine-rich repeats; PC1, polycystin 1; PKD, polycystic kidney disease domain repeat; REJ, sea urchin sperm receptor for egg jelly; RUNX2, runt-related transcription factor 2; TCF/LEF, T cell factor/lymphoid enhancer factor; WSC, cell wall integrity and stress response component.

TGF-β1 siRNA has the potential to change signaling in the mouse dura, which is responsible for suture fusion in vitro [118]. In vivo studies were also performed by using small hairpin RNA to target the mutant FGFR2 with the S252W mutation transcripts in the Apert syndrome mouse model. A restoration of the wild-type phenotype was achieved. [101].

6. Concluding remarks—Outlook

Taken together, our hypothesis can be integrated into a schematic model that incorporates PC1 and Wnt signaling (Fig. 3). We pose that the extracellular N-terminal part of PC1 lies at the osteogenic fonts of cranial sutures and acts as a mechanosensor that responds to external cues applied on sutures. The PC1 C-terminal tail (CTT) undergoes cleavage and is released intracellularly where it binds to β-catenin in the nucleus. Wnt signaling is thus triggered resulting in activation of TWIST, whereas FGF and BMP remain in an inactive state leading to suture patency. In the absence of mechanical loading, PC1 is inactive; Wnt remains silent, and FGF and BMP are over-induced and enhance premature suture fusion.

In conclusion, this review reinforces the important role of PC1 as a key molecule that modulates mechanoresponses. PC1 can, therefore, be potentially used as a diagnostic tool, suggesting that patients with craniosynostosis may also share mutations in PC1/PC2 genes.

Disclosures

None declared.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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