Genetic and Epigenetic Determinants of Skeletal Morphogenesis - Role of Cellular Polarity and Ciliary Function in Skeletal Development and Growth

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Summary: This review discusses evidence that proteins involved in establishing and maintaining polarity and/or primary cilia in cells have essential roles in skeletal morphogenesis and growth. It is argued that fibroblasts, chondrocytes and osteoblasts have a secretory polarity defined by the location of the trans-Golgi network and that the primary cilium is a good marker for the secretory apical region of the cells. It has been demonstrated that primary cilia in epithelial cells function as mechanosensors in that they can activate a Ca^{++} -permeant channel complex composed of the transmembrane proteins polycystin-1 and polycystin-2 when exposed to fluid flow or physical bending forces. It has therefore been proposed that primary cilia may also have mechanosensing functions in skeletal cells. To test this hypothesis, we have conditionally inactivated the genes encoding Kif3a, a motor protein required for cell polarity and assembly of the primary cilium, and polycystin-1. Mice carrying floxed alleles of these genes have been crossed with mice that express cre recombinase under the control of promoters that are active at an early stage in cranial neural crest cells, in early limb bud mesenchyme, or in cells of mesodermal origin of both limbs and skull.

Conclusions: The mutant phenotypes are consistent with the conclusion that protein complexes involved in cell polarity and assembly of primary cilia, including the motor protein Kif3a, are essential for hedgehog-mediated craniofacial and appendicular morphogenetic processes, and that polycystin-1 is required for normal development and postnatal function of sutures and specific synchondroses of the vertebrate skull.

Key words: primary cilia, cellular polarity, skeletal morphogenesis, hedgehog signalling, polydactyly, growth plate, synchondrosis

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INTRODUCTION

During the past two decades significant progress has been made in our understanding of the molecular and cellular regulation of the development, growth, and maintenance of the vertebrate skeleton. Many of the critical cytokines, receptors, transcription factors and extracellular matrix constituents in cartilage and bone formation have been identified, and their functions have been defined based on studies of the consequences of mutations in humans and mice. The major features of endochondral ossification, the process by which cartilage models of future bones are replaced by bone and bone marrow, have been characterized in some detail and many aspects of the regulation of endochondral bone growth are now understood. Finally, the discovery of several sources of osteochondroprogenitor cells and the development of biocompatible scaffold materials for tissue engineering are opening doors to the translation of this information for clinical skeletal tissue repair and regeneration. Unfortunately, we are still largely in the dark regarding major problems of mor-

phogenesis and growth, such as the nature of the processes that result in the formation of skeletal elements of characteristic size and shape, the cellular mechanisms by which skeletal elements respond to mechanical force, and basic cellular mechanisms of skeletal growth, maturation and aging. Addressing these problems for the craniofacial skeleton will be a major, but exciting, challenge for oral biology in the next few years.

In epithelial organs, morphogenetic processes are critically dependent on cellular mechanisms regulating cell polarity. Coupled to controls of proliferation and polarized secretion of extracellular matrix molecules, such mechanisms allow epithelial sheets to fold in distinct morphogenetic patterns. In the case of cartilage and bone development, where the cells are of mesenchymal origin, the role of cellular polarity is far less obvious, and it is generally taken for granted that cellular migration, proliferation, and matrix production/remodeling are the principal contributors to morphogenesis. Only in highly specialized cases, such as in the developing tooth dentin, is it obvious that cellular polarity is crucial for morphogenesis. However, mesenchymal cells, such as fibroblasts, chondrocytes and osteoblasts, are polarized in the sense that they have a major secretory surface defined by the position of their Golgi complex. They also have a preferred orientation of their long axis, and it has long been known that they all have a non-motile primary cilium with its basal body located close to the trans-Golgi network (Wilsman et al, 1980). Recent evidence from studies of embryonic tendon development indicate that the parallel arrangement of the collagen fibrillar scaffold in the direction of mechanical stress is determined by the polarized exocytosis of elongated packages of collagen fibrils formed within the Golgi region (Canty et al, 2004). Thus, in the developing tendon, the organized arrangement of collagen fibrils in the extracellular matrix appears to be associated with a cytoskeletal polarity, and it is tempting to hypothesize that a cellular polarity underlies the arrangement of collagen fibrils along mechanical stress lines also in other skeletal tissues, such as bone and cartilage.

The position of the primary cilium close to the trans-Golgi network makes it a good marker for the secretory polarity of mesenchymal cells. In addition, given recent evidence that the primary cilium is a mechanosensing device in epithelial cells (Praetorius and Spring, 2001; Nauli et al, 2003), it is possible that the primary cilium also may provide mesenchymal cells with mechanical information. In this review, we describe evidence suggesting that cellular polarity and/or primary cilia have essential roles in skeletal morphogenesis and growth and discuss preliminary results from experiments in which we have used conditional deletion strategies in mice to obtain support for such a role.

CELLULAR POLARITY IN LONG BONE GROWTH PLATES AND CHONDROCRANIAL SYNCHONDROSES

During endochondral ossification, mesenchymal cells condense at the sites of future bones, differentiate to chondrocytes and form avascular cartilage models of the future bones (Zelzer and Olsen, 2003). These cartilage models grow as a result of chondrocyte proliferation and production of extracellular matrix and assume shapes that are close to the shapes of the bones they give rise to. As development proceeds, chondrocytes in the center of the cartilage model cease to proliferate and differentiate to mature, hypertrophic chondrocytes; these cells express high levels of vascular endothelial growth factor (VEGF). VEGF induces sprouting angiogenesis in the perichondrium and this results in invasion of blood vessels, osteoclasts and osteoblastic progenitors into the hypertrophic cartilage (Zelzer et al, 2002). Erosion of the cartilage and apoptosis of the hypertrophic chondrocytes within this so-called primary ossification center allows bone marrow to be established and trabecular bone (primary spongiosa) to form. The cytokine Indian hedgehog (Ihh), expressed by the pre-hypertrophic chondrocytes, induces osteoblastic differentiation and the formation of a bone collar in the perichondrium around the mid portion of the cartilage, stimulates the adjacent non-hypertrophic chondrocytes to proliferate and controls the expression of parathyroid hormone-related peptide by the perichondrial cells and chondrocytes further away, closer to the developing joint at the end of the cartilage model (Kronenberg, 2003).

The continued process of chondrocyte proliferation, differentiation to hypertrophy and replacement by primary spongiosa leads to a progressive expansion of the primary ossification center, with the cartilage at each end serving as a growth center. In long bones, secondary ossification centers may form at the extreme end of the cartilage model, so that the growth center cartilage becomes a plate, the growth plate, between the primary and secondary ossification centers. Within such growth plates, proliferation and hypertrophy of chondrocytes occur primarily on the side of the primary ossification center, giving the growth plate a characteristic polarized structure. In contrast, at the base of the skull, the growth cartilages (synchondroses) are contributing more equally to the growth of the bones at each side of the cartilage and they have therefore a centro-symmetrical structure. Within the proliferative region of polarized growth plates or on each side of synchondroses at the base of the skull, the chondrocytes are elongated and tend to stack into columns oriented in parallel to the direction of longitudinal growth. In the case of long bone growth plates, it has been demonstrated that when

the cells in these stacks undergo mitosis, their mitotic spindles tend to be oriented perpendicularly to the direction of growth, so that the two daughter cells, following cytokinesis, are lying side-by-side transversely to the direction of growth (Dodds, 1930). The cells must therefore undergo a process that has been described as a 'rotation' before they reach their final position within the stacked columns. Conditional deletion of β1-integrin in mouse chondrocytes results in a defect in this postmitotic rotation, decreased proliferation, and consequently reduced longitudinal bone growth (Aszodi et al, 2003). However, the transverse orientation of the mitotic spindles does not appear to be affected. The basis for the precise orientation of the mitotic spindles in growth plate chondrocytes is unknown, but recent data linking polarity and positioning of the mitotic spindle in many different systems provide some important clues (Ahringer, 2003). By mechanisms that are conserved across a broad range of cells, aster microtubules of the mitotic spindle are anchored to defined regions within the polarized cortical protein envelope. Key components of this polarized cortex include (in epithelial cells) a trimeric complex of polarity proteins, PAR3, PAR6 and atypical PKC (aPKC), which localizes to tight junctions (Izumi et al, 1998; Joberty et al, 2000; Lin et al, 2000; Suzuki et al, 2001). PAR3 interacts with the adapter protein 14-3-3 and disruption of this interaction results in loss of cell polarity (Hurd et al, 2003).

Interestingly, there is evidence for a link between polarity control and formation of primary cilia. The PAR3/PAR6/aPKC complex interacts with another set of polarity proteins, including the protein CRB3, and CRB3, together with the PAR3/PAR6/aPKC proteins, is localized not only to the apical cortex and tight junctions but to primary cilia as well (Dougherty and Morrison, 2004). In neurons, PAR3 is transported to the tip of growing axons by kinesin II and this localization of PAR3 is necessary for neuronal polarity (Nishimura et al, 2004). The η isoform of 14-3-3 is also localized in cilia, and both 14-3-3η and CRB3 proteins have been shown to be necessary for ciliogenesis (Fan et al, 2004). This connection between formation of primary cilia and the molecular machinery of cellular polarity suggests that primary cilia may define the "apical" aspect of cells, even in cells that are not as clearly polarized as epithelial cells. Cells in skeletal tissues, including fibroblasts, chondrocytes, and osteoblasts, all fall into this category. As mentioned above, these cells are polarized in that they have a major secretory surface defined by the position of their Golgi complex and a preferred orientation of their long axis, but few or no tight junctions.

PRIMARY CILIA/INTRAFLAGELLAR TRANSPORT COMPLEXES ARE MEDIATORS OF MECHANICAL AND CHEMO-OSMOTIC SIGNALING

Primary cilia are organelles for mechanical and chemical sensing, and their functions are being recognized as essential for both organ development and functions in diverse organisms, from *Drosophila* and *C. elegans* to man (Pazour and Witman, 2003). Primary cilia are nonmotile (except for the motile nodal cilia in vertebrate embryos) and are usually present as single structures on most vertebrate cells (bone marrow-derived cells are major exceptions). They develop from the centriole, are disassembled during mitosis, and reassembled during interphase. They grow and are maintained by intraciliary/intraflagellar transport (IFT) of protein complexes (Rosenbaum and Witman, 2002). The anterograde movement towards the tip of the cilium is propelled by kinesin II (containing the motor subunits Kif3a and Kif3b); the retrograde motor is dynein.

Evidence that primary cilia can serve as mechanical sensors of extracellular fluid flow came from experiments with kidney epithelial cells in which it was demonstrated that bending of the cilium by fluid flow resulted in an increase in intracellular Ca^{++} (Praetorius and Spring, 2001); subsequent studies demonstrated that a Ca^{++} permeant channel complex, composed of the transmembrane proteins polycystin-1 (Pc1) and polycystin-2 (Pc2) and located at the base of the primary cilium, mediates the sensing of ciliary bending (Nauli et al, 2003). Inactivation of the genes encoding either Pc1 or Pc2 inactivates the channel. Based on these findings, primary cilia have been proposed as mechanosensors in osteocytes and chondrocytes (Poole et al, 1997; Whitfield, 2003). There is currently no direct evidence to support this hypothesis, but it is interesting to note that aggrecan synthesis in cartilage explants under cyclic loading is stimulated in regions of high interstitial fluid flow (Buschmann et al, 1999). Mutations in the genes (*PKD1* and *PKD2*) encoding either Pc1 or Pc2 cause autosomal dominant polycystic kidney disease in humans (Consortium, 1995; Hughes et al, 1995; Mochizuki et al, 1996). Polycystic kidneys are also the result of mutations in two other ciliary proteins, polaris and cystin. An insertional mutation in the polaris gene in the Oak Ridge polycystic kidney (*orpk*) mutant mouse causes a syndrome with cysts forming in liver, pancreas, and kidney (Moyer et al, 1994). The polaris protein is concentrated in ciliary basal bodies and within cilia (Taulman et al, 2001). Polaris is essential for the assembly of primary cilia since kidney cells with the orpk mutation do not develop normal cilia (Pazour et al, 2000). The connection between polycystic kidney disease and lack of ciliary function is further highlighted by

the postnatal development of polycystic kidney disease in mice with kidney-specific lack of cilia as the result of inactivation of *Kif3a* (Lin et al, 2003).

PRIMARY CILIA/IFT COMPLEXES ARE ESSENTIAL COMPONENTS OF MORPHOGENETIC SIGNALING PATHWAYS FOR SKELETAL DEVELOPMENT

Available evidence suggests a link between skeletal patterning and growth and proteins associated with ciliary assembly and/or function. First, mutations in *polaris* in mice cause craniofacial defects, supernumerary teeth, and abnormalities in fore- and hindlimbs, in addition to abnormalities in left-right asymmetry and polycystic kidney disease (Zhang et al, 2003). In the *polaris* hypomorph *orpk*, digit I is duplicated and mice with *polaris* null alleles die before birth with eight digits per limb. Second, seven of the eight different genes known to cause the pleiotropic disorder Bardet-Biedl syndrome (BBS) in humans code for proteins that are associated with cilia (Ansley et al, 2003; Mykytyn and Sheffield, 2004). In BBS, skeletal abnormalities include syndactyly and brachydactyly with or without polydactyly. Interestingly, with the exception of BBS6, the homologues of the Bardet-Biedl syndrome genes in *C. elegans* are expressed exclusively in the 60 ciliated neurons (out of 302 total neurons) and all contain regulatory elements for RFX - a transcription factor that modulates expression of genes associated with ciliogenesis and intraflagellar transport (Ansley et al, 2003).

On the basis of this evidence, we decided to knock out the genes encoding Kif3a and Pc1 in skeletal tissues. Homozygous *Kif3a* null mice die at midgestation with multiple abnormalities, the most dramatic of which is randomization of left-right asymmetry. The primary cilia on nodal cells are either completely absent or very short and the unidirectional "nodal flow" seen in wild-type embryos is abolished (Marszalek et al, 1999). To allow studies of the role(s) of Kif3a in skeletal development, we generated conditional knock-outs by crossing mice with floxed *Kif3a* alleles with the *Dermo1-cre* or the *Prx1-cre* deleter strains. Dermo1, a basic helix-loop-helix transcription factor, is expressed in mesenchymal condensations of the axial and appendicular skeleton, the mesodermal bones of the skull and cells at the osteogenic fronts in cranial sutures (Li et al, 1995). Prx1 (previously called Mhox) is a homeodomain transcription factor expressed in mesenchymal cells; the levels of expression are particularly high in early limb bud mesenchyme (Kuratani et al, 1994; Logan et al, 2002). *Kif3a/Dermo1-cre* mutant mice are born in a Mendelian ratio, but die shortly after birth, most likely due to respiratory failure, with abnormalities in the axial skeleton, limbs and skull. Visual inspection of the newborn mutants reveals that all four limbs are considerably shorter than in wild-type pups and have extra digits (Fig 1).

Analysis of alizarin red/alcian blue stained skeletal preparations of newborn mutant animals reveals preaxial polydactyly with duplication of digit I in both forelimbs and hindlimbs, accompanied by shortening and bending of the long bones. Generally, the bones of the hindlimb are more severely affected than those in the forelimb. Histological analysis demonstrates a defect in the formation of long bone growth plates. The typical columnar arrangement of stacked chondrocytes in the proliferating zone appears to be completely disrupted in the mutant growth plates. As can be seen in Fig 2, the mutant chondrocytes appear randomly distributed instead of being neatly stacked as in wild-type tissues. By *in situ* hybridization with probes for collagen II and collagen X, markers for proliferating and prehypertrophic/hypertrophic chondrocytes, respectively, the growth plates at each side of the primary ossification center can be readily identified in the wild-type tibia at stage E18.5, with a well-defined zone of collagen X expression flanking the ossification center (Fig 3). In contrast, the mutant tibia contains a single circular zone of hypertrophic chondrocytes in the center surrounded by cells expressing collagen II (Fig 3). Thus, it appears that Kif3a plays a role in the formation of the primary ossification center and establishment of two separate growth plates. In addition to polydactyly and long bone abnormalities, the mutant mice have striking sternal

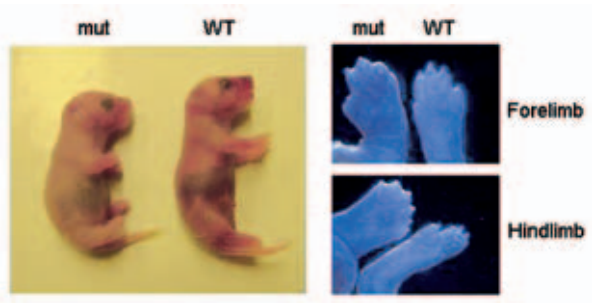

Fig 1 Left: Newborn *Kif3a/Dermo1-cre* mutant (mut) and wild-type (WT) littermate. Right: Preaxial polydactyly in newborn *Kif3a/Dermo1-cre* mouse.

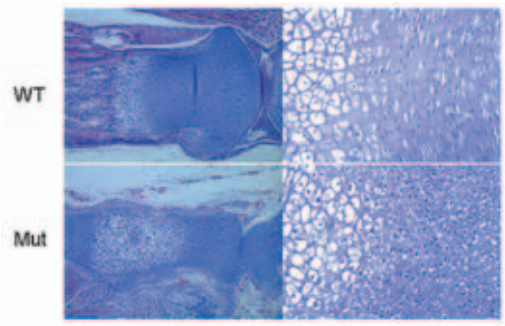

Fig 2 Left: Hematoxylin/eosin-stained proximal tibia of newborn *Kif3a/Dermo1-cre* mutant (Mut) and wild-type (WT) littermate. Right: High magnification image of proximal tibial growth plates.

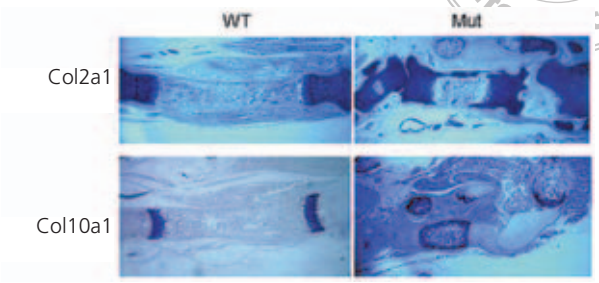

Fig 3 *In situ* hybridization with type II (*Col2a1*) and type X (*Col10a1*) collagen-specific probes of E18.5 wild-type (WT) and Kif3a deficient (Mut) tibia.

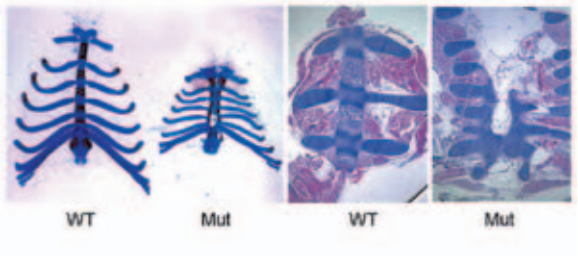

Fig 4 Left: Skeletal preparations of ribs and sternum of newborn wild-type (WT) and *Kif3a/Dermo1-cre* mutant (Mut) animals. Right: Hematoxylin/eosin-stained sections of sternum and sternocostal cartilage of newborn wild-type (WT) and Kif3a mutant (Mut) mice.

defects ranging from mildly separated caudal sternebrae to complete separation of the two sternal bands (Fig 4). During normal sternal development, two separate sternal anlagen fuse at the body midline, but the fusion is delayed and incomplete in the mutant. These early defects result in lack of normal growth plates, fusion of the sternebrae and subsequent shortening of the sternum.

In an attempt to circumvent the neonatal lethality of *Kif3a/Dermo1-cre* mutant mice, we inactivated *Kif3a* in a more restricted manner using *Prx1-cre* deleter mice; when *Prx1-cre* mice are crossed with R26R reporter mice, β-galactosidase expression is detected in limb buds, lateral plate mesoderm and dermal structures in the head region of E 11.5 embryos (Logan et al, 2002). *Kif3a/Prx1-cre* mice are born alive but have short limbs. Skeletal preparations reveal dramatic shortening of long bones in both hind- and forelimbs and a severe form of polydactyly in the forelimbs. In contrast to the polydactyly in *Kif3a/Dermo1-cre* mice, in which digit I is duplicated while the morphology of the other digits is preserved, the identity of all digits in the *Kif3a/Prx1-cre* mutants appears to be lost. As the autopods grow, bifurcation of all the digits becomes apparent. Long bone growth plates have defects that are similar to those of *Kif3a/Dermo1-cre* mutant mice.

Kif3a/Prx1-cre mice also display asymmetric pairing of the ribs, a lack of growth plates in the sternal segments and fusion of sternebrae. Other than abnormally positioned (staggered) sternocostal junctions, the ribs appear to be of normal size and morphology. This is consistent with ribs being derived from somitic mesoderm (not expressing *Prx1-cre*) and the sternum originating from lateral plate mesoderm for which *Prx1* expression has been documented. Despite severely shortened limbs, the mutant pups survive beyond weaning and feed normally. However, the overall size tends to be reduced in comparison to the control littermates.

The appendicular and sternal defects in the conditional *Kif3a* mutants are similar to what is seen following mutations in hedgehog signaling pathways, suggesting that *Kif3a* is critical for hedgehog signaling. Several types of additional experimental results support this conclusion. First, in a screen for embryonic patterning mutations induced by ethylnitrosourea, Huangfu et al (2003) identified two mouse mutants, *wim* and *fxo*, exhibiting abnormalities in the dorso-ventral patterning of the neural tube as well as other phenotypes that are characteristic of defects in sonic hedgehog (Shh) signal-

ing (abnormal brain morphology and preaxial polydactyly). The *fxo* mutant represents a hypomorphic *polaris* allele; *wim* is a mutant allele of another IFT protein that is part of a complex with polaris. Wim embryos show, like *polaris* mutants, defects in the asymmetric expression of nodal markers, randomization of left-right asymmetry and down-regulated expression of a Shh target gene in several tissues. Further experiments suggested that wild-type Wim, polaris, and Kif3a may act at the level of the hedgehog signaling component Gli3 (Huangfu et al, 2003) and Liu et al (2005) recently demonstrated that IFT proteins regulate both activator and repressor functions of Gli transcription factors. Second, mutations that affect hedgehog pathways are known to cause several types of polydactyly in humans (pre- and postaxial polydactyly, Greig cephalopolysyndactyly, Pallister-Hall syndrome) and mice (extra-toes, polydactyly Nagoya) (Reginato et al, 2001). Also, mutations in the Shh homologue, Ihh, cause human brachydactyly A1 (Gao et al, 2001). A link between Kif3a and Shh signaling during development is also suggested by the similarity between the sternal/rib phenotype in the *Kif3a/Dermo1-cre* mice and the split sternal phenotype described for extra-toes mutants (Mo et al, 1997), now known to be caused by a mutation in Gli3 (Vortkamp et al, 1992). In addition to skeletal defects, mutant *Kif3a/Prx1-cre* mice lack hair over all limbs, in the skin over the sternum and in a spot over the forehead. These hairless areas correspond to regions in which *Prx1* is expressed in the mesenchymal cells that give rise to the dermal component of the skin. It is well established that Shh, expressed in the basal cells of the epidermis, controls keratinocyte proliferation and the proliferation and downgrowth of hair follicle cells (Chiang et al, 1999; St-Jacques et al, 1998). Mature hair follicles do not develop in Shh knock-out mice because of impaired proliferation and expansion of the mesenchymal cells of the dermal papillae.

Although the phenotypic characteristics of *polaris* mutants and the *Kif3a/Dermo1-cre* and *Kif3a/Prx1-cre* mutant mice are consistent with an essential role for polaris and Kif3a in skeletal patterning and growth mediated by Shh signaling, expression analyses suggest that their absence does not significantly affect the levels of expression of downstream Shh target genes. For example, analysis of *polaris* hypomorphic limb buds indicated no changes in the expression of *Shh, Ihh, HOXD11-13, Patched, BMPs* or *Glis* (Zhang et al, 2003). Also, in *polaris* null mice or in our conditional *Kif3a* mutants there are no alterations in Shh expression, and we have seen good expression levels of *Patched* and *Gli1* in the *Kif3a* mutant limbs. The defects in these limbs are morphogenetic defects, characterized by expression of the right genes in the wrong locations.

CONDITIONAL INACTIVATION OF *Pc1* AND *KiF3a* IN CRANIOFACIAL SKELETAL PROGENITORS CAUSES ABNORMALITIES IN CRANIAL SUTURES/SYNCHONDROSES AND DEFICIENT SKULL GROWTH

To generate mice with deficiency of Pc1 and Kif3a in craniofacial skeletal elements we crossed floxed *Pc1* (generated in the laboratory of Dr. Zhou) and *Kif3a* mice with the *Wnt1-cre* deleter strain. As expected, the appendicular skeleton shows no abnormalities in the *Pc1/Wnt1-cre* mutants, but the mice show a slightly decreased ossification of the frontal bone at birth and the interfrontal suture is wide, the anterior fontanel is large and the interparietal suture is wider than normal. However, the overall shape of the skull is normal at birth, but this changes rapidly after birth. Within the first two weeks, the snout of mutant animals becomes short and broad relative to wild-type controls. The defects within the frontonasal process include a relative shortening of the nasal and premaxillary bones, resulting in pronounced Type III malocclusion. Moreover, close examination of the affected region shows a striking lack of interdigitation of the frontonasal and frontomaxillary suture as the skull grows. The outgrowth of the nasal, frontal and premaxillary bones is required for postnatal elongation of the craniofacial complex. This process is coupled to the formation of a complex pattern of interdigitation of the frontonasal and frontal-premaxillary sutures. Impaired growth at the frontonasal suture and/or inappropriate (premature) differentiation of osteogenic mesenchymal cells may therefore contribute to the snout truncation in the conditional *Pc1* knock-out mice. It has been reported that increased masticatory function promotes growth of the nasal bones (Tokimasa et al, 2000) and mechanical force induces osteogenic proliferation within the frontonasal suture (Kopher and Mao, 2003). Intriguingly, deletion of the AP-2α transcription factor using either *Wnt1-* or *AP2α-cre* lines results in a similar phenotype characterized by reduced postnatal growth and lack of interdigitation within the frontonasal suture (Brewer et al, 2004; Nelson and Williams, 2004). Therefore, we speculate that the AP-2α transcription factor, known to be expressed in the facial prominences (Schorle et al, 1996; Zhang et al, 1996), is a downstream target of a Pc1-mediated signaling pathway or regulates the expression of a critical component of this pathway.

The major contribution to the snout truncation in the *Pc1/Wnt1-cre* mutants after birth comes from an early postnatal (about day 10) ossification and complete closure of the presphenoid synchondrosis (Fig 5). This results in a severe shortening of the sphenoid. An abnormal width and defective cell proliferation can be

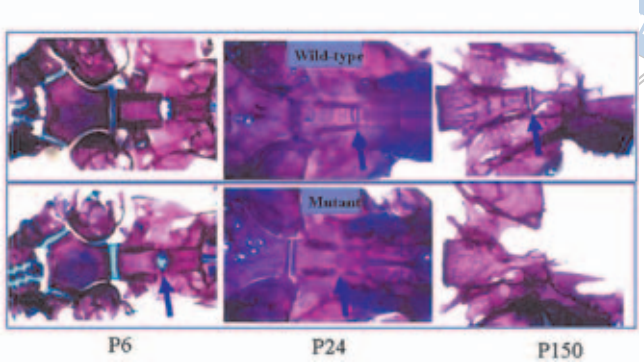

Fig 5 Early postnatal synostosis of the presphenoid synchondrosis in the *Pc1/Wnt1-cre* mutant skull base. In the wild-type control, this synchondrosis is not ossified even at postnatal day 150 (P150, see arrow). In contrast, in the mutant skull base, the synchondrosis can only be seen at postnatal day 6 (P6, see arrow); at postnatal day 24 (P24) it is completely ossified.

observed in the synchondrosis already at postnatal day 5. In contrast, the spheno-occipital synchondrosis is almost completely unaffected, showing only a subtle and transient defect in cell proliferation at an early stage. This is not surprising, since most of the cells contributing to this synchondrosis are derived from mesoderm, and a small contribution from neural crest-derived cells is seen only laterally and appears to disappear shortly after birth. However, consistent with what is known about the cellular origins of the different parts of the skull base, the spheno-occipital synchondrosis is affected in *Pc1/Dermo1-cre* mutants; in these mutants, the presphenoid synchondrosis is normal. The defect is striking in that the midline portion of the spheno-occipital synchondrosis is completely absent, forming a fenestra, at postnatal day 5, but fills in with bone. The lateral parts appear normal, but the growth of the anterior portion of the basioccipital bone is nevertheless stunted because of the early ossification of the midline fenestra. Interestingly, the mesoderm-derived synchondroses between the basioccipital and the exoccipital bones appear completely unaffected in *Pc1/Dermo1-cre* mutants, suggesting that Pc1 is not required for chondrogenesis per se. The defects seen in the presphenoid and spheno-occipital synchondroses in the *Pc1/Wnt1-cre* and *Pc1/Dermo1-cre* mutant mice, respectively, may therefore be the consequence of specific functional demands (mechanical stress?) that require Pc1 signaling at these growth centers.

Consistent with the conclusion, discussed above, that IFT complexes and associated motor proteins, including Kif3a, are essential for hedgehog-mediated morphogenetic processes, *Kif3a/Wnt1-cre* mutants exhibit lack of anterior facial structures and anterior skull base. These defects are almost identical to the defects reported for mice with *Wnt1-cre*-mediated inactivation of the gene encoding the obligatory hedgehog signaling molecule Smoothened (Jeong et al, 2004).

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