

## Chapter 19

# Craniofacial Development and Growth in Polycystic Kidney Disease

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## Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder characterized by the presence of multiple cysts in kidneys. ADPKD has been shown to be caused by mutations in the genes of PKD1 and PKD2, encoding polycystin-1 (PC1) and polycystin-2 (PC2), respectively. Polycystins are localized in primary cilia that play roles in multiple biological processes including mechanoreception, Ca<sup>2+</sup> influx and cell signalling

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pathways. Primary cilia are known to play important roles in regulating craniofacial development and growth. In this chapter, we summarize the function of Pkd1 and Pkd2 in controlling mouse craniofacial development and growth, and discuss PKD-associated molecular mechanisms.

**Key words:** Craniofacial; Development; Postnatal growth; Sutures

## Introduction

The vertebrate head is the most complex structure of a body, containing diverse organs such as the brain, bone, eye, ear, nose, tongue and teeth. Head development and growth require the co-ordination of multiple processes in time and space and many signaling pathways such as fibroblast growth factor (FGF), sonic hedgehog (Shh), bone morphogenetic protein (Bmp) and Wnt are known to play critical roles in these processes. Other processes such as mechanical force can also affect craniofacial development and growth. Mutations in the *PKD1* (encodes Polycystin-1/PC1) and *PKD2* (encodes Polycystin-2/PC2) genes have been shown to account for autosomal dominant polycystic kidney disease (ADPKD), which is characterized by the presence of renal and extra-renal cysts, cardiovascular abnormalities including hypertension and intracranial aneurysms. In this chapter, we discuss recent evidence that identifies an intriguing link between polycystic kidney disease and head development and growth.

## The mammalian head

The head is derived from all embryonic germ layers: ectoderm, mesoderm, endoderm and neural crest-derived ectomesenchyme. Neural crest cells undergo epithelial-mesenchymal transition, migrate to different locations, and differentiate into multiple cell types in the embryo (1,2).

The human skull consists of twenty-two bones, eight bones of the neurocranium that surround the brain and brainstem, and fourteen bones of the viscerocranium that supports the face (3, 4). Mesenchymal cells forming jaw and facial bones are derived from neural crest cells. The frontal bones, the medial part of the interparietal bone, and tooth-supporting tissue, alveolar bone and periodontal ligament tissue are also of neural crest origin. The parietal and the lateral parts of the interparietal bones are of mesodermal origin in mice (5, 6).

Cranial bones abut at sutures that comprise a fibrous tissue between the bones. The skull mostly enlarges along the suture margins by bone deposition. Slight movement is permitted at sutures, when the suture is still open, which is important for skull development and growth. Abnormalities in ossification in these sutures lead to craniofacial deformities (4, 7). Sutures are not just vital for the skull, but also brain development and growth, that should accommodate the growing brain (3, 4, 7).

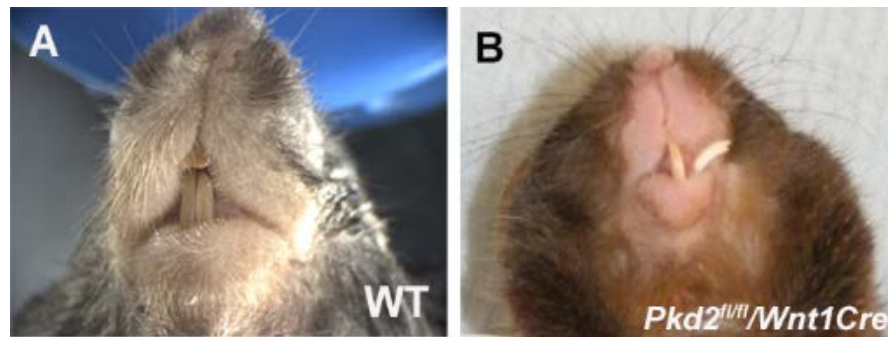
The cranial base is also an important growth center of the craniofacial skeleton where growth is controlled by synchondroses that involve the formation of either hyaline cartilage or fibro-cartilage between craniobase bones (1, 4). A synchondrosis usually temporary exists during the growth phase, and is ultimately converted into bone. Cranial base synchondroses are termed ethmoidal, intrasphenoidal, sphenoccipital or intraoccipital according to their anatomical location. Abnormalities in cranial base synchondroses result in craniofacial deformities (1, 4). In mice, both the presphenoid and phenooccipital synchondroses remain patent in adulthood (8, 9).

#### **Conditional deletion of *Pkd1/Pkd2* in mice**

Gene targeting in mice (knockout mice) is a powerful tool to determine the function of genes during development and growth. It is however difficult to investigate the relationship between *Pkd1* or *Pkd2*, and head development or growth using global *Pkd1* or *Pkd2* null mutant mice, since homozygosity for the *Pkd1* or *Pkd2* null alleles leads to early embryonic lethality between embryonic day (E) 13.5 and parturition (10, 11).

The Cre/loxP system is a commonly used tool to alter the mouse genome in a conditional manner (site- and time-specific) by deletion of loxP-flanked DNA segments. Cranial neural crest cell-specific gene targeting has been achieved by *Wnt1*-driven Cre recombinase to investigate the molecular mechanisms in craniofacial development (6, 12, 13). *Dermo1Cre* mice have been used for gene targeting skeletal tissues (14, 15, 16). *OsxCre* and *Col2a1Cre* ER\* strains can be used for deletion of the genes from osteoblasts and chondrocytes, respectively.

Mice with conditional *Pkd2* inactivation using *Wnt1Cre* (*Pkd2<sup>fl/fl</sup>;Wnt1Cre*) survive until adulthood and show a shortened snout, malocclusion, a dome-shaped skull vault and curved spine (Figure. 1) (17). Mice with conditional deletion of *Pkd1* in neural crest-derived cells (*Pkd1<sup>fl/fl</sup>;Wnt1Cre*) show similar craniofacial phenotypes to those seen in *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice (15, 18). Since no significant morphological craniofacial changes can be observed either in *Pkd1<sup>fl/fl</sup>;Wnt1Cre* or *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice at birth, it is likely that the anomalies in mutant heads occur after birth.



**Figure 1.** Adult heads of wild type and *Pkd2* conditional knockout mice showing abnormal incisor formation/occlusion. A, wild-type; B, *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice.

### Mechanical stress and *Pkd1/Pkd2* in mastication

Mastication produces strong and frequent mechanical stresses that are perceived by teeth, the periodontal ligament and the temporomandibular joints. *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice show fractures and dilacerations of teeth, alveolar bone loss and increased width of the periodontal ligament tissue. The temporomandibular joints are also compressed in *Pkd2* mutants (17). Although low levels of calcification of dentin or alveolar bone could not be detected in mutants, these results still suggested that abnormally strong occlusal forces were occurring in *Pkd2* mutants.

Mastication, including occlusion and jaw movements, is coordinated by the interaction between teeth, periodontal ligament, skin, muscle, mucosa and the temporomandibular joints. The strength of the occlusal force is known to be controlled by the central nervous system and feedback of the mechanical stress from many tissues such as periodontal tissues. Mechanical sensation can evoke the excitation or inhibition of various oral reflexes to facilitate mastication by the controlling activity of jaw-closing muscles. Inhibitory reflexes are known to be the predominant response to avoid the excess forces that would destroy tissues. Perception in the periodontal ligament has been shown to play a critical role in providing a substantial part of the information of mechanical sensation. The temporomandibular joints are also believed to control mastication through perception of the occlusal force (19). *Pkd2* is deleted only in neural crest-derived cells in *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice. Neural crest cells differentiate into periodontal ligament and the temporomandibular joints, but not into the central nervous system or muscle, suggesting that abnormal occlusion is caused by abnormal perception in the periodontal ligament and/or the temporomandibular joints due to *Pkd2* mutation.

Mechanical stimuli such as occlusal force are believed to be detected by mechanoreceptors. Primary cilia are non-motile microtubule-based organelles that are found on most cells. Primary cilia are known to sense fluid flow in many tissues including the lumen of renal tubules and blood vessels (20). Both PC1 and PC2 are localized at the plasma membrane of the primary cilia, and PC1 has been shown to function with PC2 to act as a mechanoreceptor in primary cilia in kidneys and blood vessels (21).

Primary cilia are found in cells of periodontal ligament tissues and the temporomandibular joints. The bending of primary cilia in the periodontal ligament and temporomandibular joint cells may regulate a feedback of mechanical stresses to the central nervous system to control further occlusal force. It is possible that the changes by mutation of either *Pkd1* or *Pkd2* are related to the loss of function of primary cilia as mechanocensors in periodontal ligament and/or the temporomandibular joints, which induce abnormal occlusal force.

### **Mechanical force and head morphology**

Bone is constantly remodeled by the coordinated action between bone-resorbing osteoclasts and bone-forming osteoblasts throughout life. This continuous remodeling serves to prevent and remove fatigue-related micro-damage and allows adaptation of the bone mass and structure. The balance between the amount of bone resorption and bone formation is determined by many factors including mechanical forces. Exercise results in enhanced bone formation, whereas bone loss is observed in the absence of mechanical stimulation including immobilization, disuse and exposure to low gravity. Increased mechanical stress thus stimulates bone formation.

The fibrous joints in cranial bones and the cranial base play a critical role in enlargement of the skull vault, and premature fusion by abnormal ossification in one or more cranial sutures leads to an abnormal head shape such as a dome-shaped skull. Abnormal fusion of front-maxillary and front-nasal sutures are observed in *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice. Moreover *Pkd1<sup>fl/fl</sup>;Wnt1Cre* and *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice show abnormal anterior synchondrosis in pre-sphenoid and intra-sphenoid (17, 18). Loading *in vivo* and *ex vivo* skull models results in abnormal closure of sutures (22). Mechanical stresses caused by mastication and jaw movement are known to affect craniofacial morphogenesis during postnatal growth (23, 24, 25, 26). These studies suggest that abnormal mechanical force by traumatic occlusion can alter the balance between bone resorption and bone formation in sutures in *Pkd1* and *Pkd2* mutants, resulting in premature fusion and subsequently morphological skull abnormalities including a dome-shaped skull and anterior-posterior compression of the snout.

### The relationship between *Pkd1/Pkd2* and mechanical force on bone formation

The neural crest contributes to anterior cranial base sutures including the pre-sphenoid, whereas posterior cranial base sutures including phenoooccipital synchondrosis are formed by mesodermal cells. *Pkd1<sup>fl/fl</sup>;Wnt1Cre* and *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice showed only abnormal anterior synchondrosis in pre-sphenoid and intra-sphenoid, while *Pkd1* conditional deletion using Cre recombinase regulated by the promoter of the mesoderm-specific *Dermo1* showed the formation of a ossified bridge in the phenoooccipital synchondrosis. Mesoderm conditional deletion of *Pkd1* resulted in a delay in ossification of the axial and appendicular skeleton. *Pkd1* and *Pkd2* thus play roles in both membranous (neural crest) and enchondral (mesoderm) ossification.

Mechanical stress has been shown to regulate the differentiation and proliferation of chondrocytes and osteoprogenitor cells (27, 28, 29). *Pkd1* is expressed in prechondrocytes, osteoblasts and osteocytes (30, 31). In order to clarify the interaction between *Pkd1* and mechanical force on ossification in sutures, midpalatal suture expansion has been studied as a mechanical force (32) and shown to lead to new bone formation. Osteogenic response to tensile stress is significantly reduced due to reduced proliferation, delayed differentiation and increased apoptosis of osteochondroprogenitor cells in *Pkd1* mutants. Cellular responses have been shown to vary dependent on the magnitude of mechanical force, and its frequency or duration (33, 34, 35). In addition, the stage of cellular differentiation is also likely to be involved in cellular responses to mechanical force (32). At a physiological level of mechanical stress, *Pkd1* negatively regulates the differentiation of osteoprogenitor cells to osteoblasts, whereas it accelerates differentiation to matured osteoblasts. *Pkd1* also induces matrix production by mature osteoblasts. *Pkd1/Pkd2* mutations thus directly alter the response of osteoprogenitor cells to mechanical force.

The back-and-forth movement of extracellular fluid in the bone microenvironment during exercise has been shown to bend, deform or stretch the primary cilia of osteocytes (36). Abnormal function of primary cilia due to *Pkd1/Pkd2* mutation in osteogenic cells and/or abnormal bending of the primary cilia due to traumatic occlusal force may affect bone formation in mutant skulls.

Mechanical stress also alters cell shape and cellular structures that change the influx and efflux of ions. Calcium ions play critical roles in many biological processes including exerting allosteric regulatory effects on many enzymes and proteins, and acting as a signal transducer. Conformational changes in the polycystin complex have been shown to result in  $\text{Ca}^{2+}$  entry (37). *Pkd1* and *Pkd2* mutations are known to lead to

abnormal  $\text{Ca}^{2+}$  influx (20, 37, 38). Interestingly, intracellular calcium concentration is elevated in periodontal ligament cells when they receive hydraulic pressure (39). Aberrant occlusal force is likely to change  $\text{Ca}^{2+}$  influx, which may affect bone formation in the skull.

Extracellular domains of PC1 and PC2 have been shown to play a role in cell-cell and/or cell-extracellular matrix interaction by formation of multiprotein complexes with integrins and other focal adhesion proteins including paxillin, talin, tensin, focal adhesion kinase and c-Src (20, 40, 41). Focal adhesion involving integrins is known to play a role in head development (42, 43). These complexes are often clusters of large macromolecular assemblies that transmit mechanical force and may be affected by *Pkd1*/*Pkd2* mutations.

#### **Mechanical stress-independent *Pkd1*/*Pkd2* function**

Although newborn heads show no significant changes in *Pkd1*/*Pkd2* mutants, intramembranous ossification of the skull is slightly delayed in *Pkd1<sup>fl/fl</sup>;Wnt1Cre* at birth (17, 18). Small excess bone deposition is also observed along the sutures of the snout in newborn *Pkd2<sup>fl/fl</sup>;Wnt1Cre* mice. Mesoderm conditional deletion of *Pkd1* also leads to enlarged cranial base fenestrae.

Bone-like structures are observed in *Pkd2* mutant dental pulp that is isolated from any obvious external force (17). Reparative dentin is formed in the pulp underneath dental caries or thin dentin as a reaction to external stimuli affecting odontoblasts. Odontoblasts are tall columnar cells with a polarized distribution of their cytoplasmic organelles. Reparative dentin often has a bone-like structure and is formed by odontoblasts that lose their polarity. Cells with no columnar shape are found to surround bone-like structures observed in *Pkd2* mutant dental pulp, although there is no sign of dental caries or thin dentin. *Runx2* is upregulated in *Pkd2* mutant dental pulps and mice with overexpression of *Runx2* also show bone-like structures in dental pulp (17, 44). Upregulation of *Runx2* is also observed in the nasal cartilage of *Pkd1* mutants, which shows abnormal ossification. These results suggest that *Pkd2* mutation leads to abnormal differentiation or function of odontoblasts. *Pkd1* and *Pkd2* may thus possess mechanical force-independent functions at embryonic stages, during growth, and into adulthood. Knockdown of the primary cilia protein, Polaris, resulted in abrogation of *Runx2* (45) and since primary cilia are also present in the dental pulp, it is possible that the mechanical force-independent function of *Pkd1* and *Pkd2* is related to primary ciliary function.

### Craniofacial characteristics of ADPKD patients

Experimental analysis of the roles of Pdk1 and Pdk2 in craniofacial development and growth in mice suggests that mutations of these genes in humans that give rise to kidney abnormalities, may also affect craniofacial characteristics. Rapid head growth with a bulging anterior fontanelle and sutural separation has been reported in a patient with ADPKD due to *PKD1* mutation (46). Three-dimensional photography combined with dense surface modelling was performed on ADPKD patients to analyze face morphology. Dense surface shape analysis showed individuals with ADPKD to have vertical lengthening of the face, predominantly in the upper third, and mild mid-facial hypoplasia. Linear measures derived from 3D facial landmarks showed individuals with ADPKD to have a moderate lengthening of the nose confirmed by an increase in the ratio of nasion-pronasale:nasion-subnasale (17). Although further studies are needed, it is possible that faces of ADPKD patients show subtle characteristic facial features. Since *Pkd2* mutant mice data show the possibilities of excess occlusal force, craniofacial structures may perceive supraphysiological mechanical stress in ADPKD patients.

The anatomy of the human face is different from mice and includes the presence of maxillary sinuses for example. These different structures might modify the distribution of constraints on the face. It is likely that the distribution of mechanical-related anomalies is different between humans and mouse mutants (47). These may cause different facial phenotypes between mutant mice and patients. On the other hand, in common with mutant mice, a shift of the mid-face is observed in patients (17). That is consistent with the fact that the mid-face is a susceptible region for mechanical stress during growth (22).

### Conclusion and perspective

Emerging evidence suggests that *Pkd1/Pkd2* mutations are responsible not only for kidney disease, but also for craniofacial anomalies in mice and specific facial characteristics in humans. Wnt and Shh signaling pathways are known to regulate craniofacial development and since primary cilia are involved in activation of both pathways, the function of *Pkd1/Pkd2* in craniofacial development and growth may be related to Wnt and Shh pathways (48, 49). Further studies are required to clear the precise role of *PKD1* and *PKD2* in craniofacial development and growth.

### Conflict of interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.



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