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Development of craniofacial structures in transgenic mice with constitutively active PTH/PTHrP receptor

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Abstract

Parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP) regulate calcium homeostasis, and PTHrP further regulates growth and development. A transgenic mouse carrying the constitutively active PTH/PTHrP receptor (HKrk-H223R) under the control of the mouse bone and odontoblast-specific α1(I) collagen promoter (Col1-caPPR) has been developed to demonstrate the complex actions of this mutant receptor in hard tissue formation. We have further characterized Col1-caPPR mice abnormalities in the craniofacial region as a function of development. Col1-caPPR mice exhibited a delay in embryonic bone formation, followed by expansion of a number of craniofacial bones including the maxilla and mandible, delay in tooth eruption and teratosis, and a disrupted temporomandibular joint (TMJ). These findings suggest that the Col1-caPPR mouse is a useful model for characterization of the downstream effects of the constitutively active receptor during development and growth, and as a model for development of treatments of human diseases with similar characteristics.
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Introduction

Bone formation occurs primarily through two mechanisms: endochondral and intramembranous ossification. In both cases, mesenchymal cells form condensations that prefigure the shape of, and the location of, future skeletal elements. Beyond this patterning event, in most cases, the cells of the mesenchymal condensations differentiate into chondrocytes that form the template, or anlagen, of the future bone. Through the process of endochondral ossification, this cartilaginous template is eventually replaced by bone. In other cases, cells of the mesenchymal condensations differentiate directly into osteoblasts. This pattern of intramembranous ossification occurs in a few skeletal elements, such as the lateral halves of the clavicles, parts of the skull and the periosteal collar of long bones [1].

Many signaling systems control bone formation. The parathyroid hormone/parathyroid hormone-related peptide receptor (PTH/PTHrP-R) integrates signals from both the calcium regulating hormone, parathyroid hormone (PTH) and the paracrine factor, parathyroid hormone-related peptide (PTHrP) [2]. PTH is synthesized by the parathyroid glands and acts on kidney and bone to regulate calcium homeostasis [3–5]. PTH stimulates multiple intracellular signals that include cyclic adenosine monophosphate (cAMP) [6–9], inositol phosphate, and calcium [10–15], and activates both protein kinase A [16] and C [17]. PTHrP, in contrast, is synthesized in multiple tissues with specific spatial and temporal profiles and has largely paracrine functions [4,18–21]. PTHrP has partial homology to PTH at the NH_2 terminus [22].

The PTH/PTHrP receptor binds to and responds equally to the NH_2-terminal fragments of PTH and PTHrP [3,4]. The PTH/

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PTHrP receptor is a G-protein-coupled receptor containing seven predicted transmembrane domains. Binding of ligand to the PTH/PTHrP receptor stimulates cAMP production, raises intracellular calcium, and increases levels of inositol 1,4,5-trisphosphate [4]. The PTH/PTHrP receptor mRNA is expressed in the PTH target organs, kidney, bone and mesenchymal components of teeth [23,24], as well as in organs in which PTHrP is expressed, such as extra embryonic membranes and the growth plates of bone [25,26].

Studies have demonstrated unambiguously the critical role that PTHrP plays in regulating hypertrophic chondrocyte differentiation [27]. The major phenotypic abnormality in PTHrP-deficient mice was dwarfism caused by premature differentiation of non-hypertrophic chondrocytes into hypertrophic chondrocytes. Metatarsal bones cultured in vitro in the presence of PTHrP show delayed differentiation of the hypertrophic chondrocytes [28]. Humans and mice harboring an activating mutation in the PTH/PTHrP receptor exhibit a delay in hypertrophic chondrocyte differentiation similar to what was seen in bone explants exposed to excess PTHrP [29,30]. Subsequently, gain-of-function and loss-of-function experiments in chickens and mice demonstrated that perichondrial cells and early chondrocytes express PTHrP. Expression of PTHrP is induced by Indian hedgehog (IHH), which is produced by prehypertrophic and hypertrophic chondrocytes. Ihh and PTHrP form a negative feedback loop, with PTHrP maintaining chondrocytes in a proliferative, less differentiated state. As they are displaced from the proliferative zone and begin to mature, they initiate Ihh expression, thereby further stimulating chondrocyte proliferation, and refueling the growth plate [31,32]. In further analysis, Ihh was found also to regulate the differentiation of mesenchymal cells in the bone collar into osteoblastic progenitors, and that the distance between Ihh expressing cells and PTH/PTHrP receptor expressing cells controlled the site of chondrocyte hypertrophy as well as the location of formation of the bone collar [33].

Jansen-type metaphyseal chondrodysplasia (JMC) is a rare form of short-limbed dwarfism. Genomic DNA of a patient with JMC was found to have a heterozygous His to Arg mutation at position 223 (H223R), which is found at the junction between the PTH/PTHrP receptor's first intracellular loop and second membrane-spanning helix [29]. To further explore the effects of this mutation, transgenic mice that expressed a constitutively active human PTH/PTHrP receptor (HKrk-H223R) were generated under the control of the rat α1(II) collagen promoter. The targeted expression of constitutively active PTH/PTHrP receptor in cartilage led to delayed mineralization, decelerated conversion of proliferative chondrocytes into hypertrophic cells

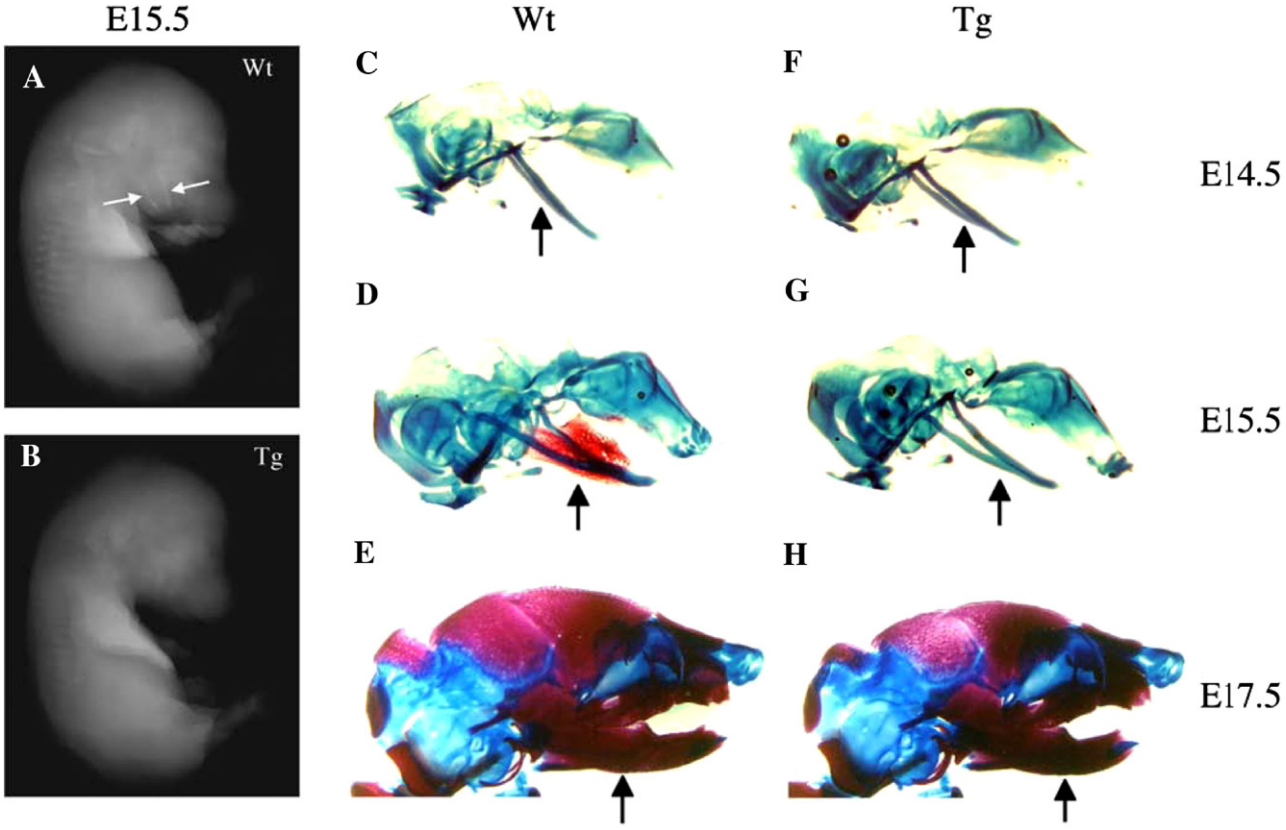

Fig. 1. Faxitron analysis of embryos at E15.5 (A, B), and fetuses stained with alizarin red and alcian blue to reveal ossification and cartilage formation at E14.5, E15.5 and E17.5 (C-H). (A) Wild type mice showed clear bone formation (arrows). (B) Clear bone structures were not seen in Col1-caPPR mice embryo. Wild type (C) and Col1-caPPR mice (F) were not stained by alizarin red at E14.5 in the mandible (arrow). At E15.5, wild type mouse (D) was stained with alizarin red in the mandible (arrow), Col1-caPPR mouse (G) was stained with alcian blue only (arrow). At E17.5, both wild type (E) mice and Col1-caPPR (H) were stained at the same intensity by alizarin red (arrows).

in skeletal segments that are formed by the endochondral process, and prolonged presence of hypertrophic chondrocytes with delay of vascular invasion. Interestingly, tooth eruption was also lacking although tooth buds had developed. However, the number of ameloblasts was reduced and the odontoblast layer was disorganized. No abnormalities were noted in other parts of the craniofacial skeleton [30]. PTHrP knockout mice were reported to have deformation of craniofacial bones [34,35], and PTHrP deficiency affected the structure and function of osteoclasts exclusively located in the vicinity of the growing tooth germ [36]. Subsequently, transgenic mice that express a constitutively active human PTH/PTHrP receptor (HKrk-H223R) were generated under the control of the mouse α1(I) collagen promoter (Col1-caPPR mice) [37]. These mice were noted for the increased numbers of osteoblastic precursors, osteoblasts and osteoclasts in trabecular bone. This constitutively active PTH/PTHrP receptor was identified as a crucial mediator of both bone-formation and bone-resorption actions of PTH. This transgenic mouse had abnormally large incisors, and other dental abnormalities [38]. The excess bone and fibrous tissue in the long bones of these mice are closely reminiscent of changes observed in fibrous dysplasia of bone (OMIM#174800) [39], caused by post-zygotic activating missense mutations of Gs alpha, a downstream mediator of PPR signaling. Furthermore, development of a myelosupportive stroma was delayed, and there was a marked loss in skeletal ("mesenchymal") stem cells [40], although it has been reported that there is an increased number of hematopoietic stem cells [41].

Our studies presented here are focused on the craniofacial development and structure in Col1-caPPR mice. This results demonstrate that the constitutively active PTH/PTHrP receptor transgenic mouse has a profound impact on form and function, not only in teeth as has been previously report [38], but also in the shape of the cranium, and in particular, on the temporomandibular joint. This mouse strain is a useful model for characterization of the downstream effects of the constitutively active receptor during development and growth, and as a model for development of treatments of human diseases with similar characteristics.

Materials and methods

Identification of transgenic mice

Male FVB/N Col1-caPPR mice generated previously [37] were mated with wild type female FVB/N mice. Genotyping was performed as described [37].

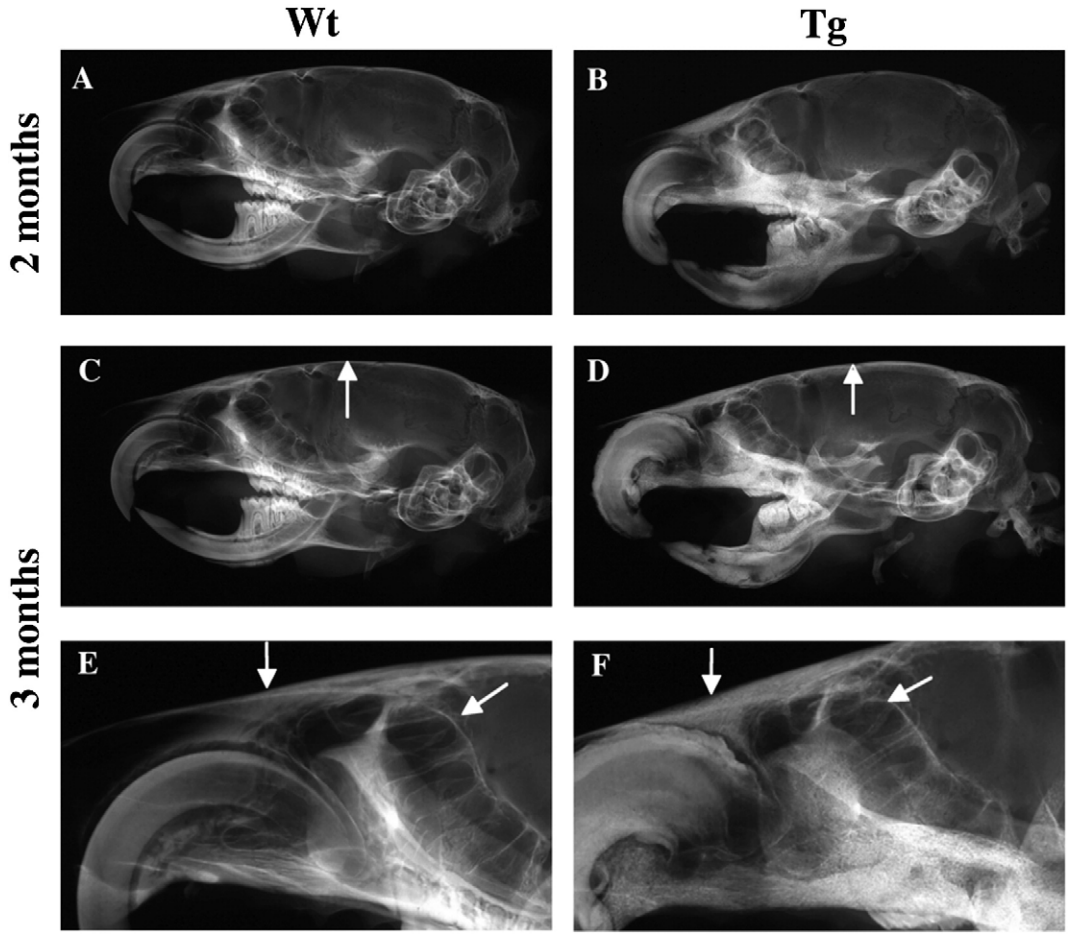

Fig. 2. Faxitron analysis of wild type mice (A) and Col1-caPPR mice (B) at 2 months after birth. At 3 months, wild type mice showed a rounded skull (C), but the skull was flattered in Col1-caPPR mice (D). (E) High magnification of the nasal bone and nasal cavity and turbinate in wild type mice at 3 months after birth. (F) Col1-caPPR mice exhibited a narrow nasal cavity and ground-glass appearance of the nasal bone 3 months after a birth (arrows).

Mouse DNA was extracted from tail clippings using a DNA extraction Kit (Qiagen, Valencia, CA). Genomic DNA (2 μl) was diluted in a 50 μl PCR reaction of PCR master mix (Roche, Indianapolis, IN). PCR was performed using the forward primer S1 from the receptor sequence: (5′-CACCTGCCCTGCTA-CAGGAAGAG-3′), (nucleotides 423–445; GenBank accession no. U22409), and reverse primer A from the pcDNA1 sequence in the transgene: (5-TTCCACCACTGCCTCCCATTCATC-3′) with the following conditions: 94 °C for 1 min, 30 s, 58 °C for 45 s 72 °C for 1 min, and an additional 3 min at 72 °C at the end of 30 cycles. PCR products were resolved by electrophoresis through 1% agarose gels, yielding bands that were 350 bp representing the targeted sequence in this transgenic mouse. All experiments were performed according to the institute's guidelines for the care and use of laboratory animals (NIDCR ASP #01-174).

X-ray analysis

Craniofacial structures were radiographed using a Faxitron MX-20 Specimen Radiography System (Faxitron X-ray Corp., Wheeling, IL, USA) at energy of 30 kV for 90 s. The images were captured with Eastman Kodak X-OMAT TL film (Eastman Kodak Co., Rochester, NY, USA). At least six mice of each genotype were X-rayed at each time point studied.

Histological and immunohistochemical analyses

Samples were fixed with fresh 4% paraformaldehyde overnight at 4 °C. After washing with PBS, the tissue was decalcified with 10% EDTA in PBS for 2 weeks. Samples were routinely embedded in paraffin. Five-micrometer-thick paraffin sections were stained with hematoxylin-eosin. For immunohistochemistry, deparaffinized sections were exposed to 3% hydrogen peroxide for 30 min to inhibit endogenous peroxidase activity, washed in PBS, and incubated with a 1:10 dilution of normal goat serum for 30 min before exposure to primary antiserum (LF-153, anti-mouse DSP, courtesy of Dr. Larry W. Fisher, NIDCR). Incubation time with primary antiserum and non-immune serum was for 2 h at room temperature. After washing in 0.01% Triton X-100/PBS, the sections were incubated with a 1:100 dilution of a peroxidase-labeled, goat affinity-purified anti-rabbit IgG antibody (Kirkegaarde and Perry, Rockville, MD) for 30 min. Immunoreactivity was revealed using the 3′,3′-diaminobenzidine reaction.

Alcian blue and alizarin red staining

For staining of cartilage and bone, mice were skinned and eviscerated, and fixed and stored in 95% ethanol. The cartilage was stained with alcian blue (0.03% in 80% ethanol/20% acetic acid) for 3 days, and the animals were washed with 95% ethanol for 6 h. After soaking in 2% potassium hydroxide for 24 h, the bone was stained with alizarin red (0.03% in aqueous 1% potassium hydroxide) for 24 h, cleared (1% potassium hydroxide/20% glycerol), and stored in 1:1 glycerol/95% ethanol [42]. At least six mice of each genotype were analyzed at each time point.

Micro-computed tomography (microCT) acquisition and analysis

Three-dimensional images generated by micro-computed tomography (microCT) were used for morphological analysis and for examining specific regions that were initially identified by X-ray. The bones were scanned by a microCT system (IMTEK Inc) and images were analyzed with software based on an Amira program (http://www.tgs.com/). 45.000000-kVp X-ray voltage was used and voxel size was 0.060 mm. Two mice of each genotype were analyzed at 3–4 weeks of age and one mouse of each genotype at 9 months of age.

Scanning electron microscope (SEM)

Samples were fixed in 4% paraformaldehyde and then transferred to absolute ethanol. Samples were then rehydrated to 0.1M cacodylate buffer, pH 7.4 and re-fixed in 4% glutaraldehyde in 0.1 M cacodylate for 3 h. Samples were rinsed, and then post-fixed in 1% osmium tetroxide for 1h. After rinsing, samples were dehydrated and critical point dried (Tousimis, Rockville MD, USA) and then viewed uncoated in a Hitachi S-3500 N scanning electron microscope. Two samples of each genotype were analyzed at 1 month of age.

Results

Craniofacial development

Type I collagen expression starts at embryonic day 8.5 (E8.5) in mesoderm, sclerotomes, dermatomes, and in the forming connective tissues [42], and at embryonic day 14.5, the 2.3 kb Col1α1 promoter is active in histologically identifiable osteoblasts [43]. After E14.5, regions of ossification showed the highest levels of the type I collagen expression, which would be controlled by bone/odontoblast-specific type I collagen promoter that drives the expression of the mutant receptor in this transgenic strain. Faxitron study revealed the occurrence of a host of calcified tissue abnormalities including morphological differences in the transgenic mice. In the craniofacial region, Col1-caPPR mice exhibited an initial bone formation delay. Although expression of the transgene was found by RT-PCR using DNase-treated mRNA extracted from the craniofacial region at E14.5 (data not shown), alizarin red staining was not yet detectable in wild type (Fig. 1C) or Col1-caPPR mice at this

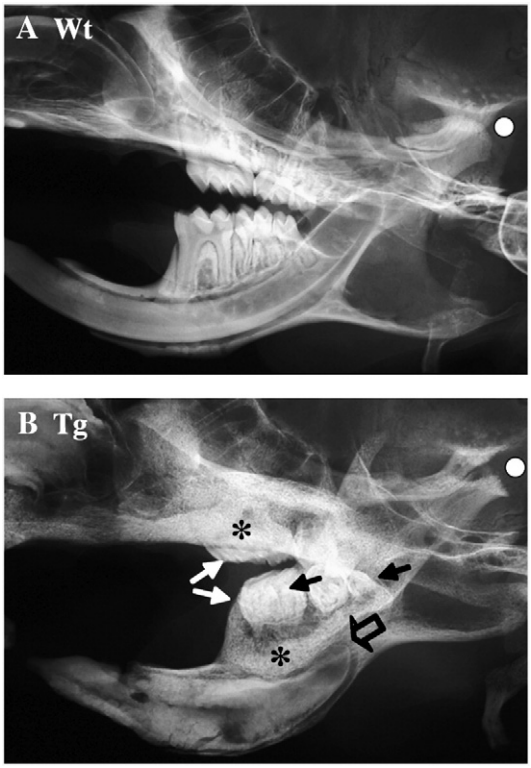

Fig. 3. Faxitron analysis of wild type (A) and Col1-caPPR (B) maxillae and mandibles at 3 months after a birth. Col1-caPPR mice exhibited mulberry-like crowns (white arrow), short roots and undetectable pulp in the teeth (black arrows). The maxillary and mandibular bone had a ground-glass bone appearance (star) in Col1-caPPR mice. In the transgenic mice, the apex position of the lower incisor (open arrow) was anteriorly displaced and disorganized, and the mandibular condyle was abnormally shaped, with a wide distance from the condyle to articular eminence (white circle) compared with wild type mice.

stage of development (Fig. 1F). At E14.5, alcian blue revealed similar levels of cartilage formation in the craniofacial region of wild type (Fig. 1C) and Col1-caPPR mice (Fig. 1F). At E15.5, normal littermates showed bone formation in the maxilla and mandible by Faxitron (Fig. 1A). No detectable ossification was noted in Col1-caPPR mice at the same stage (Fig. 1B). At E15.5, alizarin red staining confirmed this initial delay in bone formation. There was alizarin red staining in the wild type mandible (Fig. 1D) but no staining in a Col1-caPPRmouse (Fig. 1G). At E17.5, both wild type (Fig. 1E) and the Col1-caPPR mice (Fig. 1H) had the same level of staining.

Post-natal craniofacial development

There were changes in the shape of the skull as a function of age in the transgenic mice (Fig. 2). Wild type mice showed a rounded skull at all developmental stages (Figs. 2A, C), whereas the skull was flattened by 3 months in Col1-caPPR mice (Fig. 2D). The nasal bone displayed a sharp and clear morphology in wild type mice (Fig. 2E), but Col1-caPPR mice displayed a thickened nasal bone (Fig. 2F). In addition, the nasal cavity was narrow in Col1-caPPR mice (Fig. 2F). Many of the Col1-caPPR mice showed bleeding from the nose, possibly due to the narrowing of the nasal cavity leading to difficulties in breathing (data not shown).

Post-natal dental abnormalities

Col1-caPPR molars exhibited mulberry-like crowns with very short roots. Both the maxilla and mandible were expanded and had a ground-glass appearance by X-ray analysis. The incisor apex was displaced anteriorly, and was disorganized compared to wild type littermates (Figs. 3A, B). Study of tooth development and morphological differences between wild type mice and Col1-caPPR mice are shown in Fig. 4. First molars of the wild type mice erupted by 20 days after birth. The eruption of the first molars of Col1-caPPR mice was delayed compare to wild type (Figs. 4A, B). Col1-caPPR incisors had mineralization differences compared to wild type mice (Figs. 4C, D, E, F). Interestingly, there was diminished calcification in the upper incisors at 1 month (Figs. 4E); however, they were hypercalcified at 2 and 3 months (Figs. 2B, D, and 4F) compared with wild type mice (Figs. 2A, C, and 4D). MicroCT revealed an expanded size of the incisors and the turbinate structures in the transgenic mice (Figs. 4G, H, I, J).

By histological analysis of the mandible at 1 month (Figs. 5A, C), the incisors of the transgenic mice were notable by a reduced amount of enamel and dentin, and an expanded, disorganized and hypercellular pulp compared to wild type incisors. Transgenic first molars displayed the same features, along with multiple disoriented cusps. At high power (Figs. 5B, D), transgenic odontoblasts were disorganized and did not extend processes into dentinal tubules. The dentin matrix, which is acellular in wild type dentin (with the exception of odontoblastic processes), was found to contain cells, in a pattern not dissimilar from that of fibrous dysplastic bone. The abnormal dentin in the incisors and molars was found to stain positively for type I collagen, osteopontin, bone sialoprotein (data not shown), and dentin sialoprotein (DSP, Figs. 5E, F). The teeth were surrounded by excess abnormal bone in a trabeculated pattern

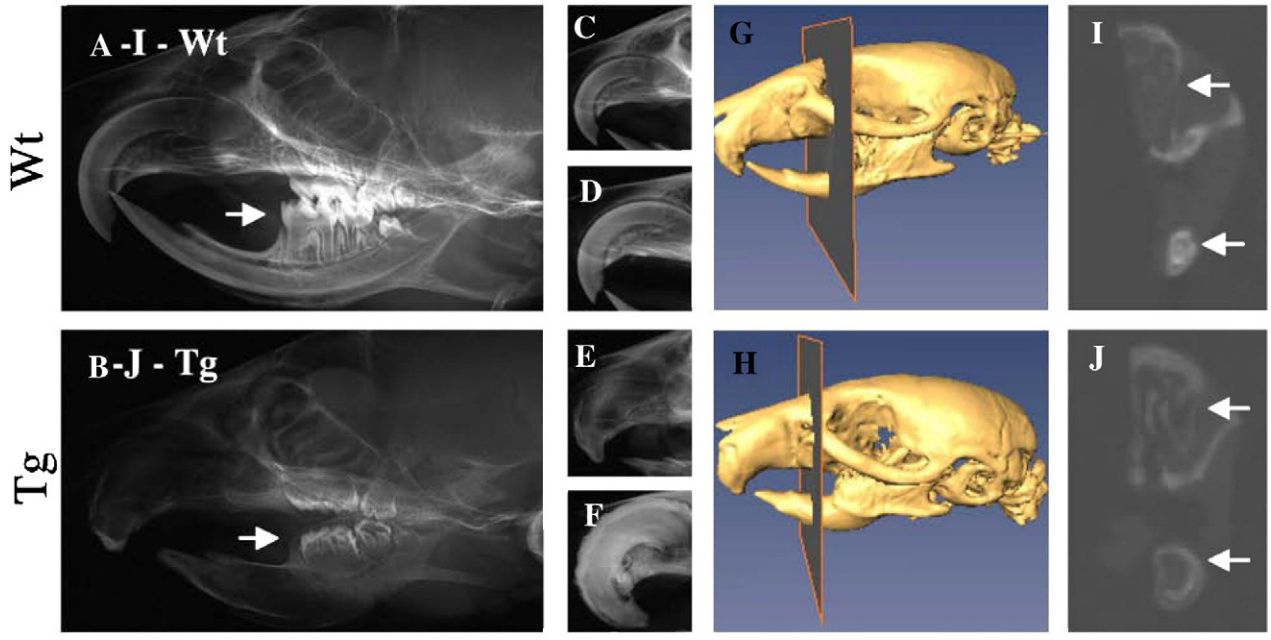

Fig. 4. Faxitron analysis (A–F) and microCT (G–J) of wild type and Col1-caPPR mice. Wild type (A), and Col1-CaPPR mice (B) at 3 weeks. Arrows show the lack of molar eruption in Col1-caPPR mice compared to wild type mice. (C) 1 month, and (D) at 3 months, wild type upper incisors. (E) 1 month, and (F) at 3 months Col1-caPPR upper incisors. Initially, transgenic incisors are hypomineralized compared to wild type, but with aging, become hypermineralized. Position of vertical microCT slices in 3–4 week old wild type (G) and Col1-caPPR (H) mice. (I) turbinate (upper arrow) and incisor (lower arrow) in wild type mice. (J) turbinate (upper arrow) and incisor (lower arrow) in transgenic mice. The turbinate and incisors are expanded in the transgenic mice, however, the incisor shows hypomineralization.

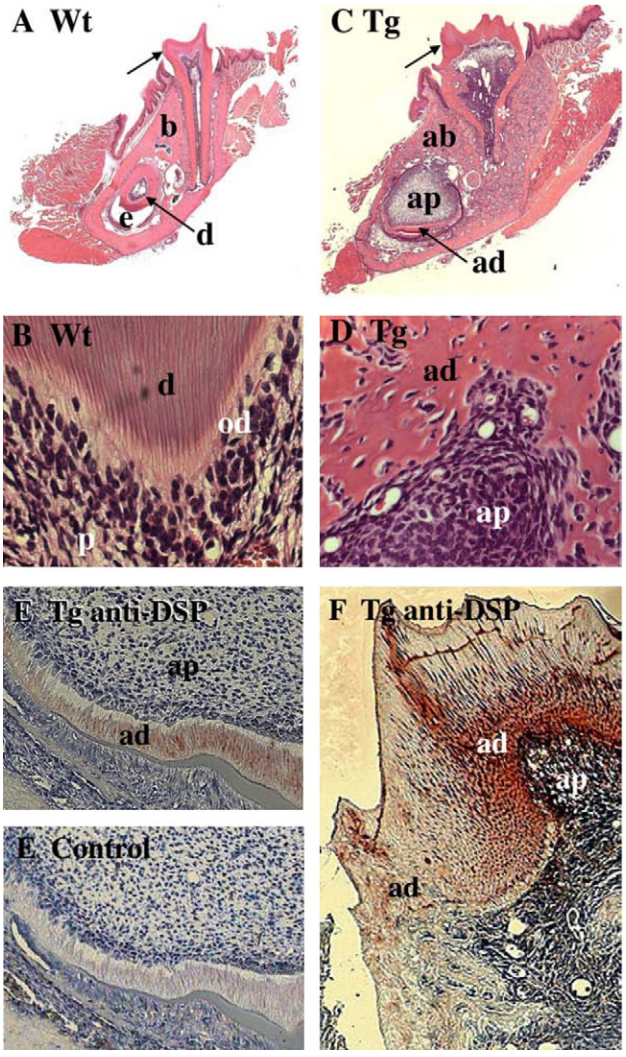

Fig. 5. Tooth and alveolar bone in wild type (A,B) and Col1-caPPR (C,D) mandibles at 1 month after birth. (A) Enamel (e), dentin (d), bone (b) and molar cusps (arrows) in normal mice. (B) In normal dentin, odontoblasts (od) extend cytoplasmic processes into dentinal tubules. Dentin does not contain cell bodies. (C) In the transgenic mandible, the incisors show an abnormally expanded pulp (ap), lack of enamel and a abnormally thin dentin (ad). The molars display similar features, and in addition have shortened roots (*) and multiple disoriented cusps (arrow). The teeth are surrounded by overabundant abnormally trabeculated mandibular bone (ab). (D) Morphologically distinguishable odontoblasts are not apparent, nor are dentinal tubules. Instead, cells are found within the abnormal dentin (ad), and the abnormal pulp (ap) is highly cellular compared to wild type pulp. Abnormal dentin (ad) in the incisors (E) and in the molars (F) stain positively for dentin sialoprotein (DSP). (G) Non-immune serum control.

similar to what has been observed in the long bones of these transgenic mice [40].

Scanning electron microscope (SEM) revealed very different surface features between upper incisors and upper molars from wild type and Col1-caPPR mice at one month of age (Fig. 6). An overview of the upper incisor in the Col1-caPPR mice revealed a rounded morphology, lacking the sharp edges of the occlusal surface and lateral walls found in the wild type mice (Figs. 6A, B). There was a distinct dentinoenamel junction in the incisors and molars of the wild type mice (Figs. 6C, I), however there were dentinoenamel junction abnormalities in all of the teeth of Col1-caPPR mice (Figs. 6E, K). Interestingly, the periodontal ligament was completely disrupted in Col1-caPPR mice (Figs. 6F, L) compared to wild type (Figs. 6D, J). The increased number of cusps and the lack of organization in the transgenic molars could be better discerned by SEM than by standard histology (Fig. 6H). Furthermore, there were many pits in the molar caps in the Col1-caPPR mice (Fig. 6H).

Abnormal growth and disruption of the temporomandibular joint in Col1-caPPR mice

In addition to the dramatic effect of the transgene on dental development, the Col1-caPPR developed a highly disrupted mandibular condyle as a function of age. At 1 month of age, Faxitron images showed an unevenness of the transgenic mandibular condyle (Fig. 7C) compared to wild type (Fig. 7A). By 2 months of age, the dysmorphia was even more pronounced, and there was a wide distance from the transgenic condyle to the articular eminence (Fig. 3B) compared with wild type mice (Fig. 3A). At 4 months, by Faxitron and microCT it was observed that the structure of the mandibular condyle in wild type mice was round (Figs. 7B, F, H), but was a concave structure in Col1-caPPR mice (Figs. 7D, J, L).

Histological analyses of the condyle revealed numerous abnormalities in the Col1-caPPR mouse (Fig. 8). As observed by Faxitron and microCT, the shape of the condyle was highly abnormal compared to wild type (Figs. 8A, B). By Faxitron and microCT, the transgenic condyle appeared concave while it is more rounded by histology. This is reconciled by the fact that the tissue was only mineralized at quite a distance away from the apex, unlike in the normal condyle, where subchondral bone lies only a short distance away from the articulating surface. The normal cellular organization was completely disrupted in the transgenic condyle. In the wild type condyle, the superficial articular layer, middle chondroblastic layer (which would be subdivided into the polymorphic cell zone and flattened cell zone in embryonic and neo-natal mice), inferior hypertrophic zone and subchondral bone were clearly discernible (Figs. 8A, C, E, G). In comparison, the Col1-caPPR condyle was hypercellular and notably reduced in morphologically identifiable chondrocytes, hypertrophic chondrocytes and associated extracellular matrices. Instead, the condyle was composed of what appeared to be immature chondrocytes and fibroblastic cells, with only an occasional island of hypertrophic cartilage. The subchondral bone displayed excessive bone formation leading to the loss of marrow, similar to what is seen in other bones (Figs. 8B, D, F, H).

Discussion

The transgenic mouse strain used in this study expresses the human mutant PPR (HKrk-H223R), under the control of the bone/odontoblast-specific 2.3-kb promoter fragment of the mouse α1(I) collagen promoter [37]. This promoter fragment has been found to be active in directing type I collagen

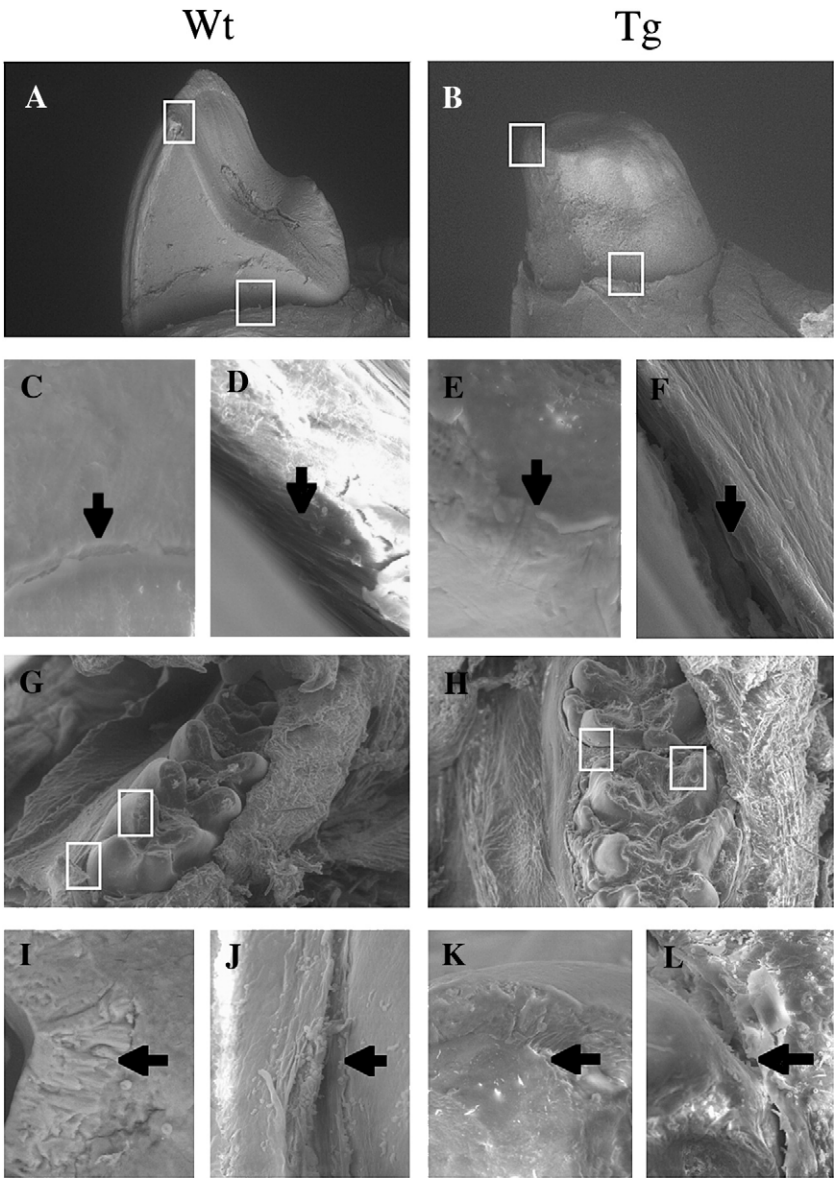

Fig. 6. Scanning electron microscopy of the upper incisor at 1 month in wild type and Col1-caPPR mice (A–F) and the upper molar at 1 month in wild type and Col1-caPPR mice (G–H). Low magnification SEM picture of wild type (A,G) and Col1-caPPR (B, H) mice. High magnification of the box regions in (A, G) of the wild type mice dentinoenamel junction (C, I) and periodontal ligament (D, J). High magnification of the box regions in (B, H) of the Col1-caPPR mice dentinoenamel junction (E, K) and periodontal ligament (F, L). (C, E, I, J) arrow — dentinoenamel junction and (D, F, K, L) arrow — periodontal ligament. The transgenic incisor lacks the sharp occlusal and lateral wall edges and is rounded in appearance. In both incisors and molars, the dentinoenamel junction is disorganized and the periodontal ligament is lacking.

expression specifically in osteoblasts starting at E14.5 [43]. Ligand-independent activity of the receptor has been shown to be driven by the bone/odontoblast-specific type I collagen promoter in this transgenic mouse [37].

The maxilla and mandible showed a complex phenotype in the Col1-caPPR transgenic mice. There are three remarkable points in common between the maxilla and the mandible. First, there was a delay in the initiation of ossification. Reduced or no alizarin red staining at E 15.5 was noted in the Col1-caPPR mice at this stage of development. While the delay in ossification could be through some other mechanism, it is also possible that initially the transgene causes a delay in osteogenesis, as it does in the development of dentin as noted previously [37], and in our study. Second, there were abnormalities in enamel and dentin formation, the position of incisor apices, and molar roots were markedly shortened. A delay in molar eruption was noted, although this was not observed in a previous study [37], most likely due to the time points that were analyzed. Third, there was a dramatic change in the maxillar and mandibular phenotype with aging with an expansion of these bones with an abnormal ground-glass appearance, reminiscent of the fibrous dysplastic appearance noted previously in the long bones of these mice [40]. These results highlight the importance of appropriate PTHrP signaling via the PPR during embryonic development as noted previously [38], and post-natal growth as we have shown here.

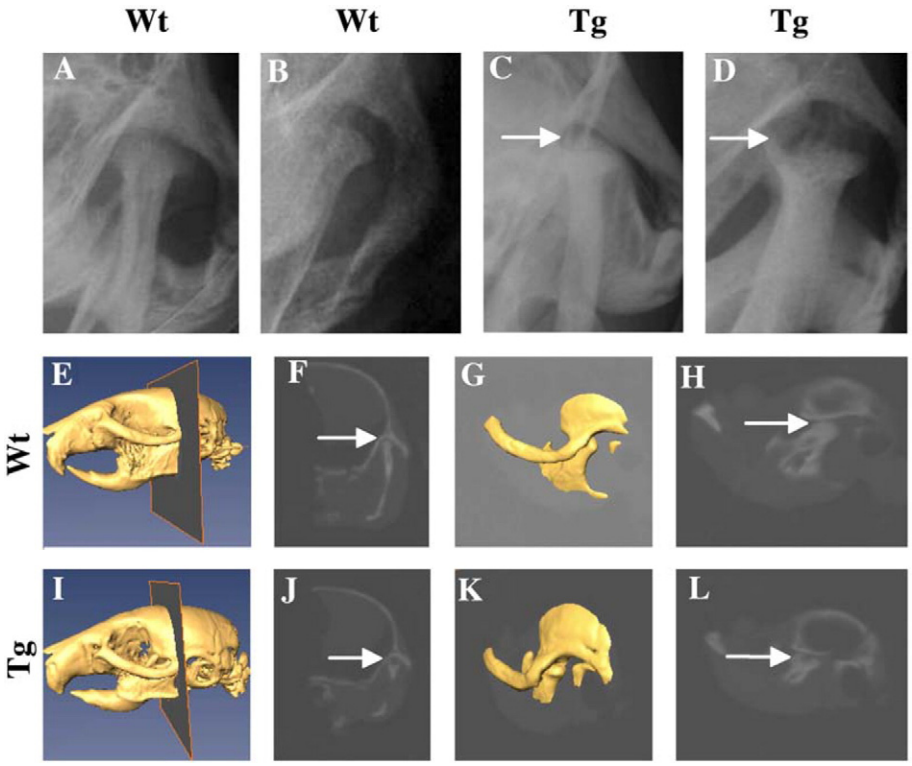

Fig. 7. Faxitron (A–D) and microCT picture (E–L). Vertical section of the wild type mandible condyle at 1 month (A), and at 4 months (B). Vertical section of the Col1-caPPR mice mandible condyle at 1 month (C) and at 4 months (D). Arrows indicate the abnormal structure of the condyle in the transgenic mouse (C, D). Position of the vertical microCT slice (E) and sagittal microCT slice (G) in 3–4 week old wild type mandible condyle (F,H). Position of the vertical microCT (I) and sagittal microCT slice (K) in 3–4 week old Col1-caPPR mandible condyle (J, L). Arrows indicate the position of the condyle, and show that the transgenic condyle has a flattened appearance.

Teeth are examples of organs that develop as appendages of the embryonic surface epithelium. The most important events during regulation of the development of all such organs are the so-called inductive interactions between the epithelial and mesenchymal tissues. These are mediated by conserved signaling pathways, and in tooth development these have been characterized in detail [44–46]. Signaling molecules have been identified that mediate interactions between the epithelial and mesenchymal tissue (paracrine), and with in each cell type (autocrine) [47]. Signaling interactions that determine the location, size, and shape of teeth take place during the early stages of tooth development [46,48].

The genetic rescue of the skeletal defects in PTHrP knockout mice, using the cartilage specific PTHrP or constitutively active PTH/PTHrP receptor transgene [30], lead to the realization that PTHrP and PTH/PTHrP receptor signaling also regulates interactions between epithelium and mesenchyme during the organogenesis of epithelial organs such as the skin, mammary glands, and teeth [49–51]. Clearly, the persistence of signaling generated by the mutant receptor in mineralizing tissues has a profound effect not only on the initiation of tooth bud formation as indicated by the abnormal morphology [38], but also further development, as shown by our study. Initially, incisors were poorly mineralized, with immature dentin and enamel surfaces as shown by Faxitron, microCT and SEM analyses, similar to the delay of mineralization observed in the mandible. The dentinoenamel junction became highly disorganized, and there was a loss of the periodontal ligament in the Col1-caPPR mice. Subsequently the incisors became hypermineralized with an obliteration of the pulp cavity, most likely due to inappropriate mineralization of the abnormal pulp cells.

Disruption of the mandibular condyle was also a pronounced feature of the Col1-caPPR mice that has not yet been recognized. The temporomandibular joint (TMJ) is a bilateral diarthrosis between the mandibular condylar and the temporal bone. During development of the murine mandibular condyle, the first event is marked by the appearance of an ectomesenchymal cell condensation on the mandibular anlagen. This condensation differentiates into cartilage and forms a growth plate-like structure. This structure is characterized by an outer most fibrous cell layer, an underlying layer of polymorphic progenitor cells, followed by a zone of flattened chondrocytes, and finally a zone of hypertrophic chondrocytes. However, unlike growth plates in long bones, the condyle elongates via appositional growth at the apical end. It is thought that primarily undifferentiated cells in the polymorphic layer proliferate and differentiate into chondrocytes in the condyle, while in other growth plates, elongation is established by the mitotic activity of chondroblasts in the proliferative zone [52].

In post-natal mice of the age used in our study, the polymorphic layer and zone of flattened chondrocytes are highly

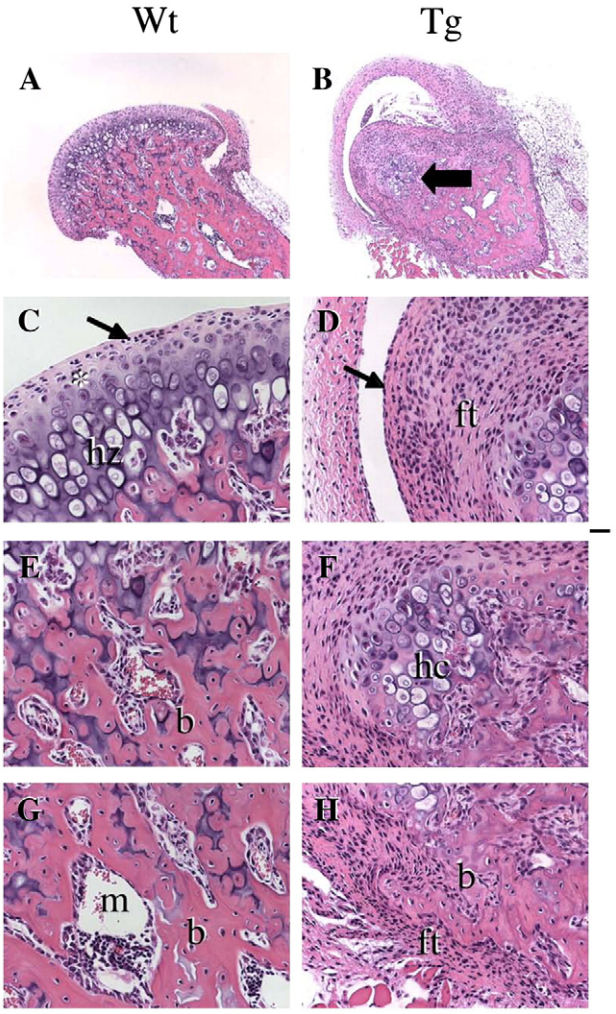

Fig. 8. Histological observation of the temporomandibular joint of wild type (A,C,E,G) and Col1-caPPR mice (B,D,F,H) at 1 month after birth by hematoxylin and eosin (H&E) staining. Low magnification of wild type (A) and Col1-caPPR mice (B). Large arrow indicates a small island of hypertrophic cartilage in the transgenic condyle. (C) Superficial articular layer (black arrow) and middle chondroblastic layer (*) and hypertrophic zone (hz) in the wild type condyle. (D) In the transgenic condyle, the surface is covered by flattened cells and the condyle is composed of a hypercellular fibrous tissue (ft). (E) The hypertrophic zone and subchondral zone were clearly discernible in wild type mice. (F) In the transgenic condyle, only occasional areas of hypertrophic cartilage are seen (hc). The subchondral bone displayed excessive bone formation leading to loss of marrow in Col1-caPPR mice (H) compared with wild type mice (G).

condensed and difficult to distinguish. However it is clear that the composition of the condyle was highly irregular in the Col1-caPPR mice. What could be discerned was that an outer articular layer and hypertrophic layer present in wild type mice was virtually absent in the transgenic mice, which instead was characterized by a highly cellular tissue composed of cells with an immature chondrocytic and fibroblastic morphology, and only occasional islands of hypertrophic chondrocytes. This pattern is completely different from what is noted in the long bone growth plates of these transgenic mice, which are normal. These observations suggest that first, the 2.3 Col1α1 promoter is active in a certain population of cells in the mandibular condyle, which is fibrocartilagenous in nature. Second, the forced expression of the caPPR in these cells leads to the extensive proliferation, but lack of maturation of a particular cell type, perhaps the elusive polymorphic cell, responsible for fueling elongation of the condyle. Further study is needed, however, this model provides insight into the differences between the mandibular condyle and the articular surfaces and growth plates of long bones.

In summary, targeted expression of the constitutively active PTH/PTHrP receptor to mineralizing tissues resulted in abnormal formation of the craniofacial bones, incisors and molars, and in the temporomandibular joint as well. Our findings suggest that maintained expression of the signaling pathway initiated by the mutant PTH/PTHrP leads to abnormal mesenchymal and epithelial interactions, and points to the importance of tight control of signaling between these cell types for normal development.

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