Original Article

Role of polycystin-1 in bone remodeling: *Orthodontic tooth movement study in mutant mice*

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ABSTRACT

Objective: To test the hypothesis that polycystin-1 (PC1) is involved in orthodontic tooth movement as a mechanical sensor.

Materials and Methods: The response to force application was compared between three mutant and four wild-type 7-week-old mice. The mutant mice were PC1/Wnt1-cre, lacking PC1 in the craniofacial region. An orthodontic closed coil spring was bonded between the incisor and the left first molar, applying 20 g of force for 4 days. Micro–computed tomography, hematoxylin and eosin staining, and tartrate-resistent acid phosphatase (TRAP) staining were used to study the differences in tooth movement among the groups.

Results: In the wild-type mice the bonded molar moved mesially, and the periodontal ligament (PDL) was compressed in the compression side. The compression side showed a hyalinized zone, and osteoclasts were identified there using TRAP staining. In the mutant mice, the molar did not move, the incisor tipped palatally, and there was slight widening of the PDL in the tension area. Osteoclasts were not seen on the bone surface or on the compression side. Osteoclasts were only observed on the other side of the bone—in the bone marrow.

Conclusions: These results suggest a difference in tooth movement and osteoclast activity between PC1 mutant mice and wild-type mice in response to orthodontic force. The impaired tooth movement and the lack of osteoclasts on the bone surface in the mutant working side may be related to lack of signal from the PDL due to PC1 deficiency. (*Angle Orthod.* 2014;84:885–890.)

KEY WORDS: Tooth movement; Mutant mice; Bone remodeling; Cilia; Polycystin-1

INTRODUCTION

Orthodontic tooth movement relies on application of force that causes remodeling of the alveolar bone through a complex process mediated by the periodontal ligament (PDL). Bone is removed by osteoclasts in areas of the PDL under compression and formed by osteoblasts in areas of the PDL under tension. While many histological and cellular aspects of the process have been studied,[1,2] it is still unclear how the applied biomechanical force is transmitted from the tooth to the PDL to activate the remodeling process. Several theories (neurological response, bioelectric fields, fluid flow, mechanical cellular perturbation, and inflammation) have been proposed.[3–7] The inflammatory theory, including changes in PDL vascularity that lead to localized inflammatory responses involving cell recruitment, apoptosis, and changes in biochemical signaling, have been studied extensively.

Our hypothesis is based on the fluid flow theory.[5] According to this theory, the PDL acts as a continuous hydrostatic system, and the fluid in the PDL has the potential to stimulate sensing mechanisms and

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mediate a cellular response. The bony wall of the dental socket contains fenestrations that allow compressed fluid in the PDL to be expressed into the marrow spaces. Thus, changes in fluid flow are not only felt by PDL cells but also by osteocytes located in the adjacent bone, and cells in the marrow spaces could mediate a cellular response. Fluid flow dynamics have also been implicated in the stimulation of a number of cell types via primary cilium.[8] This raises the possibility that the link between the extracellular matrix, fluid flow, and cellular mechanotransduction may be the primary cilium.

A previous study[9] suggested that primary cilia, with their associated ion channels and signaling molecules, play an important role in fluid detection by kidney epithelial cells. A subsequent study[10] demonstrated that a Ca^{2+} channel complex, composed of the transmembrane proteins polycystin-1 (PC1) and polycystin-2, located at the base of the primary cilium, mediates the sensing of cilia bending. This discovery offered a detailed insight into the way cells sense dynamic fluid movements and transmit signals by means of calcium influx into the cells. Based on these findings, primary cilia have been proposed to act as mechanosensors in osteocytes[8] and chondrocytes,[11] capable of regulating bone remodeling. Numerous studies of kidney cells describe a direct mechanical stimulation of primary cilia triggered by fluid flow dynamics. Recent reports[12,13] suggest that cultured osteoblastic cells respond to fluid flow by altering gene expression. As such, fluid flow is one of the most potent mechanical stimulators for bone cells.[14] It has also been suggested[8] that bone and kidney cells might share certain similarities in flow detection mechanisms. Current studies[15–19] specific to the effect of primary cilia on skeletal growth and mechanical stress indeed showed promising results in support of the PC1 sensor role hypothesis.

If PC1 is indeed essential to bone remodeling, then one may expect a major effect on orthodontic tooth movement in PC1-deficient mice. In this study we compare tooth movement in wild-type and PC1-deficient mice to test the hypothesis that PC1 partakes in orthodontic tooth movement as a mechanical force sensor.

MATERIALS AND METHODS

Seven-week-old female mice, 20–23g in weight, were subjected to orthodontic force for 4 days. After anesthesia with intraperitoneal injection of ketamine (87 mg/kg) and xylazine (13 mg/kg), the mice were subjected to orthodontic tooth movement according to the method described by Pavlin and Gluhak-Heinrich.[20] Our study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Harvard Medical Area Standing Committee on Animals (Protocol 02074). To carry out dental procedures, mice were held and restrained using a custom-made holder (Figure 1).

Figure 1. Custom-made holder to restrain the mouse.

All treatment procedures were performed under a dissecting microscope. The orthodontic force was applied by an 0.0056 × 0.022-inch Elgiloy coil spring (Rocky Mountain Orthodontics, Denver, Colo) that was bonded between the incisor and the left first molar using a light-cured adhesive (3M Unitek, Monrovia, Calif) (Figure 2).

The force magnitude used in the experiments was 20 g. The right side was used as a non-force control in this study. We compared four wild-type to three PC1/Wnt1-cre mutant mice.

The PC1/Wnt1-cre mutant mice have been bred as follows:

$$\begin{aligned} &\mathrm{Wnt1-cre} + \mathrm{PC1^{(flox/flox)}} \\ &\rightarrow \mathrm{PC1^{(flox/WT)}-Wnt1-cre} \\ &+ \mathrm{PC1^{(flox/WT)}-Wnt1-cre} \\ &\rightarrow \mathrm{PC1^{(flox/flox)}-Wnt1-cre}(25\%) \end{aligned}$$

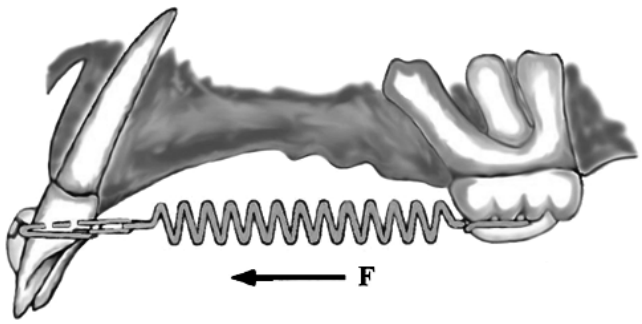

Figure 2. Spring activation—application of orthodontic force.

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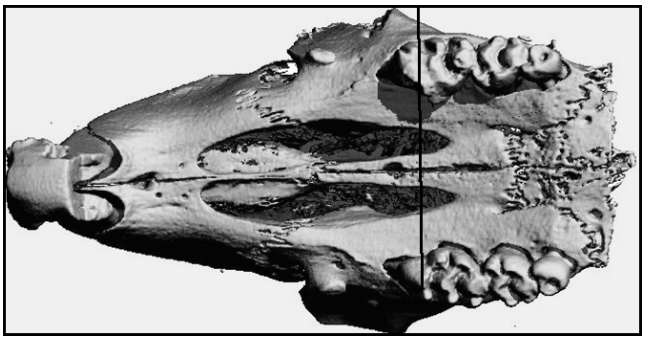

Figure 3. Three-dimensional micro-CT reconstruction of a wild-type maxilla: Mesial movement of the molar on the left side (with orthodontic force). No movement of the molar on the right side (without orthodontic force).

The conditional knockout mice lack the transmembrane protein PC1 in the craniofacial region. The Pkd1 gene is floxed. Since the Wnt1 promoter is expressed in cranial neural crest cells, cre recombinase removes the Pkd1 gene in neural crest–derived cells. Mice carrying floxed alleles of Pkd1 were described previously.[21] The mice were fed a sugar diet for 4 days and then sacrificed using carbon dioxide. The maxillae were dissected out from both the control and experimental mice immediately after the animals were euthanized.

Micro–Computed Tomography

A desktop micro–computed tomography (μCT) 40 system (Scanco Medical AG, Brüttisellen, Switzerland) was used for scanning. The intact maxillae were placed in a holder and scanned with the palate perpendicular to the image plane producing a three-dimensional stack of images. Scans were taken every 16 μm.

Histology

Samples were fixed in 4% (w/v) paraformaldehyde overnight and demineralized in 0.5 M ethylenediamine tetraacetic acid (EDTA) for 14 days at 4°C before they were embedded in paraffin. The maxillae were then hemisected along the midpalatal raphe, embedded in paraffin, and sectioned into 6-μm sections parallel to the mesiodistal plane of the tooth. For histological analysis, paraffin-embedded sections were stained with hematoxylin and eosin and then microphotographs were taken. Tartrate-resistent acid phosphatase (TRAP) staining to identify osteoclasts was carried out as follows: Samples were fixed in 4% paraformaldehyde, decalcified in EDTA, and then sectioned after paraffin embedding. After deparaffinization, TRAP staining was carried out using a Sigma (St Louis, Mo) Diagnostic kit, images were obtained, and the TRAP-positive cells were identified.[16]

In all experiments, we compared the following four samples:

1. Wild-type mice, left side (with orthodontic force);
2. Wild-type mice, right side (without orthodontic force);
3. Mutant mice, left side (with orthodontic force); and
4. Mutant mice, right side (without orthodontic force).

Comparison between samples 1 and 2 and between samples 3 and 4 was made in order to isolate the effect of the applied force, while comparison between samples 1 and 3 and between samples 2 and 4 was made to isolate the effect of PC1.

RESULTS

Micro-CT analysis was used to assess the extent of tooth movement, as well as to image changes in PDL width. A reference line, perpendicular to the midpalatal suture, through the mesio-occlusal cusp tip of the first molar, was used as a reference in the measurement of the molar movement. In sample 1 (force applied to wild-type mice), the bonded first molar moved mesially (Figure 3, left side), and the PDL was compressed on the compression side (mesial side of distal root: Figure 4a). In sample 2 (wild-type without orthodontic force) there was no movement of the molar (Figure 3, right side), and the PDL had a uniform width (Figure 4b). In sample 3 (mutant mice with orthodontic

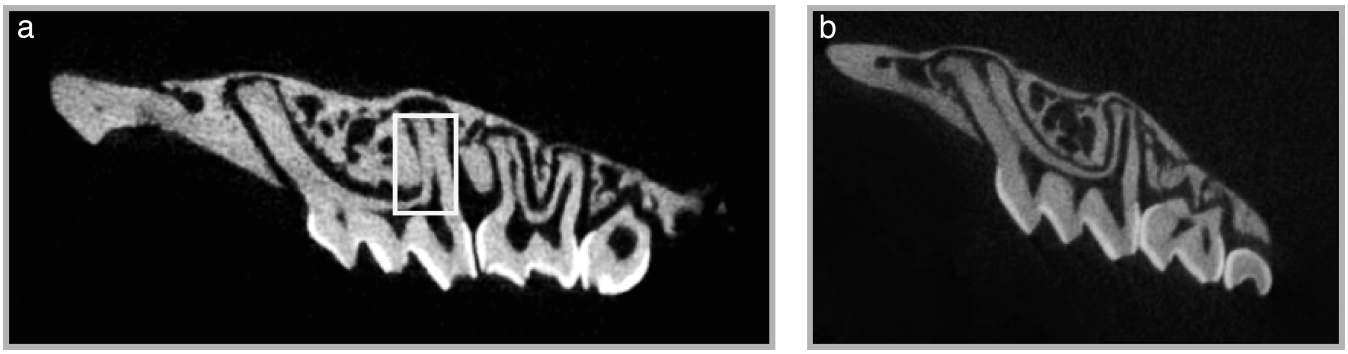

Figure 4. Micro-CT slices of a wild-type mouse. (a) With orthodontic force: compressed PDL on the compression side. (b) Without orthodontic force: uniform width of the PDL.

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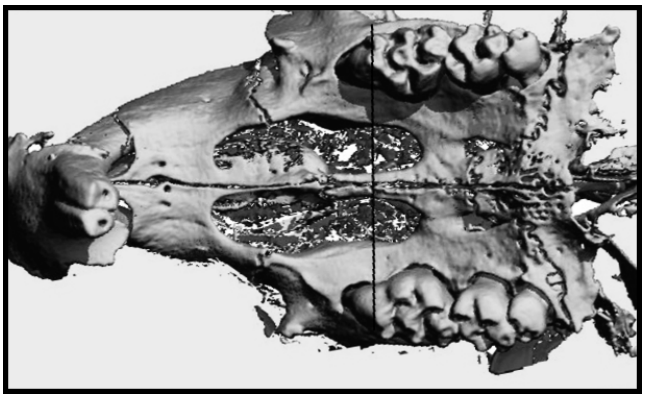

Figure 5. Three-dimensional micro-CT reconstruction of a mutant maxilla: no movement of the molar on the left side (with orthodontic force); palatal tipping of the incisors.

force), the molar did not move, the incisor tipped palatally (Figure 5), and there was a slight widening of the PDL in the tension area (distal side of distal root: Figure 6a). In sample 4 (mutant mice without force) the PDL had a uniform width (Figure 6b).

Histological Analysis

Utilizing hematoxylin and eosin staining, a hyalinized zone with loss of cells was demonstrated in sample 1 (compression side of the wild-type mice, with orthodontic force, Figure 7a). Using TRAP staining, osteoclasts were identified on the bone surface of the compression side (Figure 7b, purple staining).

In sample 2 (right side of the wild-type mice, without orthodontic force), the PDL was intact (Figure 8a), and osteoclasts were identified, using TRAP staining, on the distal area of the distal root (Figure 8b), in accordance with the distal drift in mice.

In sample 3 (left side of the mutant mice, with orthodontic force, Figure 9a), osteoclasts were not seen on the bone surface or on the compression side. Osteoclasts were only observed on the other side of the bone—in the bone marrow (Figure 9b). Each of the results presented is typical of all the animals in its group.

The morphology of the mutant molar is different from the morphology of the wild-type molar. The sections were taken along the long axis of the root in both cases. Although the figures may appear to have different orientations, they represent the result of an identical procedure applied to teeth with different morphologies.

DISCUSSION

If PC1 is indeed a mechanosensor in bone development, one should expect a difference in bone remodeling between PC1-deficient and wild-type mice. In this study, we induced bone remodeling using tooth movement, providing stress across the periodontal ligament. Using this mouse model, we show that in the PC1-deficient mice no movement of the molar tooth was observed.

PC1-deficient mice exhibit premature ossification of the presphenoid synchondrosis at the skull base and retarded postnatal growth of the anterior craniofacial complex.[17] In our study, the hypoplastic maxilla and the

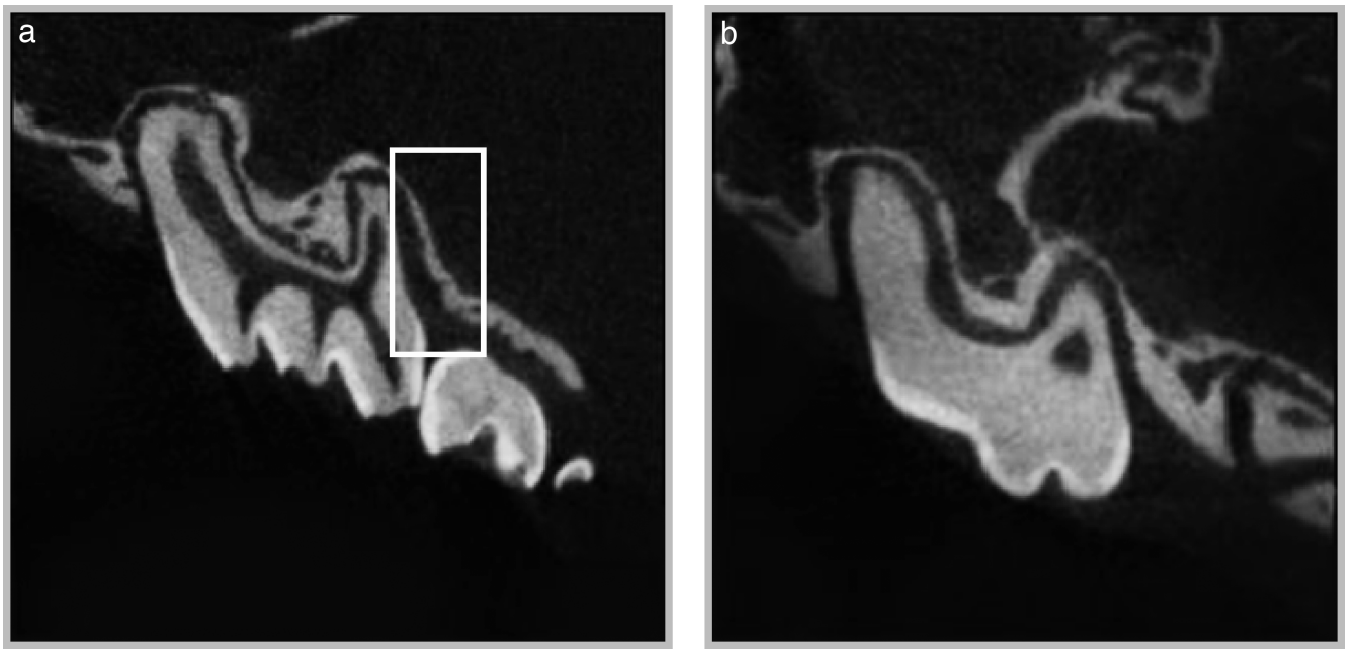

Figure 6. Micro-CT slices of a mutant mouse. (a) With orthodontic force: slight widening of the PDL on the tension side. (b) Without orthodontic force: uniform width of the PDL.

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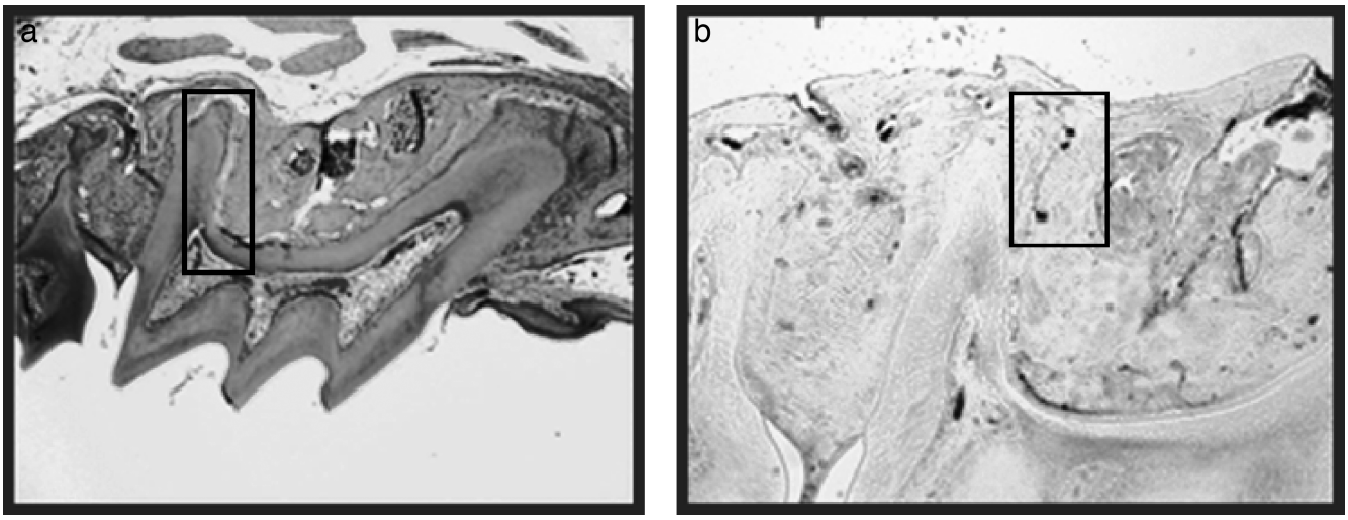

Figure 7. Histological analysis of a wild-type mouse with orthodontic force. (a) Hematoxylin and eosin staining: hyalinized zone with loss of cells on the compression side. (b) TRAP staining: osteoclasts on the bone surface of the compression side (purple staining).

subsequent skeletal Class III could be associated with the palatal tipping of the upper incisors we observed in the mutant mice. Based on the analysis of the skull phenotype observed in Pkd1-deficient mice, Kolpakova-Hart et al.[17] hypothesized that tensile force within the growing viscerocranium is essential for skeletal growth and that PC1 is an important mediator of this process, as it controls proliferation of mesenchymal cells at the osteogenic fronts. Later on, Hou et al.[18] demonstrated that these mutant mice exhibited an impaired response to tensile force. Our findings support these previous studies, as we observed impaired tooth movement in the mutant mice.

In addition, we found a difference in osteoclast activity in the PC1-deficient mice, which has not been reported before. This different osteoclast activity could be due to lack of signal from the PDL attributed to PC1 deficiency. This finding might suggest that PC1 is involved in regulating osteoclast formation.

Orthodontic tooth movement in mice is a challenging procedure, mainly because of the miniscule size of the molar tooth. The challenge is worthy, though, since the model enables studying the effects of different mutations on bone remodeling.

Our study was a pilot study, aimed at establishing a mouse model for orthodontic tooth movement and at testing the effect of PC1 deficiency on bone remodeling. Future studies, including a larger sample, would be helpful in determining the specific mechanism of PC1 in tooth movement and in bone remodeling.

This study was designed to further the understanding of tooth movement. The findings can serve orthodontists interested in bone biology. By studying the possible role of PC1 in tooth movement in mice,

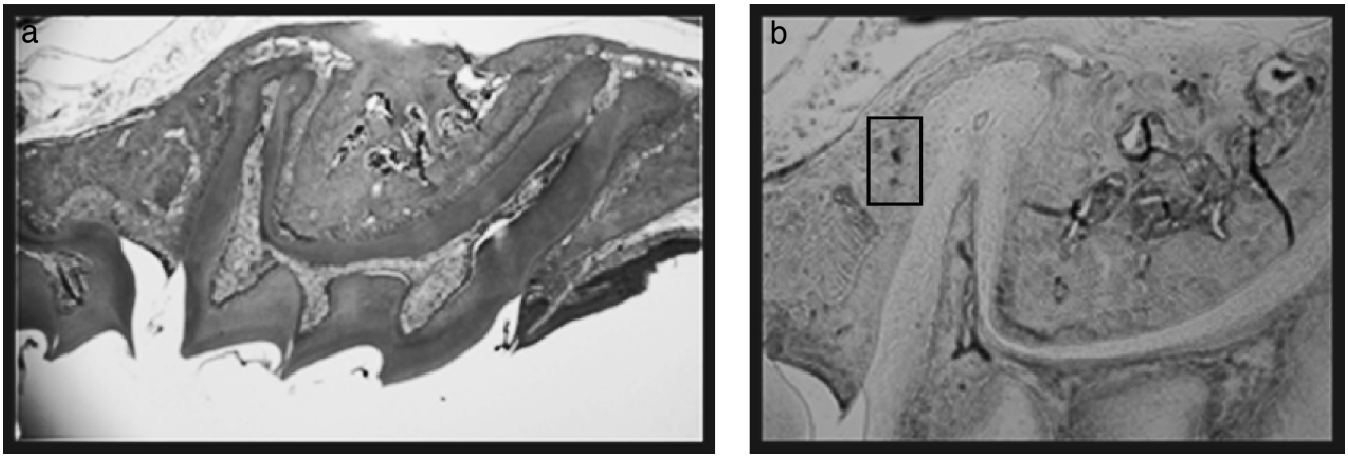

Figure 8. Histological analysis of a wild-type mouse without orthodontic force. (a) Hematoxylin and eosin staining: normal PDL. (b) TRAP staining: osteoclasts on the distal area of the distal root.

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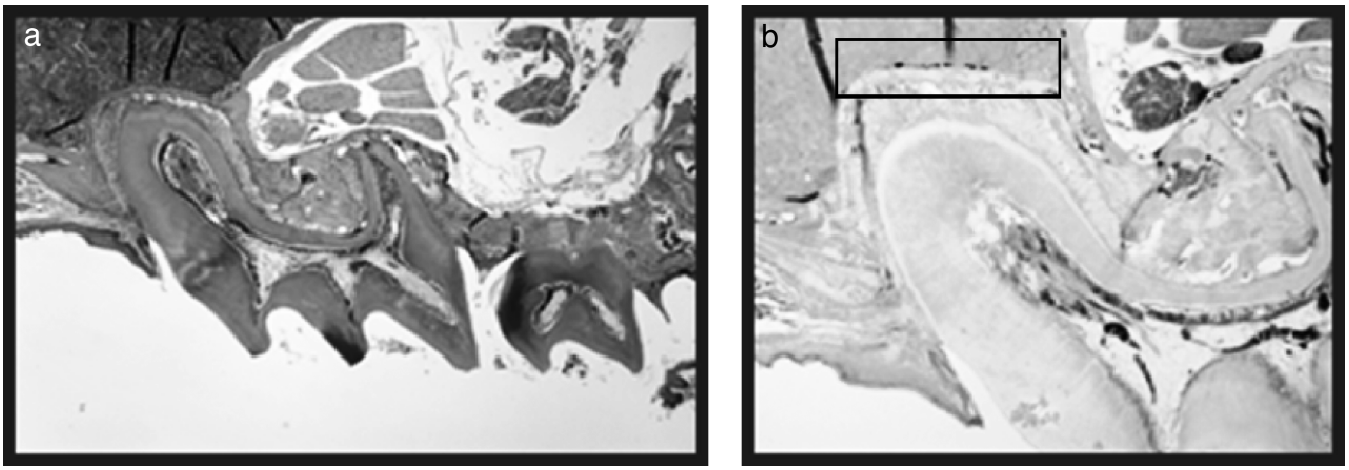

Figure 9. Histological analysis of a mutant mouse with orthodontic force. (a) Hematoxylin and eosin staining: no hyalinized zone. (b) TRAP staining: osteoclasts are observed in the bone marrow.

the molecular mechanism behind bone remodeling can be further clarified.

CONCLUSIONS

- These results suggest a difference in tooth movement and osteoclast activity between PC1 mutant mice and wild-type mice in response to orthodontic force.
- The impaired tooth movement and the lack of osteoclasts on the bone surface in the mutant working side may be related to lack of signal from the PDL due to PC1 deficiency.

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