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Hand in glove: brain and skull in development and dysmorphogenesis

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Abstract

The brain originates relatively early in development from differentiated ectoderm that forms a hollow tube and takes on an exceedingly complex shape with development. The skull is made up of individual bony elements that form from neural crest- and mesoderm-derived mesenchyme that unite to provide support and protection for soft tissues and spaces of the head. The meninges provide a protective and permeable membrane between brain and skull. Across evolutionary and developmental time, dynamic changes in brain and skull shape track one another so that their integration is evidenced in two structures that fit soundly regardless of changes in biomechanical and physiologic functions. Evidence for this tight correspondence is also seen in diseases of the craniofacial complex that are often classified as diseases of the skull (e.g., craniosynostosis) or diseases of the brain (e.g., holoprosencephaly) even when both tissues are affected. Our review suggests a model that links brain and skull morphogenesis through coordinated integration of signaling pathways (e.g., FGF, TGFβ, Wnt) via processes that are not currently understood, perhaps involving the meninges. Differences in the earliest signaling of biological structure establish divergent designs that will be enhanced during morphogenesis. Signaling systems that pattern the developing brain are also active in patterning required for growth and assembly of the skull and some members of these signaling families have been indicated as causal for craniofacial diseases. Because cells of early brain and skull are sensitive to similar signaling families, variation in the strength or timing of signals or shifts in patterning boundaries that affect one system (neural or skull) could also affect the other system and appropriate co-adjustments in development would be made. Interactions of these signaling systems and of the tissues that they pattern are fundamental to the consistent but labile functional and structural association of brain and skull conserved over evolutionary time obvious in the study of development and disease.

Introduction

Nowhere is the complexity of evolution and development more evident than in the head, a composite structure made up of a multitude of cell types, tissues, organs and spaces that originate separately but develop in tight synchrony guaranteeing structural and functional coherence within each organ and integration across cranial tissues. Remarkable accommodation and conformity of brain and skull, two organs that together form most of the head, is evident across living and extinct vertebrates [42, 136] and throughout development of extant vertebrate species. These tissues, and their coordination in development, are fundamental to our evolution as a species. Though evolutionary changes specific to brain

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and specific to skull are pronounced, change in one tissue accommodates change in the other. Here, we suggest that the integration of these tissues occurs at the level of the cell.

Persistent accommodation between brain and skull shape is revealed in the study of craniofacial and neural tube anomalies. Anencephaly describes several types of neural tube defects in which the brain is partially or totally absent [107] with associated malformations of the skull (holoacrania or meroacrania) consistent with the specific brain anomaly. Holoprosencephaly refers to a spectrum of disorders in which the prosencephalon fails to completely divide resulting in a range of anomalous conditions [15, 144]. Associated skull defects vary with the severity of the disorder, but often include the absence or malformation of the midline facial bones and malformation of the sphenoid bone [23, 75].

Correspondence in brain and skull is also apparent in normal development and in evolution, irrespective of any specific trajectory. Despite the fact that the brain takes shape before mineralization of the earliest developing cranial bones, once initiated skull and brain morphogenesis occur in temporal and spatial accord. Later growth of the cerebral hemispheres is associated with dynamic expansion of cranial vault skeletal elements whose contours track the changing shape of the hemispheres. How is this accomplished? How, over evolutionary and ontogenetic time, do skull and brain preserve exquisitely sculpted, perfectly paced compliance with one other?

In this review, we briefly consider evolutionarily important genetic signaling pathways that have been shown to be required for individual development of brain and of skull. Though members of these signaling families represent only a small fraction of the thousands of genes active in development of the head, we use them as known examples of genes whose expression affects patterning and development of craniofacial tissues. We build an argument that integration of brain and skull phenotypes is based on the response of cells to signaling systems and communication between cells and tissues, and these signaling families provide examples where known changes in gene expression have been shown to affect morphogenesis. Though cells of the formative brain and skull each respond to an orchestrated set of currently inestimable signals fundamental to the individual patterning of each tissue, the superb synchrony of brain and skull requires a form of direct or mediated communication between cells that comprise the two tissues. This involves direct signaling between cells of the two organs, coordinated response of tissue-specific cells to similar signals, the ability of cells of one tissue to indirectly respond to changes in the other, or a combination of these mechanisms. We summarize the major stages of brain and skull embryology that is driven, at least partially, by these major signaling networks and review what is known of the interaction and integration of their development. Finally, by interrogating a specific clinical condition, craniosynostosis, we provide further insight into the basis of cell-to-cell signaling that underlies the co-development of these important tissues of the head.

Common signaling pathways used by brain and skull in development

Distinct signaling pathways allow cells that have the competence to take on cartilaginous, osseous or neural fates to receive, process, and respond to information. As cells differentiate they take on new duties, but also acquire new abilities to send and receive signals within their immediate surroundings. Recognizing that 80 % of all genes are expressed in the brain [85] and that our research into developing mice has revealed over 95 % of all genes are expressed in the head [149] we highlight members of five important gene families that have been shown in separate analyses to be involved in patterning and morphogenesis of the brain and of the skull (Table 1). Each of these gene families are ancient in origin, with homologs identified in metazoans as basal as sponges [109] and cnidaria [32]. The number of members

in these gene families has increased and taken on new functions during evolution of the vertebrates as a result of major genome duplications [22]. Additional duplications and diversification (by mutation, modification of regulatory sequences, etc.) of individual genes and gene clusters have produced further functional changes.

Gene families active in brain and skull evolution and development

Hedgehog—The Hedgehog (hh) gene family is found across the Metazoans [96]. Desert hedgehog (Dhh) was produced by the first ancient genome duplication, while Indian hedgehog (Ihh) and Sonic hedgehog (Shh) were born of the second duplication [79]. Hedgehog signaling in Drosophila and vertebrates begins with the attachment of a Hedgehog ligand to a transmembrane domain receptor (Patched) which represses another receptor (Smoothened) that activates transcription factors [96]. Hedgehog signaling plays multiple roles in development, and is known to regulate cell fate specification, cell proliferation, and survival in different contexts [57] including multiple regions of the head. Signaling through Ihh is required for osteoblast differentiation in endochondral ossification [86].

Wnt—Wnt signaling is an ancient and highly evolutionarily conserved pathway, with members of this gene family present in every sequenced animal genome, from cnideria to humans [19]. The Wnt family currently comprises more than 100 homologous genes operating in several different pathways. Wnt pathways generally involve the interaction of a Wnt ligand with a Frizzled receptor, which activates the cytoplasmic effector Disheveled, at which point the gene cascades of these pathways diverge. Wnt signaling functions in early development [110] and is critical to brain and skull formation (Table 1).

Notch—The Notch signaling pathway consists of a highly conserved set of genes that are transmembrane receptors and function as transcription factors that regulate cellular interactions, proliferation, differentiation, cell fate, and apoptosis [139]. Two Notch receptor gene duplication events produced Notch2 and Notch3 from Notch1, with a later duplication event producing Notch4 in the evolution of mammals [139]. Notch signaling contributes to the control of bone formation, neural crest cell production and neurogenesis (Table 1).

TGFβ—The appearance of the transforming growth factor b (TGFβ) gene family coincides with the advent of the first animal species [54] and like most gene families already discussed, this family evolved and expanded through consecutive cycles of gene duplication. The TGF beta superfamily of ligands includes bone morphogenetic proteins (BMPs). The TGFβ signaling pathway is a versatile signal transduction engine, playing fundamental roles in development, organogenesis, stem-cell control, immunity and cancer [54]. After binding to a receptor, TGFβ activates signaling cascades that modulate gene transcription to control numerous cellular responses (i.e., proliferation, differentiation, apoptosis, migration). As we are finding for most signaling systems, TGFβ signaling can evoke diverse signals in varying tissues that differ according to the derivation of the responding cell and the temporo-spatial cellular context [152].

FGF—The Fibroblast Growth Factor (FGF) family is a set of small proteins that act as mitogens. They are signaling factors that interact with cell surface receptors to initiate cellular processes. FGFs are classified as canonical and non-canonical based on their evolutionary history and interactions with fibroblast growth factor receptors (FGFRs). Canonical interactions with heparin or heparin sulfate proteoglycans cause FGFRs to homodimerize, and activate and phosphorylate multiple cytoplasmic signaling cascades [27, 62, 114]. Non-canonical FGFs either act independently of FGFRs or lack the high-affinity heparin binding sites [63].

The 22 genes coding for FGFs in vertebrates, and four coding for FGFRs were generated from early metazoan and later vertebrate whole genome duplications. FGF/FGFR signaling is highly conserved but novel functions have been added through the accumulation of genes and of splice variants with different ligand binding specifications [63, 124]. FGFs play major roles in basic cellular processes in many tissues including bone, blood vessels and brain [43, 116, 117, 140, 142] but the specific activities vary depending on the cell type and timing [21, 116, 131].

Members of these gene families contribute to head development by initiating, terminating, intensifying and diminishing signals, or by detecting signals to which a cell should respond. These processes are not controlled exclusively by members of these families, but comprise interactions involving an elusive but large number of additional genes and processes (e.g., RNA interference that regulates gene expression). Because these five gene families have been studied extensively, specific aspects of their role in the generation of neural and cranial skeletal tissues is known (Table 1). Importantly, none of these are genes “for” head development [8], as they are involved in many networks that control the development of other tissues. Moreover, additional genes (unnamed here) may play equally important roles in cranial morphogenesis, in that if they are mutated, development is disrupted.

Crosstalk among several signaling systems in the integrated development of brain and skull is highlighted by recent evidence that a signaling center in the forebrain, the Frontonasal Ectodermal Zone (FEZ), mediates signals between the brain and face, profoundly impacting the shape of the face, including the facial skeleton [89]. Local and global aspects of signaling interactions that drive development in centers like the FEZ are context specific, hierarchical, spatiotemporally sensitive, and dynamic. The demonstration that perturbation in long-range Hedgehog signaling disrupts signaling of Fgf, Bmp and Erk leading to abnormal patterning and extensive apoptosis within diverse craniofacial primordial composed of differing tissues provides another example of tissue interactions through signaling as a basis for integration of brain and skull dysmorphogenesis [23].

To provide a background for further examination of the interaction of these tissues in development and in disease, we first describe critical aspects of the development and morphogenesis of each organ.

Brain development

Neurulation

Our knowledge of human central nervous system (CNS) development derives largely from the analysis of several species including mouse, chick and zebrafish. The following description makes use of relevant information from CNS development in several species. CNS development begins with the process of neurulation where a sheet of ectodermal epithelial cells thicken, invaginate, and fuse to form the neural tube, establishing the basic structure of brain and spinal cord. The process of neurulation is divided into four stages: formation, shaping, bending, and closure [16] (Fig. 1). Shaping begins with apical restriction of neural plate cells immediately dorsal to the notochord causing the neural plate to invaginate anteroposteriorly, extend along the AP axis and narrow mediolaterally. Bending of the neural plate raises the lateral edges until the neural folds meet and fuse at several distinct locations along the midline eventually forming the neural tube [16].

Regionalization of the CNS

While the neural tube as a whole is being organized into separate brain regions (see following section), the inner structure of the neural tube is being patterned into specialized regions containing specific neurons that reflect adult CNS anatomy. Two signaling centers,

the floor plate (ventrally located) and the roof plate (dorsal), run the entire length of the neural tube [11]. The floor plate forms from the ventral portion of the neural tube in response to Shh signals from the notochord and once present, the floor plate also becomes a signaling center for Shh [91]. Shh expression in the floor plate, and in the ventral forebrain, induces the differentiation of neuroepithelial cells into ventral neurons and oligodendrocyte precursors by activating and repressing several transcription factors in a dose-dependent fashion [91]. Formed of cells that originate in the neural folds as a result of BMP signaling from the adjacent epidermal ectoderm [99], the roof plate provides signals that pattern the dorsal aspect of the neural tube, though it does not operate independent of the floor plate. Specific functions of secreted BMPs in dorsalization of the neural tube remain uncertain as different BMPs appear to function redundantly in various regions of the dorsal neural tube [12]. Once formed, the roof plate secretes BMP and Wnt signals that contribute to proper specification and proliferation of dorsal interneurons [13].

During the third week of human development the cephalic flexure appears marking the level of the formative mesencephalon (Fig. 2a) and by week 5 the neural tube can be divided into the forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon) by a series of between-region constrictions (Fig. 2b) [128]. Continual folding of the cephalic portion of the embryo results in: (1) the appearance of the cervical flexure at the future boundary between the spinal cord and rhombencephalon; (2) further partitioning of the forebrain into the telencephalon and diencephalon; and (3) subdivision of the hindbrain into the metencephalon and myelencephalon (Fig. 2c). These subdivisions, along with the mesencephalon, give rise to every major structure in the brain.

Hindbrain—The developing hindbrain is notable for its segmental structure, which includes a series of discrete bulges called rhombomeres that emerge around the time when the cephalic flexure occurs [128]. Hox genes play important roles in the positional establishment of rhombomeres, with lower numbered Hoxa and Hoxb genes expressed in more cranial rhombomeres, and higher numbered Hox genes being expressed more caudally [87]. In zebrafish, rhombomere 4 (r4) is the first rhombomere to form and it serves as an important signaling center for development of the rest of the hindbrain [97]. That portion of the neural tube destined to become rhombomere 4 expresses FGF ligands (FGF3, FGF8) before the appearance of r4 boundaries, which in turn establishes r3 and r5 [97]. Adult derivatives of the hindbrain include the medulla, the cerebellum, and the pons, which contain fibers that connect the cerebral and cerebellar cortices with the spinal cord.

Midbrain—The isthmic constriction or midbrain–hindbrain boundary (MHB), located between the midbrain and hindbrain is an important signaling center for specification of the midbrain and anterior hindbrain regions [87]. The MHB is an evolutionarily ancient positional organizer of the nervous system, present in vertebrates and in protochordates [145]. Before its appearance, the position of the MHB is defined in vertebrate embryos by the junction of expression of Otx2 (cranial) and Gbx2 (caudal) transcription factors. The relative position of the Otx2/Gbx2 boundary is determined by Wnt signaling from the lateral mesoderm that represses Otx2 expression in the caudal neuroectoderm and induces Gbx2 expression. The region of Otx2 expression (which also slows Wnt1 expression just cranial to the MHB) induces formation of the mesencephalon, while the region of Gbx2 expression marks the future site of the metencephalon with FGF8 expressed just caudal to the MHB [153].

Forebrain—In addition to its role in craniocaudal patterning in the midbrain and hindbrain, FGF8 is also critical to patterning of the forebrain. FGF8 is expressed by the anterior neural ridge (ANR), a forebrain signaling center that consists of a section of non-neural ectoderm that surrounds the cranial extent of the neural plate where the telencephalon will form [11]

and eventually gives rise to the olfactory and hypophyseal placodes, and the olfactory bulbs [6]. FGF8 secreted by the ANR is necessary for cell survival and maintenance of pattern formation in the telencephalon, but does not directly induce pattern formation in this region [11].

Skull development

The secretion and mineralization of bone matrix is an important characteristic of vertebrate evolution, the details of which have been published previously [40, 74, 118]. Humans, like all vertebrates depend on mineralized tissue for internal skeletal support produced essentially by calcification [74] so that information from other vertebrates can be used to inform us of human bone development. Cells that form the mesenchyme that will become bones of the skull (either through endochondral or intramembranous ossification) come from two embryonic sources: mesoderm and neural crest [98, 112]. Cephalic paraxial mesoderm provides the cells for bones of the neurocranium though lateral mesoderm also contributes cells [112]. The neural crest, a pluripotent population of cells that appeared evolutionarily with the emergence of the vertebrates [24, 39], delaminate from the neural plate–ectoderm junction as the neural folds rise but before they fuse to form the neural tube (Fig. 1d). Neural crest cells undergo an epithelia-to-mesenchymal transition as they migrate toward target sites to differentiate into a diversity of cell and tissue types [25, 40, 84]. Important for our discussion, cranial neural crest cells form chondrocytes of primary and secondary cartilage, osteoblasts and osteocytes of intramembranous and endochondral bone, and odontoblasts of dentine [40, 74].

The identification of the embryonic source of various skeletal elements in vertebrate species (i.e., mesoderm or neural crest) has profound implications for understanding vertebrate evolution and diseases of the craniofacial complex [52, 92]. Evolution of the neural crest cell population along with gene diversification (by duplication, mutation, translocation, addition of splice variants, and modification of regulatory sequences) are associated with the rise of the vertebrates. The neural crest comprises part of the raw material that enabled the acquisition of a coordinated set of characters fundamental to the evolving mobile predator lifestyle that required improvements in systems for detection and capture of prey. Essential changes included specializations in exchange and distribution of respiratory gasses, modifications of the CNS and peripheral nervous system, special sense organ circuitry, pharyngeal anatomy, and new designs for a protective skeletal system. The neural crest enabled, or contributed to the development of most of these systems in the evolution of vertebrates. Knowing the cell population from which different bones and other tissues of the head derive in modern species can help delineate the timing of developmental insults pushing research forward and suggest directions for the development of therapeutics.

Bone in modern vertebrates can form in two developmentally distinct ways. Endochondral ossification begins when cells of either neural crest or mesodermal origin accumulate as chondroblasts to form a cartilaginous “model” which is eventually invaded by osteoblasts that replace cartilage with bone [40, 73, 78, 98]. Intramembranous ossification occurs when bone forms directly from undifferentiated mesenchymal cells without passing through a cartilaginous stage [40, 69].

Endochondral ossification of skull bones

Endochondral ossification is largely understood through analysis of developing long bones, but it is likely that the process is similar in endochondrally forming bones of the skull (e.g., ethmoid, basi sphenoid, basi occipital, petrous temporal). Early in development undifferentiated mesenchymal cells group together ventral to the brain to form prechondrogenic mesenchymal condensations. These condensations represent the

parachordal and additional cartilages that together form the anlagen of bones of the cranial base. Shh, Bmp (Bmp 2-5, Bmp7), Fgf, Hox genes and others are involved in a cascade of signaling and response that lead step by step to the positioning and shape of endochondral condensations [41, 69]. In general, the shape of a cartilage model roughly establishes the shape of the endochondrally ossifying bone [17].

In mice, parachordal cartilage formation at embryonic day 11 (E11) marks the earliest appearance of the chondrocranium [98]. Cells within each condensation differentiate into chondroblasts and then chondrocytes. While chondrocytes of each condensation hypertrophy, a thin outer layer of cells flattens to become the perichondrium. The bone collar, the precursor of the cortical region of endochondrally developing bones, forms within the perichondrium. Formation of the bone collar involves inhibition of chondrocyte maturation by Runx2 and induction of mature osteoblasts in the adjacent perichondrium by Ihh signaling [69].

The perichondrium is thought to signal vasculature invasion of the cartilaginous model, a process necessary to ossification of the bone collar and of the cartilaginous model. Vasculature intrusion is accomplished largely by signaling through vascular endothelial growth factor (VEGF) that enables subsequent invasion of the cartilaginous model by osteoblasts that synthesize osteoid, which eventually calcifies to form bone [17]. Upon endochondral ossification, most cranial cartilages become bones of the skull but others never mineralize and remain cartilage while others regress completely without undergoing mineralization.

Intramembranous ossification of skull bones

Bones of the facial skeleton (e.g., nasals, maxillae, premaxillae, zygomatic, mandible) and the cranial vault (e.g., frontal, parietal, and squamous temporal) form primarily by intramembranous ossification. Intramembranous ossification occurs when undifferentiated cells aggregate to form initial condensations and continue to proliferate [69, 80, 141]. Cells closest to the site of the initial condensation begin to differentiate along an osteogenic path as indicated by the expression of Runx2 which then induces the expression of Osterix in osteogenic cells [86]. These cells then synthesize type I collagen, bone sialoprotein, osteocalcin, and finally bone matrix (hydroxyapatite and calcium carbonate) [59, 69, 130, 141]. BMPs are active in regulating the early differentiation of osteogenic cells while the later stages of differentiation are dependent on signaling by BMP, Ihh and parathyroid hormone-related proteins (PTSrP, PHLH) that negatively regulate differentiation of osteoblast progenitors into osteoblasts.

Bone matrix is initially deposited in the form of trabeculae that fuse to form a lattice-like appearance (Fig. 3). Spicules of the original lattice thicken and the entire complex expands in size and changes in shape. Vascularization is present at the earliest stages of intramembranous ossification and as bone matures, vascular canals provide cells within the already mineralized matrix with metabolic support. The expanding surfaces of these bony lattices contain proliferating presumptive bone cells that add new bone. Since bone can only grow appositionally (not interstitially), its formation requires the continual recruitment and/or differentiation of additional osteoprogenitor cells to the expanding bone mass. Current knowledge suggests that these cells are recruited from the expanding lattice [141] and not from surrounding tissues. Once embedded in the mineral matrix, osteoblasts become osteocytes and control maintenance of many bone properties communicating via canaliculi within the mineralized matrix [30, 40, 74]. Canaliculi provide entryways for osteoprogenitor cells and osteoclasts, both necessary to the breakdown and rebuilding of bone (remodeling), and optimizing bone strength.

Regions of the skull

The mammalian skull is commonly described as consisting of three major parts. The facial skeleton or splanchnocranium is phylogenetically the oldest part of the skull, originally formed as support for pharyngeal slits in our protochordate ancestors [70]. The cranial base (chondrocranium) and cranial vault (dermatocranium) are considered phylogenetically and developmentally separate components that combine to form the neurocranium.

Facial skeleton—The facial skeleton is derived primarily from cranial neural crest cells [18] that physically interact with surface and neuroectoderm, mesoderm and endoderm as they migrate to a multitude of target sites to shape a series of complex skeletal elements [18] formed primarily by intramembranous ossification (Fig. 4).

Several facial processes form as swellings of mesenchymal cells whose differential growth, rearrangement, and fusion bring together various aspects of the upper and mid face. In addition, a series of bar-like ridges (the pharyngeal arches) form on the ventro-lateral surface of the embryonic head and neck region. Migration of neural crest cells is controlled by differential expression of regulatory signals along the closing neural tube such that a differential contribution of specific populations of neural crest cells to specific facial processes and pharyngeal arches is known. Each pharyngeal arch consists of a core of mesenchyme surrounded by neural crest that is externally covered by ectoderm and internally by endoderm. The first pharyngeal arch gives rise to elements of the upper and lower jaws that articulate with the rest of the facial skeleton that forms within the facial processes. The remaining pharyngeal arches produce small bones and cartilages of the ear and pharyngeal apparatus. The relative movements of facial processes and pharyngeal arches are controlled by strict temporal and spatial signaling, so that initially separate mesenchymal condensations come together to form bones that eventually articulate into the face and palate.

As the bones of the face grow in size and the edges of facial bones approximate one another, sutures are induced to form between the bones. Sutures are the fibrous tissues uniting the bones of the skull and are characterized by the presence of immature, rapidly dividing mesenchymal progenitor cells [115]. Most facial sutures remain patent during facial growth but eventually fuse.

Cranial base—Bones of the cranial base underlie the brain and form via endochondral ossification of cartilage precursors [73, 78] (Fig. 4). The number of paired cranial base cartilages is consistent within species (e.g., there are 14 paired cartilages in mouse [98] and 8 in human [134]), as is the relative location and timing of their formation and the source of the cells. In general, the derivation of the more anterior components of the cranial base is neural crest while the more posterior components are derived from mesoderm [98].

Cranial base cartilages form on the ventral surface of the brain and their organization enables passage of major neurovascular bundles and cranial nerves that connect the head with postcranial anatomy. Fusions of separate cranial base cartilages and mesenchymal condensations can combine to form single bones of the skull that cross cranial base-cranial vault boundaries (e.g., occipital bone, sphenoid bone, temporal bone) so that some skull elements represent the amalgamation of multiple chondrocranial cartilages and intramembranous growth centers [98].

Cranial vault—Bones of the cranial vault protect the dorsal and lateral aspects of the brain. Cranial vault bones form intramembranously from one or more mesenchymal condensations that proliferate and then differentiate into osteoblasts. Mesenchymal condensations of the frontal and parietal bones first localize just above the eye [60] and extend apically toward

the dorsal aspect of the head as sheet-like structures that lie between the brain and surface ectoderm. Fewer osteogenic mesenchymal cells differentiate into osteoprogenitor cells within areas between growing vault bones referred to as ‘presumptive sutures’ [116, 117]. Though cranial sutures have long been characterized as ‘intramembranous growth sites’ [115], undifferentiated mesenchymal cells do not appear to have intrinsic growth potential, so that “growth” of bones occurs on the edges of opposed bones at the sutural margins (Fig. 5) [115, 141]. Differentiation of osteoblasts and bone deposition (growth) of the ossification front at the sutural edge is thought to be regulated by signaling interactions between the mesenchyme, the osteogenic fronts, and the dura mater [31, 102, 106, 115, 151].

This short summary of brain and skull provides at least a suggestion of the complexity of their individual development. The cells of each tissue derive from different cell populations and the timing of major developmental events differs within each tissue. Still, any snapshot of development reveals tight synchrony in their changing shapes. Below we summarize mechanisms proposed to contribute to this coordination.

The “How” of brain and skull integration

Patterning and morphogenesis of the skull and of the brain are initiated, driven and supervised by vast signaling networks and cell–cell signaling behaviors specific to each of these tissues. There is evidence that signaling that appears to regulate a specific tissue can have direct influence on other tissues. For example, a number of studies linking bone phenotypes in mouse models defined for neuropeptides and neurotransmitters have shown the negative regulation of osteoblast proliferation by the sympathetic nervous system [71, 73]. This phenomenon is probably more the norm than the exception as no signal preferentially targets one cell or another; instead cells are either equipped or not equipped to recognize and respond to a signal. Cells do not “know” that they are destined to form one tissue or the other, but they do “know” to what signals they are sensitive.

One candidate for mediating signaling between brain and skull is quasi-static tensile strain on developing cranial bones by pressure produced by the growing brain. The pressure applied fosters morphological remodeling of the neurocranium to reduce strain by mirroring the shape of the expanding brain (Fig. 6) [44]. The roots of this idea were formalized by Moss [104, 105] as the functional matrix hypothesis, but a mechanism for the transfer of information from mechanical loads to changes in cell behavior was not specified. It is now known that mechanical forces can deform the cell membrane and/or cytoskeleton producing changes affecting cell signaling, cell differentiation, proliferation, and the production of extracellular matrix molecules [88]. The conversion of mechanical stress applied to the cell into a chemical response by the cell suggests an interaction between extra cellular matrix (ECM) molecules and the internal cytoskeleton [56, 138]. Cell shape is provided by the cytoskeleton, a balanced system of tension- and compression-resistant forces produced by an intricate structure of microtubules and microfilaments [56]. The ECM is integrated into the stress network of the cytoskeleton at regions of physical connection between the cell surface and ECM substrates called focal adhesion protein complexes that are composed of integrin receptors, growth factor receptors and cytoskeleton-coupling proteins [56, 123]. Stress-based deformation of the ECM alters the cytoskeleton and deforms the cell through stress applied at these focal adhesion sites [56]. The precise mechanisms through which the transmission of stress between the ECM and cytoskeleton alter patterns of gene expression and chemical signaling require further investigation. However, it has been demonstrated in vitro that the application of strain to preosteoblasts results in an accumulation of β-catenin in the cell cytoplasm and nucleus, which increases expression of target genes in the Wnt/b-catenin pathway, including Wisp1 and Cox2 [10]. It is suggested that connections between the cytoskeleton and the nucleus enable mechanical forces to alter chromatin binding

patterns and change gene transcription patterns [7]. It is well known that cartilage cells are supplied by diffusion aided by flexion or compression and for much of development cranial vault bones are not yet mineralized (or are incompletely mineralized), so it is easy to think about transmission of these forces to cells within non-mineralized matrices of osteoprogenitor cells. Once mineralization occurs, the reaction of osteocytes to strain might occur through the network of dendritic canaliculi embedded in the mineralized bone matrix that transports nutrients, nervous signals, blood, and waste products to and from the cells [1]. Osteocytes respond to mechanical stimulation by altering the expression of several molecules, including IGF-I, IGF-II, and osteocalcin [127].

The hypothesis that strain produced by the growing brain results in changes in neurocranial size and shape has been difficult to test owing to the close-fitting morphological configuration of brain and skull, and the difficulty in measuring relatively low levels of strain that the growing brain is likely to apply to the cranium. The physical interface between brain and skull occurs at the meninges which consist of three layers (Fig. 6). The pia mater is a thin vascular membrane that tightly adheres to the brain by following the intricate contours of all gyri and sulci. The arachnoid mater is separated from the pia by the subarachnoid space in which cerebral spinal fluid flows and consists of two parts: a trabecular layer bridging the pia to the dura mater through the subarachnoid space and a continuous membrane adherent to the inner surface of the dura mater. The dura mater is the outermost meningeal layer made of dense fibrous tissue, the outer surface of which functions as the endosteum of cranial vault bones [143].

Dura mater is known to express many osteogenic growth factors, osteogenic cytokines and ECM molecules critical for ossification and morphogenesis of cranial vault bones [143] with immature dura mater showing substantial increases in these substances relative to mature dura mater [34, 35]. Rat dura mater is under tensile strain that is significantly greater in immature relative to mature rats and is disproportionately distributed in a pattern that reflects directions of brain growth [45]. In vitro application of tensile strain to immature rat dura mater significantly increases the expression of TGF-β1, FGF-2, Ca^{++}, osteopontin and plasma membrane permeability [29, 156], suggesting that tensile strain produced by brain expansion could reasonably account for at least a portion of the difference in gene expression between immature and mature dura mater. Immature dura mater has been shown to play a critical role in the regenerative capacity of calvarial bones of children younger than 2 years of age [50]. Soluble factors secreted by dura mater are important in the regulation of osteogenesis within the suture and therefore contribute to mechanisms underlying cranial vault suture patency [115]. Together, these observations provide strong evidence that the ability of dura mater to regulate osteogenesis is age dependent and potentially highly localized [115].

The interaction between the meninges and cranial vault bones is established early in head development. Initial mineralization of each frontal bone of the cranial vault begins just above the eye and proceeds apically as the head grows (e.g. [61, 141, 143]). Initiation of apical growth of the frontal and parietal bones and differentiation of meningeal precursors into three distinct layers begin in parallel in laboratory mice and progress jointly toward the apex of the head [143]. Importantly, in the congenital hydrocephalus (ch) mouse strain where a mutation in *Foxc1* (a forkhead/winged helix transcription factor) occurs spontaneously, meningeal development initiates correctly but the mature, three-layered meninges are never established [143]. Mineralization of the cranial vault bones initiates normally in these mice but apical growth does not, demonstrating an intimate developmental association between establishment of the properly layered meninges and apical growth of the frontal and parietal bones. That this relationship exists before the three-layered meninges

are established points to further age-dependent mechanisms in the co-development of brain, meninges and skull.

The meninges covering the mammalian brain are derived from two sources. Meninges that develop with and cover the cerebral hemispheres (forebrain) are of neural crest origin, whereas meninges of the mid- and hindbrain derive from the cephalic mesoderm [68, 155] (Fig. 2d). The internal surface of mammalian dura mater and the outer face of the arachnoid mater derive from a common precursor cell that also has two sources, being of mesoderm origin at the skull base and midbrain and of neural crest origin at the telencephalic region [68]. Neural crest cells are unique to vertebrates and their appearance accompanied fundamental evolutionary changes in the vertebrate head including the emergence of novel rostral cranial tissues (e.g., neurogenic placodes, rostral brain protected by a well-developed endoskeleton; cephalic sense organs and associated circuitry) [33, 39, 70, 113]. The neural crest-derived portion of the meninges evolved in parallel with the rostral portion of the brain and skull during the fast paced phenotypic change of the heads of early vertebrates. This correspondence in derivation and proximity could mean that the rostral brain, meninges and cranial vault evolved in part by responding to shared signals (biochemical or tensile strain) of development. It is likely that the protochordate ancestor of the vertebrates had the beginnings of a genetic program for neural crest formation [51] which was augmented in the earliest vertebrates in association with the definitive characteristics of the neural crest including the remarkable ability of neural crest cells to individually migrate through embryonic tissues and differentiate into an extraordinarily varied range of cell types. Though the details are not at all clear, the co-option of existing gene networks and signaling pathways by a novel cell population (neural crest) equipped with slightly varying receptor characteristics and response mechanisms could produce novel cranial tissues (neural crest-derived meninges and bone) and contribute to the rapid production of cranial variation in the evolution of the heads of early vertebrates.

A critical difference that needs to be underscored in discussing the relevance of meninges to cranial vault bone development is the distinct role of meninges in morphogenesis and early development, relative to later developmental events. Perturbations of development that occur early (as in the Foxc1 mutant) suggest interdependence or coupling of the signals involved in the process of meningeal and bone cell differentiation (and potentially proliferation). Once established, the meninges and cerebral spinous fluid serve to protect the brain and spinal cord but also provide a potentially continuous envelope connecting brain and vault bone surfaces capable of transmitting signals either as a mechanical consequence of local changes, or as a medium of cell-to-cell signaling. There are congenital cranial pathologies in which brain, bone and meninges differentiate and form, but grow along abnormal ontogenetic trajectories that also reveal interdependence of cells that form brain, meninges and skull. Next, we offer a summary of a craniofacial condition where mutations in genes of major effect are known and brain, meninges and skull differentiate properly, but dysmorphic phenotypes are produced.

Brain and skull integration in craniosynostosis

Craniosynostosis, defined as the premature fusion of sutures that form between cranial vault bones, is a serious and common craniofacial birth defect (3–6/10,000 births [14]). The premature closure of a suture is typically associated with abnormal cranial vault shape requiring early reconstructive surgery, within the first few months of life (Fig. 7). Isolated or non-syndromic occurrences of premature suture closure are common and a small fraction of these cases are accounted for by known genetic variants (e.g. [67]), while about 30 % of syndromic craniosynostosis cases are explained by mutations in at least 11 genes [14, 120]. Another 16 % of these craniosynostosis cases are related to chromosomal abnormalities

(e.g., duplications, deletions, copy number variants) [120]. The diverse molecular pathologies for craniosynostosis conditions suggest the influence and interaction of many, assorted molecular pathways.

Though dysmorphogenesis of the cranial base has been proposed as the primary defect [103, 108], craniosynostosis has long been regarded as a disease of the cranial vault sutures. The corresponding dysmorphology of brain and skull in craniosynostosis conditions is customarily described as a result of compensatory growth, a change in local growth trajectories that result when the brain is forced to grow in directions perpendicular to patent sutures where skulls bones are not resisting. This raises concerns that premature suture closure impacts the growing brain by limiting intracranial volume and increasing intracranial pressure. A large proportion of craniosynostosis research has consequently focused on the suture and the prevailing treatment regime includes surgical suture release and reconstruction of the neurocranial vault. Surgical outcomes are mixed for reasons that are not fully understood [28, 119, 132, 133] but will certainly be traced to variation in disease etiologies among craniosynostosis patients.

With the detection of contributing mutations, a group of craniosynostosis syndromes already characterized as separate clinical entities were discovered to be caused by mutations in FGFR1, FGFR2, and FGFR3 [14, 20, 65, 120, 150]. Much work has been done to further explain the consequences of impaired FGFR1-3 gene function in these syndromes with reference to the cranial vault and sutures [38, 55, 58, 146-148]. Most analyses of genetic networks recruited up- and downstream of these FGFR mutations have carried the implicit assumption that the identified mutations primarily and directly affect the behavior of mesenchymal and osteogenic cells local to the affected suture.

Research conducted in our laboratory has been driven by the overriding hypothesis that craniosynostosis is a consequence of a larger developmental assembly with a broad evolutionary and genetic basis involving the integration of regulatory networks among gene pathways shared by cells that contribute to cranial tissues other than the suture. To test this hypothesis, we have investigated the potential effects of mutations in this signaling system on additional regions of the skull and on other cranial organs in humans and in mouse models for these conditions. Statistical comparisons of 3D cranial shape in human infants with various craniosynostosis conditions have demonstrated that all parts of the skull (i.e., cranial vault, cranial base, facial skeleton) are affected in craniosynostosis [47, 48, 126, 157]. Statistical analysis of brain shape in infants with specific craniosynostosis conditions reveals distinct brain phenotypes with some differences in brain shape anticipated based on corresponding skull morphology and suture closure patterns, but other aspects of subcortical dysmorphology not reflected in skull shape [3, 4, 125]. Because these human studies are limited to the analysis of postnatal ages, we cannot determine from these data whether shape changes of the cranial vault, cranial base, facial skeleton, or brain are primary or secondary to the prematurely closed cranial vault sutures.

Further tests of this hypothesis using mouse models for some of the FGFR-related craniosynostosis syndromes reveal additional impacts of perturbed FGF/FGFR signaling on head development. Apert syndrome, one of the FGFR-related craniosynostosis syndromes, is caused by one of two neighboring mutations of FGFR2: Ser252Trp or Pro253Arg. The *Fgfr2*$^{+/S252W}$ and *Fgfr2*$^{+/P253R}$ Apert syndrome mouse models carry the mouse orthologs of these FGFR2 mutations and enable the study of head development at any point in prenatal ontogeny [146, 145]. Though the coronal suture is patent in the skulls of some mice carrying these mutations at embryonic day 17.5 (E17.5), significant differences in skull shape are apparent in the morphometric comparison of mutant mice and their unaffected littermates at this age. All parts of the skull are dysmorphic relative to unaffected littermates at birth (P0)

with the facial skeleton being the most dysmorphic [94]. Partial or complete closure of the coronal suture is evident in nearly all *Fgfr2*$^{+/S252W}$ and *Fgfr2*$^{+/P253R}$ Apert syndrome mice at birth, but is not strongly correlated with skull dysmorphology [94].

Our analysis of mouse models for additional FGFR-related craniosynostosis syndromes has demonstrated generalized regulatory shifts in rates of bone volume growth [122], variation in cranial vault and facial suture patency, and significant reductions in nasopharyngeal and eye volumes [93, 94]. These findings provide an expanded catalog of clinical phenotypes in craniosynostosis conditions caused by aberrant FGF/FGFR signaling and attest to the broad role of FGF/FGFR signaling in development of various cranial tissues derived from different embryological origins.

Detailed analysis of data from magnetic resonance microscopy images of newborn *Fgfr2*$^{+/S252W}$ and *Fgfr2*$^{+/P253R}$ Apert syndrome mice and of *Fgfr2c*$^{C342Y/+}$ Crouzon syndrome mice reveals localized, subcortical changes in brain morphology that appear to be primary targets of these *Fgfr2* mutations rather than secondary responses to premature closure of vault sutures or of cranial vault dysmorphogenesis (Fig. 8) [2, 93]. Study of early postnatal growth [from P0 to postnatal day 2 (P2)] in *Fgfr2*$^{+/P253R}$ Apert syndrome mice and their unaffected littermates indicates that although the brain and skull physically correspond to one another in overall shape at each age, directions and magnitudes of growth of the skull from P0 to P2 are different from those recorded for brain growth over the same interval [49]. The demonstration of divergent, altered patterns of development in brain and skull in the *Fgfr2*$^{+/P253R}$ mouse model for Apert syndrome suggests some level of independence in the pattern of growth for the two tissues rather than one tissue directing development of the other.

That brain and skull are both primarily affected in the FGFR-related craniosynostosis syndromes is fairly certain. Though we know details of the complex signaling involved in cranial vault formation (e.g., [64, 80, 115, 141]), bone development (e.g. [69, 117]), suture formation [46, 115], formation of the meninges (e.g., [64, 68, 143], and sensory placode and brain development (e.g., [11, 16, 135, 137]), we do not know how these processes come together to form a head. As a simple example, it is still unclear whether a crania vault suture is a ‘thing’ that exists in some pre-patterned sense, is established as the consequence of two bony fronts that approach one another but do not fuse, or is an area that remains void of mineralized tissue due to signals emanating from underlying dura mater or other neighboring tissues.

The relative location of the initial mineralization sites of bones of the mammalian skull and identification of the cells from which they derive is fairly well established (e.g., [36, 77, 98, 101, 141]). Many cranial vault bones begin as two or more “ossification centers” (areas where mineralization is initiated) that fuse early to form a single bone. In these cases, the amalgamation or “fusion” of these ossification centers is a normal part of skull development and is not considered pathological or premature [125]. These consolidation events are likely accomplished by signaling between cells and in large part determine the shape of individual cranial bones, the pattern of suture arrangement, and the overall shape of the skull. Premature closure of cranial vault sutures occurs due to perturbation in this signaling system. Current experimental approaches might reveal the effect of a given mutation on the morphogenesis of a single tissue indicating whether the processes of migration, differentiation or proliferation are affected in the cells of interest, but they do not allow us to know how these mutations affect the complex set of interactions among genes and regulatory networks that drive communication among cells and tissues in the making of cranial morphology.

Modeling morphogenesis as information exchange

The causes of abnormalities of the skull and brain have been traced to mutations in many genes, and the dissection of their effects has helped define the role of these genes in normal developmental processes. But beyond these genes of major effect, the role of additional components of the vast genetic architecture underlying normal craniofacial development (large numbers of transcription factors and signaling pathways contributing to individually small, variable effects) is unknown. The processes by which coherence between skull and brain shape persist through both ontogenetic and evolutionary time have not been addressed molecularly, yet the bits of knowledge derived from individual studies of brain, meninges and skull morphogenesis inch us toward the formulation of appropriate questions of this highly cooperative process. Building on the existing knowledge of the genetic bases of disease and development of these tissues while turning our focus to signaling between cells and on what those signals are inducing cells and tissues to do can lead to a broader appreciation of the interdependence of cells of developing cranial tissues. As we have summarized from our review of typical as well as atypical cases: the brain and bones of the skull derive from different cell populations; the timing of major events in the development of brain and of skull do not appear dependent upon one another; and the patterning required for the development of each structure can be neatly described without reference to the other. Yet brain and skull develop in rather exquisite harmony. The meninges associate brain with skull developmentally and physically but the nature and mechanisms of communication that underlie their interaction are unknown.

Understanding the roles of different signaling molecules in the production of brain, meninges and bone and the relative role of these tissues in the production of head shape presents great difficulty in part because we do not yet have a full list of genes that contribute to the production of these tissues, but also because changes in cells or in their immediate environments result in changes in cell sensitivity and responsiveness to genetic and mechanical signals. Interdependence of development of tissues can be visualized as a series of rings like ripples on a pond radiating from the site of the initial signal initiating cell differentiation (Fig. 9). Imagine signals causing differentiation of brain and of skull vault bones as pebbles dropped onto the surface of a still pond in relative proximity to each other and in temporal order corresponding to the anatomical location and relative timing of their differentiation. As these tissues differentiate and take shape, signaling that fosters morphogenesis and is produced by further development emanates from the cells like ripples spreading across the pond. Beyond the immediate impact of these signals among similar cells that are destined to contribute to the tissue of interest, these signals will have the next greatest impact on cells destined to become the more proximate tissues. Each ripple ring represents the signals contributing to tissue-specific cell behavior as the signal spreads through the system. Though characterized here as equal in all directions, the signal may spread unevenly, or at varying speeds along different directions. Signal strength is represented in the height and periodicity (distance between concentric rings) of the individual waves. The amplitude of a ring (radius) represents the extent of the impact of these signals. Though there is some overlap between these ideas and those of standard reaction-diffusion (Turing processes) equations [76], they are not explicitly included here, but could be added. Turing process is a logical candidate for explaining dynamics of passing from one morphogenetic process to the next or the critical points in development where a change in a signal can nudge cells and/or tissues toward an alternate phenotypic outcome.

In the current model, strength of signal may diminish with distance from the source, or be amplified when contacting “alternate” signals. New signals in the form of response of cells to the original signals can initiate at the center, or can be added to the contents of each wave as the signal emanates through the system via cell-to-cell communication. As tissues expand

in size, take on properties like mass, elasticity, hardness, and come into physical contact, alternate signaling potential is added. This occurs for two reasons. First, as tissues grow and approach final size and shape, they may naturally become more/less resistant to mechanical perturbation. Second, as cells differentiate their capacity to respond to certain genetic signals changes.

Conclusions

We have reviewed the role of several major signaling systems in the development of brain and skull. The targeted nature of these signaling systems in the separate development of brain and of skull appears more and more incomplete as evidence of the interaction of these signaling systems and others in the development of multiple cranial tissues begins to accumulate (e.g., [23, 154]). The failure of simple models of genetic causation to explain other highly heritable complex traits (e.g., [5]) should serve as a warning of the inadequacy of current experimental paradigms to identify sufficient parameters and interactions to explain development of the head.

A model coupling the process of brain and skull morphogenesis via a common set of signaling pathways whose impact is perhaps mediated, or simply includes signaling via cells of the meninges provides a potential approach to understanding the correspondence in brain and skull morphology. With the evolution of the vertebrates this complex adaptation based on cell-to-cell communication of signals could contribute to survival and reproduction by tolerating or perhaps enabling rapid change in brain morphology and complexity while ensuring the development of a perfectly fitting protective helmet by simply mineralizing a surface whose shape mirrors brain morphology. The evolution of the neural crest-derived meninges enveloping the expanding forebrain and providing a surface for endosteal development provided a 3D design through which signals, however induced, could be transmitted and on which osteoprogenitor cells fated to form the skull might differentiate and begin the important process of mineralization.

Developmental events are comprised of cellular processes (e.g., migration, adhesion, proliferation, differentiation) that are employed over and over during morphogenesis, initiated by genetic signals and generally controlled by communication among cells. We now know that novel traits can be built using old genes wired in novel ways [9] but it is still a mystery whether novel traits evolve when genes are rewired de novo one at a time into new developmental networks, or whether clusters of pre-wired genes are co-opted into the development of the new complex traits [100]. Creating separate signaling systems for development of rostral brain, meninges and skull at the dawn of the vertebrates would require linking many genes within each system. For any specific genetic change to spread through a population, each of these genes would either have to be fixed by genetic drift or provide an immediate selective advantage at every intermediate step. While not impossible, this lengthy process seems unlikely given the relatively rapid evolution of the vertebrate head. The prolonged process could be avoided if brain and skull development were coupled. This linkage would involve a common set of signaling pathways that cooperatively build both brain and skull. This co-option of signaling systems could involve the meninges that function proximately as a protective unit, but that also develops the capability of transmitting signals sensed by brain and skull, all the while providing a local scaffold on which clusters of osteoprogenitor cells could take shape as brain and bone follow their own developmental trajectories.

The integration of signaling networks in the composite development of brain and skull provides multiple chances for errors in development to occur (and to be rescued), but also many potential opportunities for mutation effects of varying genes, cis-regulatory elements,

or transcriptional targets to produce phenotypically diverging, yet viable, integrated phenotypes. Importantly, such a system would not act individually on developing organs, but would cooperatively impose order by supervising the integration of many tissues. Relevant gene families and the signaling systems in which they operate likely evolved together along with the morphological and structural variation that they foster. The unique qualities of brain, meninges and skull are produced by many of the same signaling networks and we propose that those networks also administer the complementary fit of the shape of these tissues by cooperatively managing their development. Though not understood nor even fully described, the signaling that supervises the connection between skull, meninges, and brain development predicts a fundamental property that enabled rapid evolution of cranial phenotypes at the dawn of the vertebrates and explains the coordination of these tissues in development and disease.

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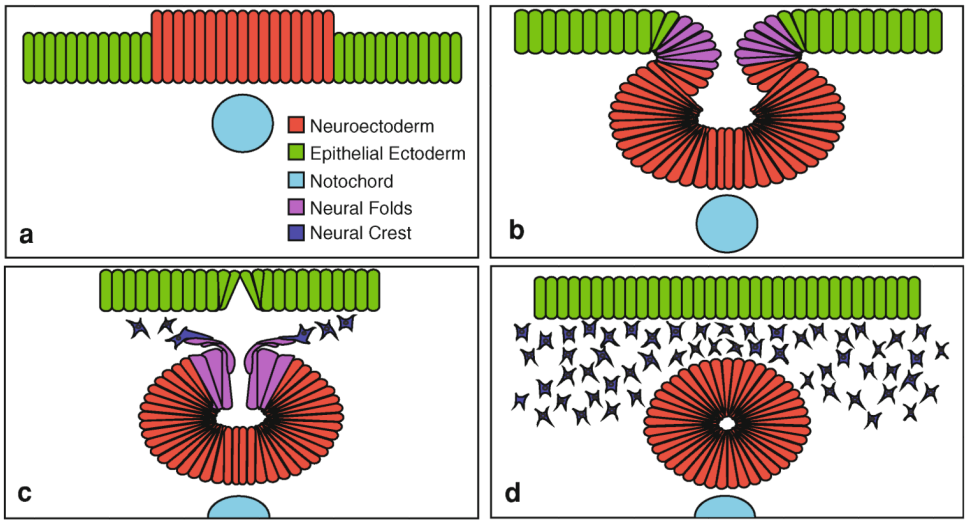

Fig. 1.

Stages of neurulation (based on [16]). **a** A section of ectodermal cells thicken to become neuroectoderm as a result of signaling from an organizing tissue. **b** The most lateral edges of the neural plate nearest to the epithelial ectoderm, the neural folds, begin to undergo changes that will form the neural crest. **c** The neural folds separate from the epithelial ectoderm, and the neural crest cells undergo epithelia to mesenchymal transition and migrate away from the forming neural tube. **d** The neural tube and epithelial ectoderm fuse separately while neural crest cells begin to migrate away from the neural tube

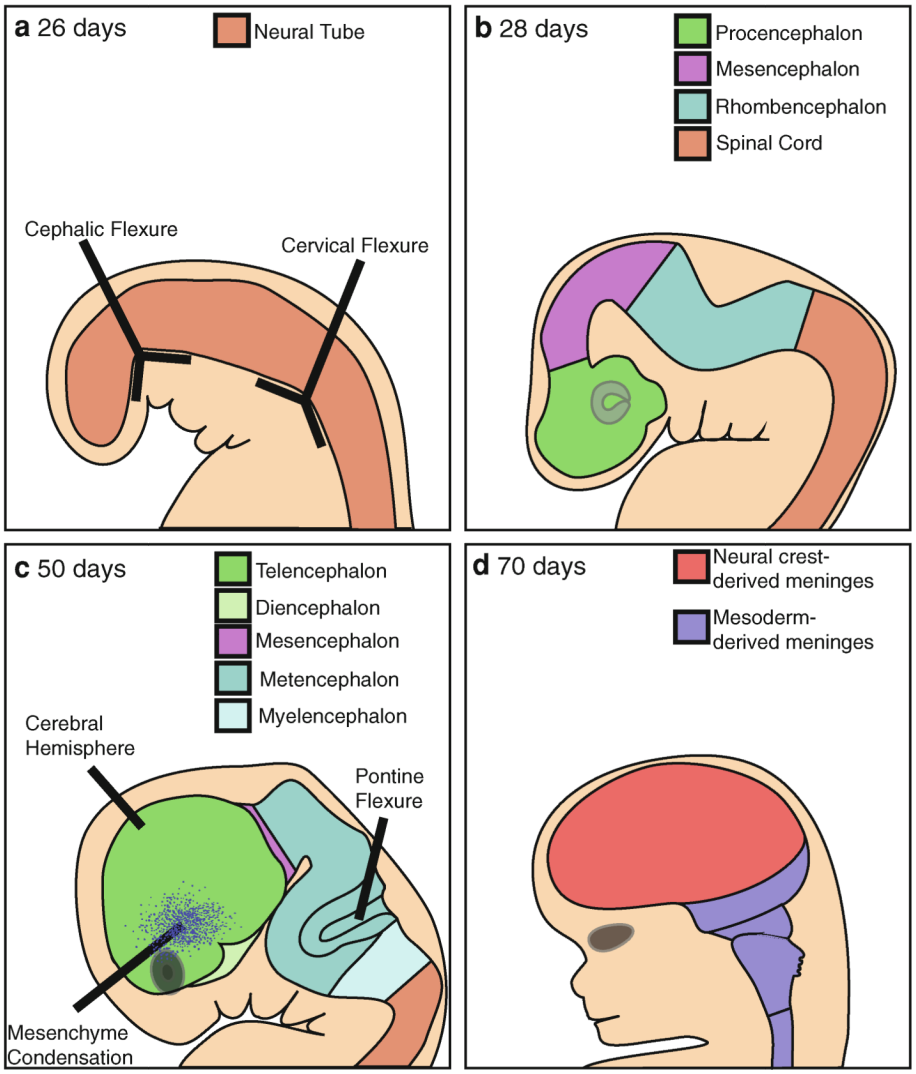

Fig. 2.

Human neural tube development (based on [82]). **a** At 26 days of development, the cephalic and cervical flexures are present; **b** at 28 days, the prosencephalon, mesencephalon, and rhombencephalon are distinguishable; **c** at 50 days much of the future structure of the brain is present, with the cerebral hemispheres beginning to overlap the rest of the brain. Also present is the pontine flexure that forms the barrier between the metencephalon and myelencephalon, and the cell condensations forming the frontal bone (*blue dots*); **d** at 70 days, the meninges derived from neural crest cover the telencephalon, while meninges derived from mesoderm cover more caudal CNS structures, though the boundary between the two is indeterminat (ossified elements are not pictured)

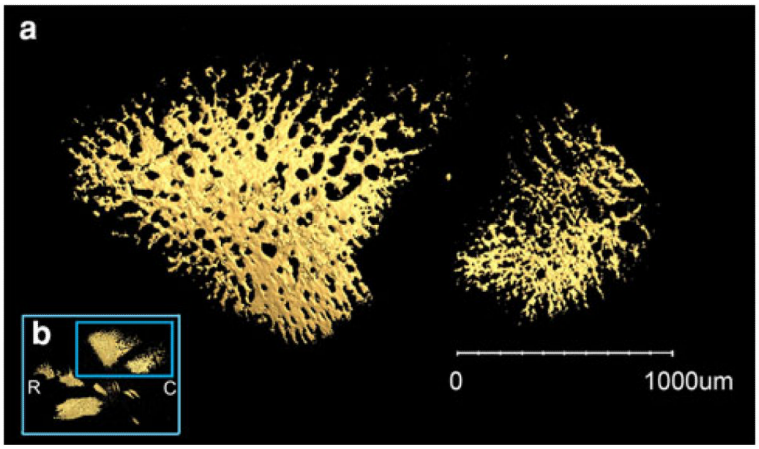

Fig. 3.

a Forming bones of the cranial vault reveal a lattice-like appearance. Lateral view of the forming cranial vault bones of a mouse at embryonic day 16.5 (E16.5) as visualized by ultra-high resolution micro-computed tomography (HRμCT) by XRadia. Frontal bone is at *left*, parietal is at *right*. **b** Lateral view of HRμCT scan of entire mineralized skull at E16.5 in *larger blue box* with frontal and parietal bones outlined in *smaller blue box*. Developing facial skeleton is rostral (*R*), parietal bone is caudal (*C*)

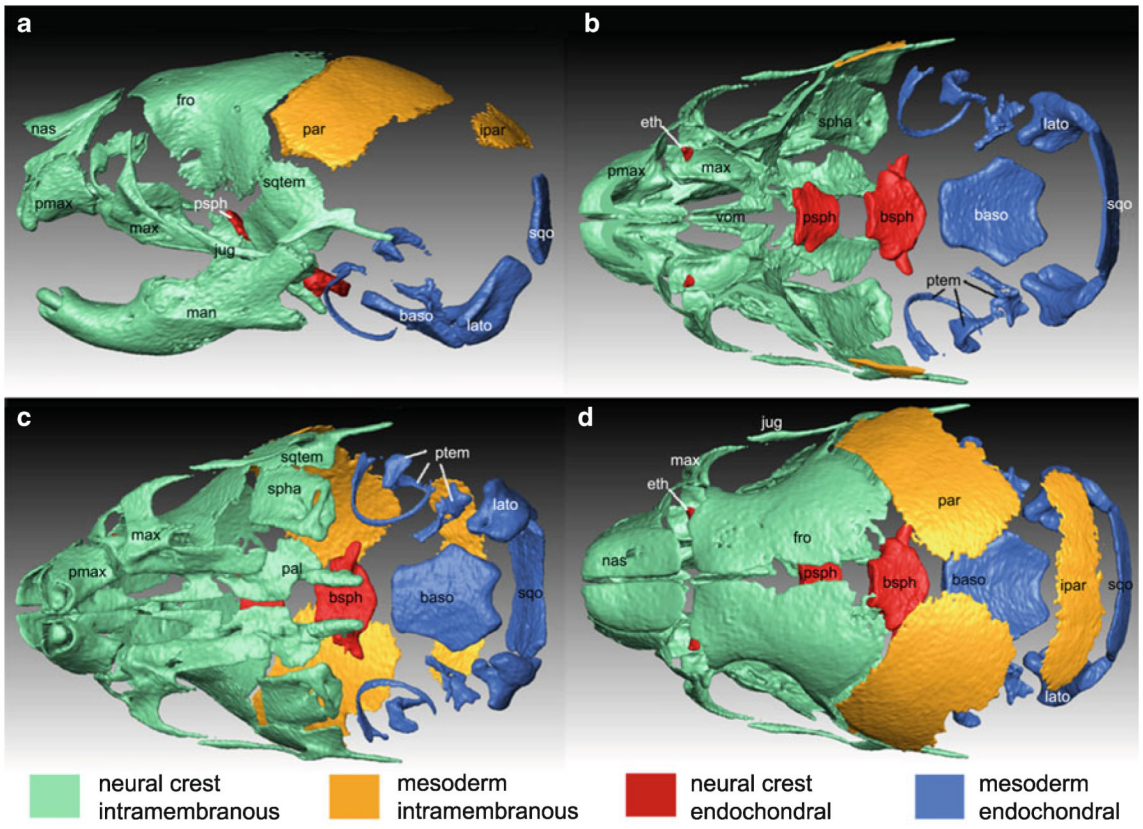

Fig. 4.

Contribution of cells and type of ossification of bones of the skull. 3D reconstruction of high-resolution micro-computed tomography images of a newborn mouse head segmented to visualize mineralized bone. Individual cranial elements are *color coded* to represent the contribution of neural crest or mesoderm and whether the bone forms intramembranously or endochondrally. In all views rostral is to left and caudal is to right. Views are lateral (**a**) superior with cranial vault removed (**b**) inferior with mandible removed (**c**) superior (**d**). Abbreviations of individual bones are as follows: facial skeleton: *max* maxillae, *jug* jugal, *nas* nasal, *pmax* premaxillae, *vom* vomer, *pal* palatine (includes pterygoid), *man* mandible; cranial vault: *fro* frontal, *par* parietal, *ipar* interparietal, *sqtem* squamous temporal; cranial base: *lato* lateral occipital, *sqo* squamous occipital, *baso* basi occipital, *psph* presphenoid, *spha* sphenoid alae, *bsph* basi sphenoid, *ptem* petrous temporal, *eth* ethmoid. Abbreviations for bones that occur bilaterally are given only on one side. Though primarily of mesoderm origin, the center of the interparietal (*ipar*) receives small numbers of neural crest cells and the presphenoid (*psph*) receives small numbers of neural crest cells between the sphenoid alae and base. At P0 the petrous temporal (*ptem*) and the ethmoid (*eth*) are mostly cartilaginous; only those regions that mineralize early are shown. In our 3D reconstruction, the lacrimal bones are segmented with the maxillae and the pterygoids are segmented with the palatine bones. Sources: [64, 98, 112]

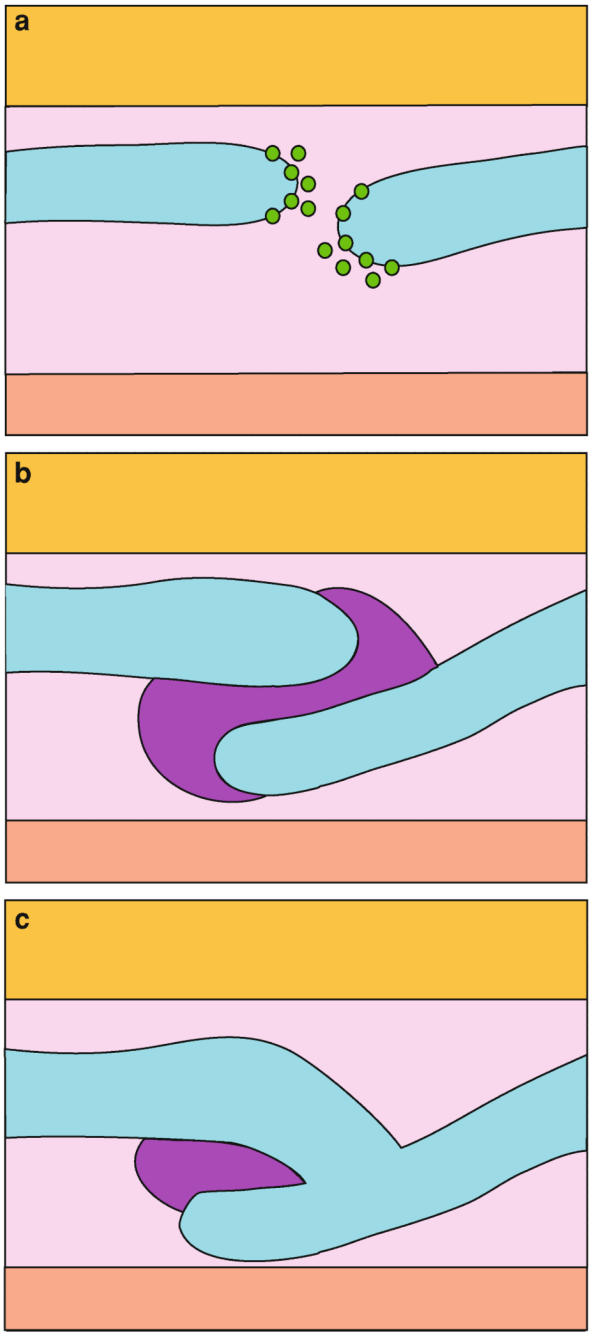

Fig. 5.

Formation and fusion of the coronal suture (after [115]). **a** Opposing bone fronts (*blue*) within a layer of mesenchyme (*pink*) between the dura mater (*red*, *below*) and periosteum (*orange*, *above*) approach each other by localized deposition of bone matrix by osteoblasts (*green*). The undifferentiated mesenchyme between the bone fronts comprise the suture. **b** As the opposing bone fronts begin to overlap, a mesenchymal 'blastema (*purple*) forms between the bone fronts. For a time, signaling from the sutural mesenchyme, bony fronts or other tissues (e.g., the dura mater), prevents differentiation of osteoblasts and mineralization of the suture; **c** in normal suture fusion and in craniosynostosis, osteoblast activity is no

longer inhibited in the suture region, resulting in fusion of the bone fronts and obliteration of the suture

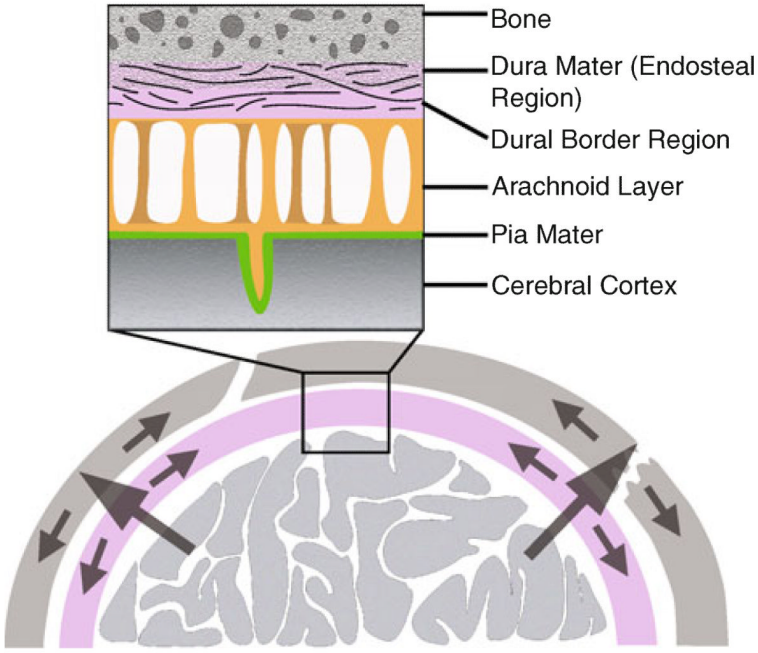

Fig. 6.

Schematic representation of the biomechanical interaction of growing brain, meninges and skull (*below*) and detail of meningeal layers (*above*). As the brain develops, it expands in all directions (*large arrows*), which applies tensile strain (*small arrows*) to the dura mater (*violet*) and the calvarial bones (*gray*). The transmission of force from the growing brain through the dura mater and neurocranium signals for the calvarial bones to expand to conform to the size and shape of the brain. The physical interface between brain and intramembranous bones of the cranial vault occurs at the meninges, that consist of three layers, shown in the *inset* (*above*). The pia mater is a thin vascular membrane that clings to the contours of the external surface of the brain. The arachnoid mater consists of a trabecular layer that bridges the pia to the dura mater and includes the subarachnoid space and an unbroken membrane adherent to the inner surface of the dura mater (dural border region). The dura mater is the outermost meningeal layer whose outer surface functions as the endosteum of cranial vault bones

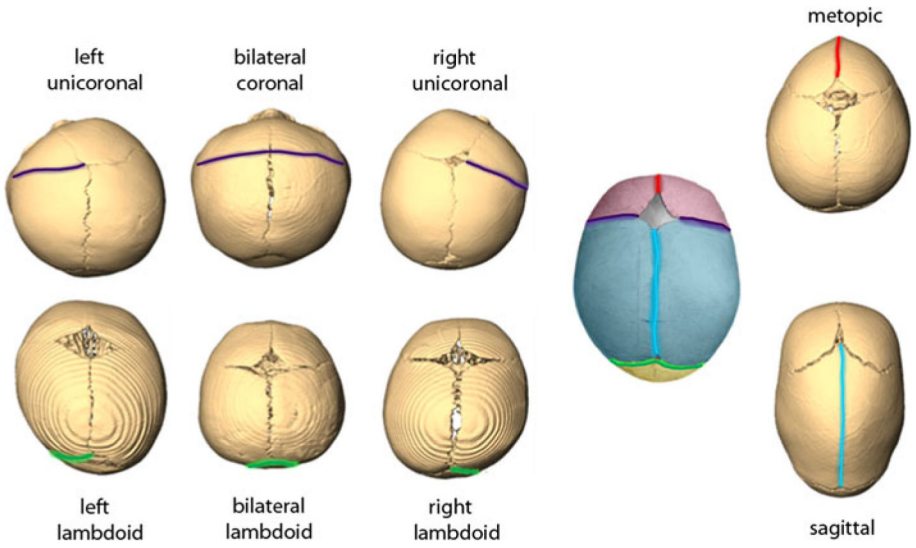

Fig. 7.

Superior view of 3D computed tomography (CT) reconstructions of human infant skulls with forehead toward *top of figure*. Individual bones and sutures of the typically developing infant cranial vault are *color coded*: metopic suture (*red*) between the two frontal bones (*rose*); sagittal suture (*blue*) between the two parietal bones (*blue*); coronal suture (*purple*) between the frontal and parietal bones, and lambdoid suture (*green*) between the occipital (*yellow*) and parietal bones (*blue*). Coronal and lambdoid sutures occur bilaterally. Typical craniosynostosis skull phenotypes for bilaterally occurring sutures are shown at *left* while craniosynostosis phenotypes for midline sutures are shown at *right*. Concentric rings reveal varying slice thicknesses of CT slice images for cases stored in our image archive

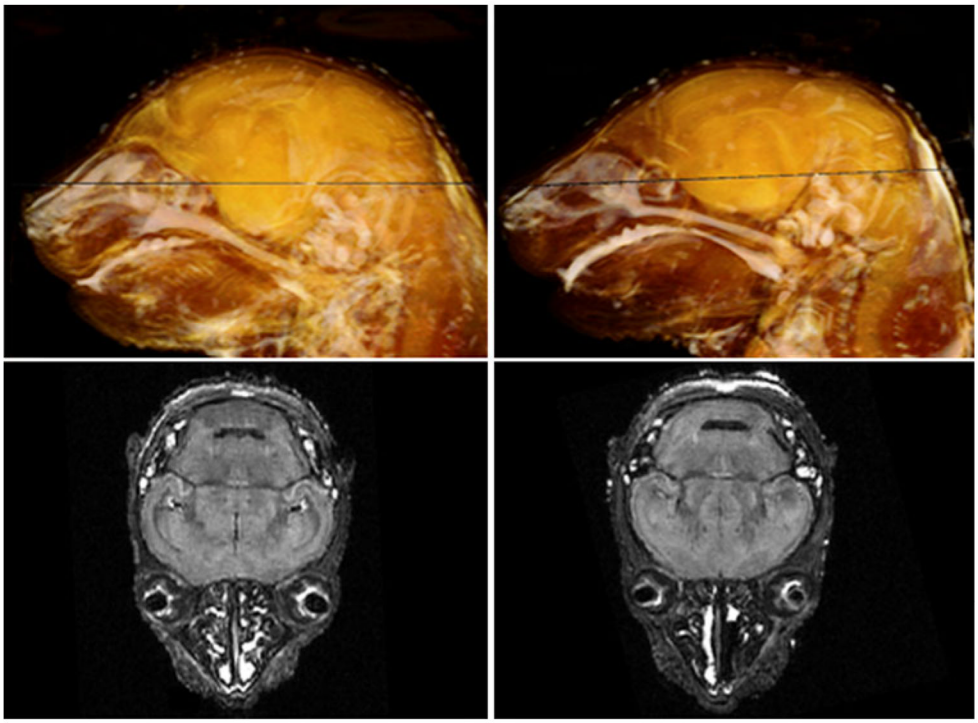

Fig. 8.

3D reconstruction of magnetic resonance microscopy (MRM) images (*top row*) of newborn *Fgfr2*^{S252W} Apert syndrome mouse (*left*) and unaffected littermate (*right*) and axial section of MRM (*bottom row*). *Horizontal line* in reconstruction shows approximate plane of axial slice (*bottom row*). *Intense white areas* show the contrast medium [2 % Magnevist (Bayer Health Care, Wayne, NJ) phosphor-buffered solution] used to reduce the T1 and T2 relaxation time during MRM imaging. Obvious neural changes in *Fgfr2*^{S252W} Apert syndrome mice relative to unaffected littermates include: more globular, domed forebrain, deeper extension of the lateral cerebrum on either side of the posterior pharynx, change in shape of fourth ventricle, generally wider cerebrum

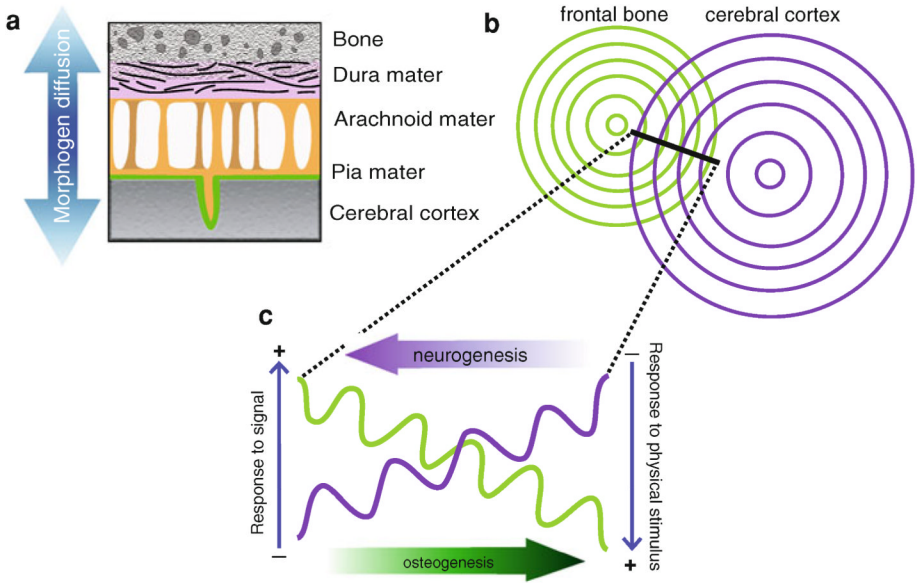

Fig. 9.

Cells in developing cranial tissues receive signals from diffusible molecules that form gradients across tissues and from the physical properties of other cells and tissues. **a** Anatomical relationships of brain, meninges and vault bone with hypothetical diffusion fronts traveling away from meninges toward frontal bone and the cerebral cortex. Cells are simultaneously sensing signals and reacting or not reacting according to their position with reference to the morphogen gradient. **b** Tissues expand in size by adding new cells and by increasing the distribution of the tissue as the organism grows, such that cells of different types sense each other's presence by one of many potential mechanisms [81]. **c** The strength of cellular response to a morphogen depends on the position of the cell with reference to the morphogen gradient (e.g., molecules spreading by diffusion), but cells also respond to signals generated by tissue structure and expansion (e.g., pressure, strain). In our highly simplified model, the cellular response to the morphogen precedes its response to physical pressure during growth. Once established, cells within differentiated tissues simultaneously respond to both types of signals as they continue to expand in size, constantly changing their relative location to morphogen gradients and generating and sensing changes in pressure

Table 1

Summary of some of the known functions of signaling pathways in the development of brain and skull

Pathway name	Receptor	Role in brain development	Role in bone development[a]
FGF	Fibroblast growth factor receptors: FGFR1, FGFR2, FGFR3, FGFR4	Signaling centers (r4/MHB [153]/ANR [11]), NC cell induction [111], varied early patterning processes in early CNS and neocortex development [43]; cell survival and apoptosis in CNS [37]	Cell condensation and proliferation [69]; cell growth [117]; differentiation [86, 90, 117, 140]; apoptosis [90, 117, 140]
TGFβ	TGFβ receptor	Neural induction (BMP antagonists) [129], NC cell induction [111], cell differentiation within neural tube [95]	Cell condensation and proliferation [72, 103]; proliferation [41]; apoptosis [117]
Wnt	Frizzled (Canonical)	A–P axis formation in neural tube [110], MHB [153], NC cell induction [111], neuronal maturation and synapse formation [121]	Differentiation [53, 72, 86]; osteoblast proliferation, mineralization [66]; promotes osteogenesis through stimulation of Runx2 [66]; reduces osteoblast apoptosis and osteoclast differentiation [66]
Hedgehog	Patched	Oligodendrocyte precursor induction, neural progenitor proliferation, motor neural differentiation, axon guidance of retinal ganglion cells [91]	Cell condensation and proliferation [41, 69, 72]; differentiation [72]
Notch	Notch	Neural stem cell proliferation, differentiation and apoptosis [83]; NC cell induction [111]	Differentiation [26, 72]; inhibition of osteoblast differentiation [86]

Pathway signaling can involve extra cellular ligands, secreted molecules or cell–cell contact and the role of the signaling family can involve negative (inhibitive) or positive regulation. The references included here represent review articles that summarize what is known, or original articles where a specific role for these signaling molecules in specific functions of brain and bone development has been demonstrated. Details of these processes and the additional pathways activated by those mentioned here depend on the particular receptor, ligand and cellular context, which are also dependent upon the developmental and metabolic state of the cells and input from other signaling pathways

[a]Role in bone development includes intramembranous and endochondral ossification. For pathways specific to chondrogenesis, see [72]