Periodontal Defects in the A116T Knock-in Murine Model of Odontohypophosphatasia

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Abstract

Mutations in *ALPL* result in hypophosphatasia (HPP), a disease causing defective skeletal mineralization. *ALPL* encodes tissue nonspecific alkaline phosphatase (ALP), an enzyme that promotes mineralization by reducing inorganic pyrophosphate, a mineralization inhibitor. In addition to skeletal defects, HPP causes dental defects, and a mild clinical form of HPP, odontohypophosphatasia, features only a dental phenotype. The *Alpl* knockout (*Alpl⁻/⁻*) mouse phenocopies severe infantile HPP, including profound skeletal and dental defects. However, the severity of disease in *Alpl⁻/⁻* mice prevents analysis at advanced ages, including studies to target rescue of dental tissues. We aimed to generate a knock-in mouse model of odontohypophosphatasia with a primarily dental phenotype, based on a mutation (c.346G>A) identified in a human kindred with autosomal dominant odontohypophosphatasia. Biochemical, skeletal, and dental analyses were performed on the resulting *Alpl⁺/A116T* mice to validate this model. *Alpl⁺/A116T* mice featured 50% reduction in plasma ALP activity compared with wild-type controls. No differences in litter size, survival, or body weight were observed in *Alpl⁺/A116T* versus wild-type mice. The postcranial skeleton of *Alpl⁺/A116T* mice was normal by radiography, with no differences in femur length, cortical/trabecular structure or mineral density, or mechanical properties. Parietal bone trabecular compartment was mildly altered. *Alpl⁺/A116T* mice featured alterations in the alveolar bone, including radiolucencies and resorptive lesions, osteoid accumulation on the alveolar bone crest, and significant differences in several bone properties measured by micro–computed tomography. Nonsignificant changes in acellular cementum did not appear to affect periodontal attachment or function, although circulating ALP activity was correlated significantly with incisor cementum thickness. The *Alpl⁺/A116T* mouse is the first model of odontohypophosphatasia, providing insights on dentoalveolar development and function under reduced ALP, bringing attention to direct effects of HPP on alveolar bone, and offering a new model for testing potential dental-targeted therapies in future studies.

Keywords: alkaline phosphatase, hypophosphatasia, bone, cementum, dentin, periodontium

Introduction

Loss-of-function mutations in *ALPL* result in hypophosphatasia (HPP), an inborn error of metabolism that features defective mineralization of the skeleton and dentition (Whyte 2012). *ALPL* encodes tissue nonspecific alkaline phosphatase (TNAP; alternately, TNSALP), an enzyme that reduces local concentrations of the mineralization inhibitor, inorganic pyrophosphate (Millán 2013). Skeletal complications of HPP include rickets and osteomalacia, with clinical severity ranging widely from profound skeletal hypomineralization that is lethal at birth to dental defects alone. Dental manifestations include cementum deficiency, tooth loss, thin dentin, widened pulp chambers, malformed roots, and enamel alterations (Foster, Nociti, et al. 2014; Foster, Ramnitz, et al. 2014). Dental hard tissues seem exceptionally sensitive to HPP, as the clinical form odontohypophosphatasia affects only the dentition (Reibel et al. 2009).

The *Alpl* knockout (*Alpl⁻/⁻*) mouse recapitulates the metabolic and skeletal phenotype of severe infantile HPP (Narisawa et al. 1997; Fedde et al. 1999). We demonstrated in *Alpl⁻/⁻* mice

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defects in cementum, dentin, alveolar bone, and enamel (McKee et al. 2011; Foster et al. 2012; Yadav et al. 2012; Foster, Nagatomo, et al. 2013; Zweifler et al. 2015). However, these mice die by 2 to 3 wk of age, preventing analysis of milder forms of HPP at advanced ages, including studies to rescue dental tissues.

We aimed to create a knock-in mouse model of odontohypophosphatasia. The *Alpl* c.346G>A mutation, predicting an A116T substitution, was selected for knock-in based on homology with the predominantly dental phenotype of a large well-characterized HPP kindred (Hu et al. 2000) and dominant-negative effect in vitro of the causal mutation (Lia-Baldini et al. 2001; Fauvert et al. 2009; Ishida et al. 2011). We hypothesized that a mouse heterozygous for the A116T mutation ($Alpl^{+/A116T}$) would phenocopy odontohypophosphatasia and serve as a model for further studies of HPP dental disease.

Materials and Methods

Knock-in Vector Design and Synthesis

Mouse experiments were approved by the Institutional Animal Care and Use Committee of the Sanford Burnham Medical Research Institute (La Jolla, CA, USA). Methods for vector design and in vitro expression analysis are detailed in the Appendix. The targeting construct to introduce the c.346G>A base change into exon 5 of *Alpl* is shown in Figure 1A. Sequencing confirmed germline transmission (Fig. 1B). The $Alpl^{A116T}$ mouse line was maintained by breeding wild-type (WT) mice with heterozygote mice ($Alpl^{+/A116T}$). $Alpl^{+/A116T}$ and WT mice were analyzed at 27 d postnatal (dpn) and 4 and 14 mo.

Plasma Chemistry Analysis

Blood was collected by cardiac puncture, transferred into lithium-heparinized tubes (Becton, Dickinson & Co., Franklin Lakes, NJ, USA), and plasma was separated by centrifugation at $3,000 \times g$ for 10 min. Alkaline phosphatase (ALP) activity, phosphorus, and calcium were measured using a VetScan Comprehensive Diagnostic Profile rotor (Abaxis, Union City, CA, USA).

Radiography

Radiographs of skeletons were obtained with an MX-20 Specimen Radiographic System (Faxitron X-ray Corp., Chicago, IL, USA) and inspected by 2 blinded independent analysts familiar with HPP skeletal disease. Femoral length was measured using MicroDicom software (Sofia, Bulgaria). Hemimandibles were scanned in a cabinet X-ray (Faxitron X-ray Corp.) at 30 kV for 40 s.

Micro–computed Tomography

Methods for micro–computed tomography (micro-CT) analysis are detailed in the Appendix. Femora were scanned on a Skyscan 1076 micro-CT scanner (Kontich, Belgium), and

regions of interest were determined using established guidelines (Bouxsein et al. 2010). Skulls were scanned on an eXplore Locus SP micro-CT scanner (GE Healthcare, London, ON, Canada), and regions of interest for the parietal and frontal bones were determined as described previously (Liu et al. 2013) and measured using established algorithms (Meganck et al. 2009; Umoh et al. 2009). For dentoalveolar analysis, dissected mandibles were scanned on a Scanco Medical μCT 35 (Scanco Medical AG, Brüttisellen, Switzerland). DICM files were reoriented using ImageJ software (1.48r), with coronal, sagittal, and transverse planes of section chosen for comparison.

Parameters analyzed by microCT included total cross-sectional tissue area (Tt.Ar), cortical bone area (Ct.Ar), cortical area fraction (Ct.Ar/Tt.Ar), cortical thickness (Ct.Th), tissue mineral density (TMD), tissue volume (TV), bone surface (BS), bone volume (BV), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), trabecular number (Tb.N), structure model index (SMI), and bone mineral density (BMD), bone mineral content (BMC), and tissue mineral content (TMC) (Bouxsein et al. 2010).

Three-point Bone Bending

Three-point bone bending was performed to determine the mechanical properties of the femur, using an Instron 3342 material testing machine (Instron, Norwood, MA, USA) fitted with a 100-N load cell. The span was fixed at 10 mm, and the cross-head was lowered at 1 mm/min. Load (N) and extension (mm) were recorded every 0.2 s until fracture. Maximum stiffness, work to fracture, and failure and fracture points were calculated from load-extension curves as described previously (Aspden 2003).

Histology

Mandibles used for histology were decalcified in AFS (10% v/v glacial acetic acid, 4% v/v neutral buffered formalin, and 0.85% w/v sodium chloride in water) by stirring at 4 °C for 3 to 4 wk, then processed in paraffin to make serial 6-μm sections. To evaluate periodontal ligament collagen fiber organization, sections were stained by the picrosirius red method with 0.2% phosphomolybdic acid hydrate, 0.4% Direct Red 80, and 1.3% 2,4,6-trinitrophenol (Polysciences, Inc., Warrington, PA, USA), as described previously (Foster 2012). Staining for tartrate-resistant acid phosphatase to identify osteoclasts was performed following the manufacturer's instructions (Wako Chemicals, Japan). Briefly, deparaffinized sections were incubated at 37 °C for 60 min with staining solution containing sodium tartrate, followed by counterstaining with nuclear stain, air-drying, and mounting.

Immunohistochemistry was performed on histologic sections using an avidin-biotinylated peroxidase-based kit (Vectastain Elite, Vector Labs, Burlingame, CA) with a 3-amino-9-ethylcarbazole substrate (Vector Labs) to produce a red product. Primary antibodies included rat monoclonal anti-human ALPL (R&D Systems, Minneapolis, MN, USA;
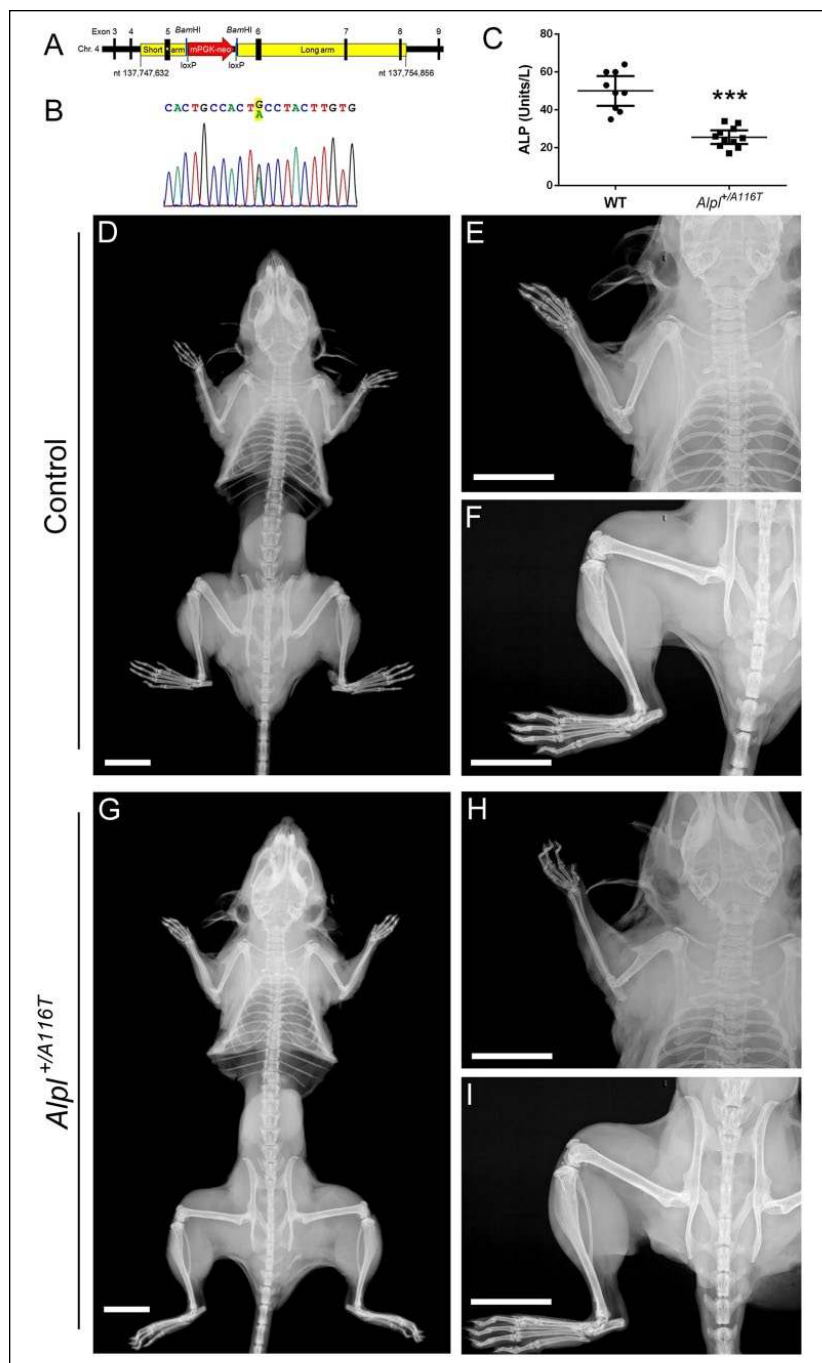
Figure 1. Postcranial bone phenotype is not altered by the *Alpl* A116T mutation in mice. (**A**) Schematic representation of the targeting construct used to introduce the c.346G>A (A116T) mutation into the *Alpl* locus. The construct consists of short and long arms (yellow) separated by a neomycin gene under the control of the mouse phosphoglycerol kinase promoter (red), flanked by loxP recognition sites (green lines), inserted into a *Bam*HI restriction site (blue lines) in intron 5 of *Alpl*. The nucleotide positions (nt) of the ends of the construct on chromosome 4 are given. (**B**) Electropherogram of DNA extracted from knock-in mice showing successful knock-in of the c.346G>A (highlighted) mutation into the *Alpl* locus. (**C**) Plasma collected from wild-type (WT; *n* = 7) and *Alpl*[+/A116T] (*n* = 9) mice at 120 d postnatal showed a 50% decrease in mean alkaline phosphatase (ALP) activity in *Alpl*[+/A116T] mice (***P < 0.001 by 2-tailed Student's *t* test). Values are reported on the scatter plot as individual measurements (mean ± 95% confidence interval). (**D–I**) Radiography of WT and *Alpl*[+/A116T] heterozygous male mice at 120 d postnatal showing whole animals (D, G), left forelimbs (E, H), and left hind limbs (F, I). No overt skeletal pathology was detected in *Alpl*[+/A116T] mice at this age. Scale bar indicates 10 mm for all panels (C–H).

Zweifler et al. 2015), rabbit polyclonal anti-mouse bone sialoprotein (Dr. Renny Franceschi, University of Michigan, Ann Arbor, MI, USA; Foster, Soenjaya, et al. 2013), rabbit polyclonal LF-175 anti-mouse osteopontin (Dr. Larry Fisher, National Institute of Dental and Craniofacial Research, Bethesda, MD, USA; Foster 2012), goat polyclonal anti-mouse receptor activator of nuclear factor kappa-B ligand (RANKL; R&D Systems), and rabbit polyclonal anti-human periostin (POSTN; Abcam Inc., Cambridge, MA, USA).

Samples used for undecalcified sectioning were fixed in paraformaldehyde, embedded in methylmethacrylate, and von Kossa stained as described previously (Foster, Soenjaya, et al. 2013).

Histomorphometry

Acellular cementum width was measured on lingual and buccal surfaces of the first mandibular molar mesial root 100 μm from the cementoenamel junction and on lingual surfaces of incisors. Mean values for WT (*n* = 5 to 6) and *Alpl*[+/A116T] (*n* = 7 to 9) cementum thickness were compared by the independent-samples *t* test. Serum ALP levels were correlated to incisor and molar acellular cementum thickness using Pearson's *r* coefficient. Cellular cementum area was measured using ImageJ software. Alveolar bone height was quantitated as distance from cementoenamel junction to the alveolar bone crest on the lingual and buccal aspects. Statistical analyses were performed using GraphPad Prism 6.01 (La Jolla, CA, USA).

Results

Alpl A116T Mutation in Mice Reduces Their Plasma ALP Activity

No significant differences in litter size, survival to weaning, or survival to 120 dpn were observed between WT and *Alpl*[+/A116T] male mice (data not shown). Body weight did not differ significantly between genotypes (*P* > 0.05), averaging 31.6 ± 1.4 g for WT and 30.4 ± 0.6 g for *Alpl*[+/A116T] male mice at 120 dpn.

Plasma biochemistry analysis indicated no differences between genotypes in calcium and phosphorus concentrations (Appendix Table 2). However, ALP activity was significantly reduced in *Alpl*[+/A116T]

versus WT mice (Fig. 1C, Appendix Table 2). Mean ALP was reduced by 50% in $Alpl^{+/A116T}$ mice (25.6 ± 5.4 vs. 50 ± 10.2 U/L in WT), with ALP of individual heterozygotes ranging from 34% to 68% of the WT mean. This range in plasma ALP in $Alpl^{+/A116T}$ mice reflects a similarly broad range reported in the human kindred (Hu et al. 2000). Notably, ALP in $Alpl^{+/A116T}$ mice was higher than the activity observed in vitro in cotransfected cells (with WT and mutant alleles), which produced only 32.5% of WT activity (Appendix Table 3).

Postcranial Skeletal Phenotype Is Not Altered in Alpl$^{+/A116T}$ Mice

By radiography, no differences were observed between postcranial skeletons (i.e., the skeleton lying posterior to the skull) of WT and $Alpl^{+/A116T}$ mice at 120 dpn, including no evidence of malformations, fractures, rickets, calcific periarthritis, chondrocalcinosis, or pseudogout (Fig. 1D–I). Femur length did not differ ($P > 0.05$) between groups (17.1 ± 0.1 for both WT and $Alpl^{+/A116T}$). Similarly, radiographic analysis at age 14 mo did not reveal any skeletal abnormalities (data not shown). Micro-CT analysis of femora indicated no differences in cortical or trabecular bone structure or density (Appendix Table 4). Three-point bending analysis indicated no differences in failure or fracture loads, work to fracture, or stiffness between WT and $Alpl^{+/A116T}$ femurs (Appendix Table 5).

Alterations in Cranial Bones in Alpl$^{+/A116T}$ Mice

Frontal bones of $Alpl^{+/A116T}$ mice were not different from WT mice (Appendix Table 6). Parietal bone mineral content, bone mineral density, tissue mineral content, and BV/TV were not different from controls. However, BS/BV and trabecular number were increased, while Tb.Th was decreased ($P < 0.05$ for all) in $Alpl^{+/A116T}$ compared to WT mice.

Alpl$^{+/A116T}$ Mice Feature Alveolar Bone Defects

$Alpl^{-/-}$ mice manifest mineralization defects in alveolar bone, dentin, cementum, and enamel (Millán et al. 2008; McKee et al. 2011; Foster et al. 2012; Yadav et al. 2012; Foster,
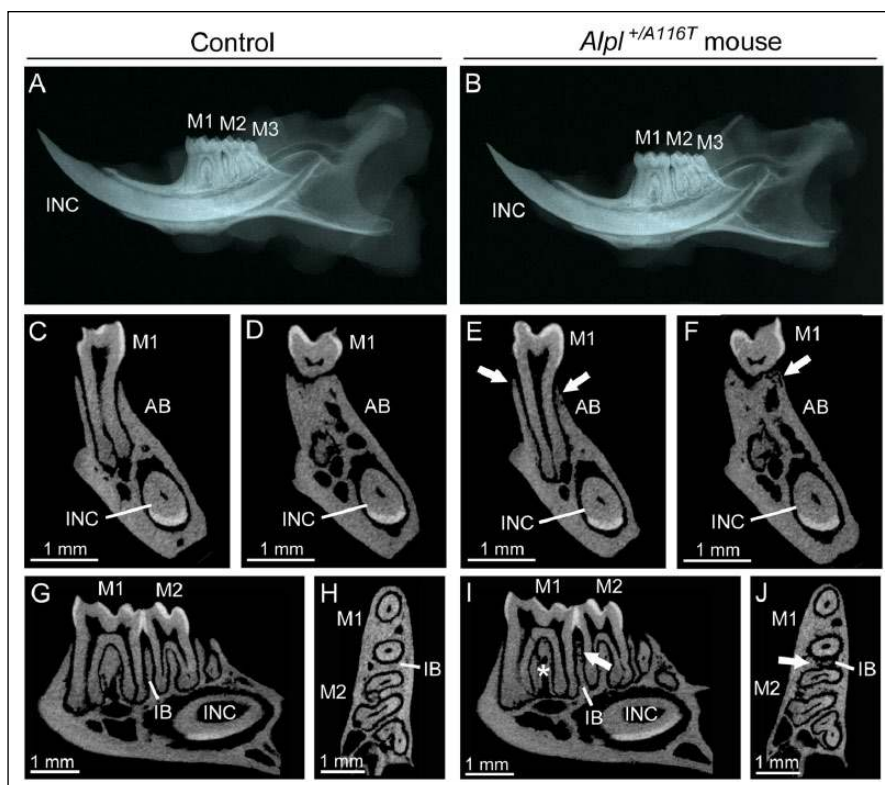
Figure 2. $Alpl^{+/A116T}$ mice feature alveolar bone (AB) defects. (**A**, **B**) Radiography reveals no overt differences in wild-type control versus $Alpl^{+/A116T}$ mandibles, molars (M1 to M3), or incisors (INC) at 120 d postnatal. (**C**, **E**) Micro–computed tomography (micro-CT) cut sections in the coronal plane at the first molar mesial root indicate radiolucency (white arrows in E) at the alveolar bone crest in $Alpl^{+/A116T}$ versus wild-type controls. (**D**, **F**) Micro-CT coronal plane sections at the first molar root furcation region reveal lesions consistent with osteoclastic resorption (white arrows in F) in the alveolar bone of $Alpl^{+/A116T}$ mice. (**G**, **I**) Micro-CT sagittal plane sections show alterations in alveolar bone in the furcation region (white asterisk in I) and resorptive type lesions in interproximal bone (IB) between first and second molars of $Alpl^{+/A116T}$ mice (white arrow in I). (**H**, **J**) Micro-CT transverse plane sections located 500 μm apical to the cementoenamel junction show extensive loss of interproximal bone between first and second molar roots (white arrow in J).

Nagatomo, et al. 2013). The Appendix Figure is included for reference to dental defects in $Alpl^{-/-}$ mice.

No gross differences in $Alpl^{+/A116T}$ versus WT mandibles were indicated by radiography at 120 dpn (Fig. 2A, B). Micro-CT revealed radiolucency in alveolar bone surrounding the first molars of $Alpl^{+/A116T}$ mice, while molars and incisors appeared unaltered (Fig. 2C vs. E). $Alpl^{+/A116T}$ mouse alveolar bone featured regions consistent with resorptive lesions in lingual and interproximal bone surrounding first and second molars (Fig. 2D vs. F, G vs. I, H vs. J).

Quantitative micro-CT analysis was performed on 2 regions of alveolar bone associated with first molars (Table). In the furcation region, $Alpl^{+/A116T}$ mice featured significantly reduced alveolar bone tissue mineral density ($P < 0.01$) indicating bone hypomineralization and decreased Tb.Th ($P < 0.05$) and increased BS/BV ($P < 0.05$) indicating alterations in trabecular bone (similar to patterns noted for parietal bone in Appendix Table 6) compared with WT. In lingual alveolar bone, $Alpl^{+/A116T}$ mice featured significantly reduced TV ($P < 0.05$), BV ($P < 0.01$), and BV/TV ($P < 0.05$), reflecting reduced bone in this region. Micro-CT analysis of dental tissues revealed reduced

Table. Micro–computed Tomography Analysis of Alveolar Bone.

Parameter	Furcation		Lingual Aspect	
	WT	*Alpl*[+/A116T]	WT	*Alpl*[+/A116T]
TV, mm³	0.31 ± 0.03	0.30 ± 0.02	0.13 ± 0.01	0.12 ± 0.01[a]
BV, mm³	0.20 ± 0.02	0.19 ± 0.01	0.11 ± 0.01	0.09 ± 0.02[b]
BV/TV, %	64.78 ± 5.56	62.57 ± 2.90	82.09 ± 3.50	73.65 ± 8.81[a]
TMD, g HA/cm³	1.07 ± 0.02	1.04 ± 0.01[b]	0.98 ± 0.02	0.96 ± 0.02
Tb.N, 1/mm	6.68 ± 0.53	7.16 ± 0.92	N/A	N/A
Tb.Th, mm	0.15 ± 0.02	0.13 ± 0.01[a]	N/A	N/A
Tb.Sp, mm	0.19 ± 0.02	0.17 ± 0.03	N/A	N/A
BS/BV, mm²/mm³	19.55 ± 1.87	21.55 ± 1.07[a]	N/A	N/A

Alveolar bone associated with first mandibular molars was compared at 120 d postnatal in wild-type (WT; $n = 7$) and *Alpl*[+/A116T] mice ($n = 9$). Values are reported as means ± SD.
BS, bone surface; BV, bone volume; Tb.N, trabecular number; Tb.Sp, trabecular spacing; Tb.Th, trabecular thickness; TMD, tissue mineral density; TV, tissue volume.
[a]$P < 0.05$. Independent-samples *t* test.
[b]$P < 0.01$. Independent-samples *t* test.

BV/TV for *Alpl*[+/A116T] mouse molar enamel and root dentin but no other differences (Appendix Table 7).

Delays in *Alpl*[+/A116T] mouse alveolar bone mineralization were indicated by accumulation of osteoid (approximately 10 to 30 μm thick) on alveolar bone crests (Fig. 3A–D), consistent with osteomalacia in the radiolucent regions observed by micro-CT. Previously, we reported increased osteopontin expression as a causative factor in mineralization defects in *Alpl*^{-/-} mice (Harmey et al. 2004; Harmey et al. 2006; McKee et al. 2011; Foster, Nagatomo, et al. 2013). In *Alpl*[+/A116T] mice, however, osteopontin deposition appeared unaltered in teeth and surrounding bone (Fig. 3E–H). TNAP distribution by immunohistochemistry also appeared normal in the periodontium of *Alpl*[+/A116T] mice versus controls (Fig. 3I vs. K). Because alterations in mineralization of tooth or bone, including HPP, can lead to defective periodontal attachment, picrosirius red staining was performed to evaluate collagen fiber organization. PDL fibers were observed to be well organized, with no insertion defects identified at the tooth or bone surfaces of *Alpl*[+/A116T] mice (Fig. 3J vs. L).

Areas of apparent bone resorption were also determined on histologic sections. *Alpl*[+/A116T] mice at 120 dpn featured localized alveolar bone resorption associated with numerous tartrate-resistant acid phosphatase–positive osteoclast-like cells (Fig. 3M, O). RANKL was identified by immunohistochemistry in some cells bordering areas of resorption, but the levels were not remarkable in *Alpl*[+/A116T] versus WT tissues (data not shown). Areas of extensive resorption featured a mix of connective tissue with fibroblasts, blood vessels, and marrow space, indicated by periostin immunostaining (Fig. 3N, P). Alveolar bone resorption was not observed in *Alpl*[+/A116T] mice by micro-CT or histology at 27 dpn (data not shown).

In light of an apparent increase in resorption in *Alpl*[+/A116T] alveolar bone, histomorphometry was performed to search for changes in alveolar bone height. No differences were identified in alveolar bone height in *Alpl*[+/A116T] compared to WT (data not shown; $P = 0.74$ and 0.78 for lingual and buccal aspects, respectively).

Acellular Cementum Thickness Is Correlated with Serum ALP

HPP causes absent or reduced acellular cementum formation (as in the *Alpl*^{-/-} mouse; Appendix Fig.; Foster, Nociti, et al. 2014). We confirmed presence of both acellular and cellular cementum by histology (Fig. 4A–H) and immunohistochemistry (e.g., Fig. 3F, H). However, acellular cementum appeared thin in *Alpl*[+/A116T] mouse molars and incisors, and histomorphometry suggested a nonsignificant trend toward decreased thickness compared to WT mice ($P = 0.09$ for incisor cementum; $P = 0.07$ and 0.14 for molar lingual and buccal acellular cementum, respectively; Fig. 4I). Cellular cementum featured hypomineralized regions (cementoid), confirmed by von Kossa staining at 27 dpn (Fig. 4G inset vs. 4E inset). Cellular cementum area was not altered in *Alpl*[+/A116T] versus controls ($P = 0.46$ and 0.40 for buccal and lingual sides, respectively; Fig. 4J). Plasma levels of circulating residual ALP activity were correlated positively with incisor acellular cementum thickness in WT and *Alpl*[+/A116T] mice ($P = 0.03$), with nonsignificant "trends" ($P = 0.05$ and 0.09) in molar acellular cementum thickness (Fig. 4K, L).

Discussion

To define the long-term effects of relatively mild HPP on the mineralized dentoalveolar tissues, we generated the first knock-in mouse model of HPP. Our model featured a c.346G>A point mutation in exon 5 of *Alpl*, resulting in an A116T amino acid substitution. *Alpl*[+/A116T] mice featured a mean 50% reduction in plasma ALP, with individual values ranging from 34% to 68% of WT. Postcranial skeletal elements were unaffected in *Alpl*[+/A116T] mice, and parietal bones in the skull were mildly affected in diploe (trabecular) measurements. The A116T mutation caused hypomineralization of mouse alveolar bone and cellular cementum, with a trend of reduced acellular cementum. Acellular cementum of molars and incisors was correlated with plasma ALP activity.
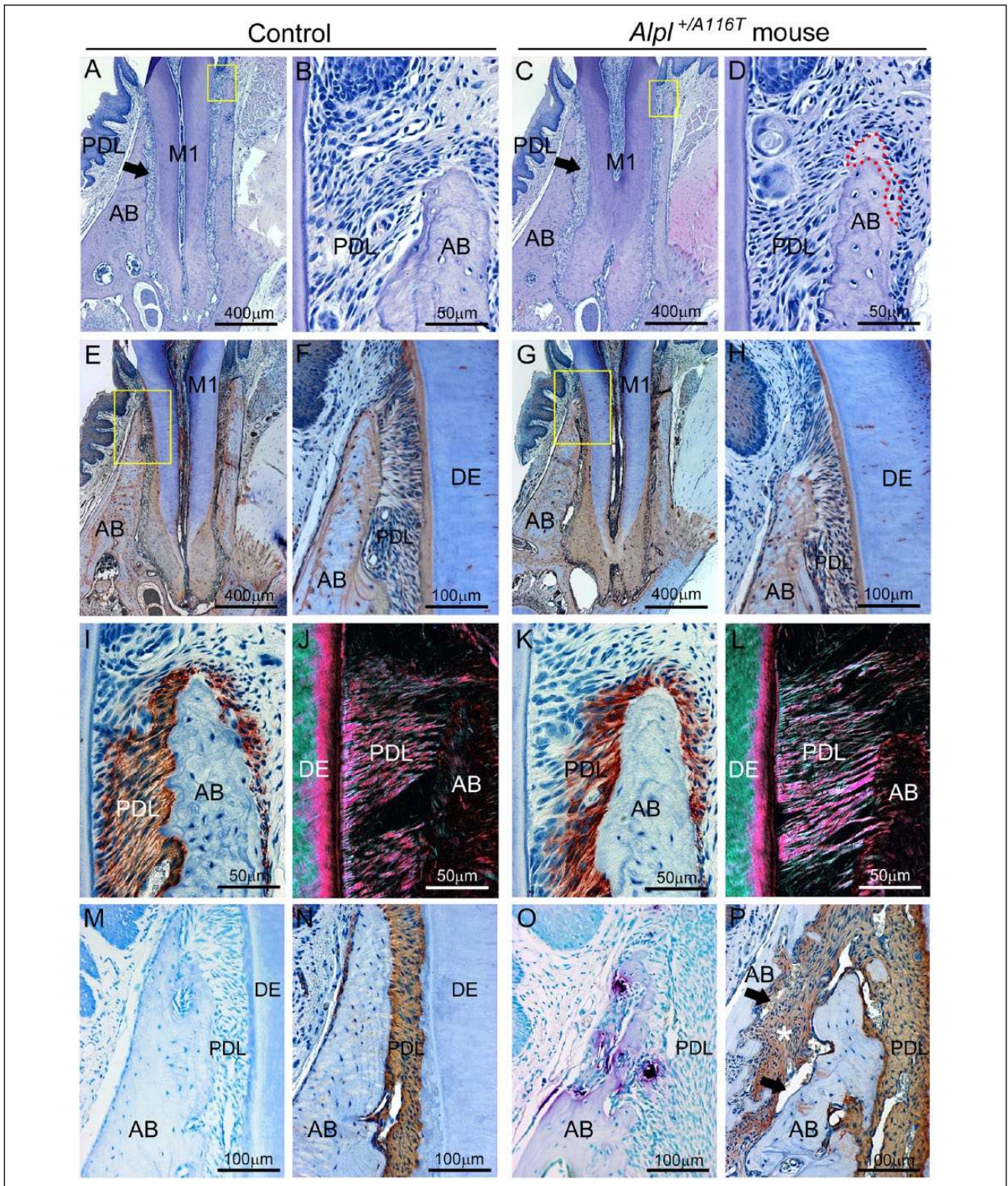
Figure 3. Delayed mineralization and increased resorption in alveolar bone of $Alpl^{+/A116T}$ mice. (**A–D**) Histologic sections of mandibular dentoalveolar tissues from 120 d postnatal mice feature overtly normal first molars (M1), while $Alpl^{+/A116T}$ mice feature accumulation of osteoid (area outlined in dotted red line in D) at the alveolar bone (AB) crest. Yellow boxes in A and C represent regions shown in B and D, respectively. (**E–H**) Immunohistochemistry (IHC) reveals no differences in osteopontin localization in tooth or bone of $Alpl^{+/A116T}$ mice versus wild-type controls. Yellow boxes in E and G represent regions shown in F and H, respectively. No differences in $Alpl^{+/A116T}$ versus wild type are noted for (**I, K**) tissue nonspecific alkaline phosphatase intensity or localization by IHC, or (**J, L**) collagen organization of the periodontal ligament (PDL), as shown by picrosirius red staining under polarized light microscopy. (**M, O**) Regions of bone resorption in $Alpl^{+/A116T}$ mice are associated with numerous TRAP-positive osteoclast-like (purple-red, multinucleated) cells on the bone surface. (**N, P**) IHC for periostin indicates that regions of extensive alveolar bone resorption in $Alpl^{+/A116T}$ tissues feature a mix of connective tissue (white star) and blood vessels (black arrows). DE, dentin.
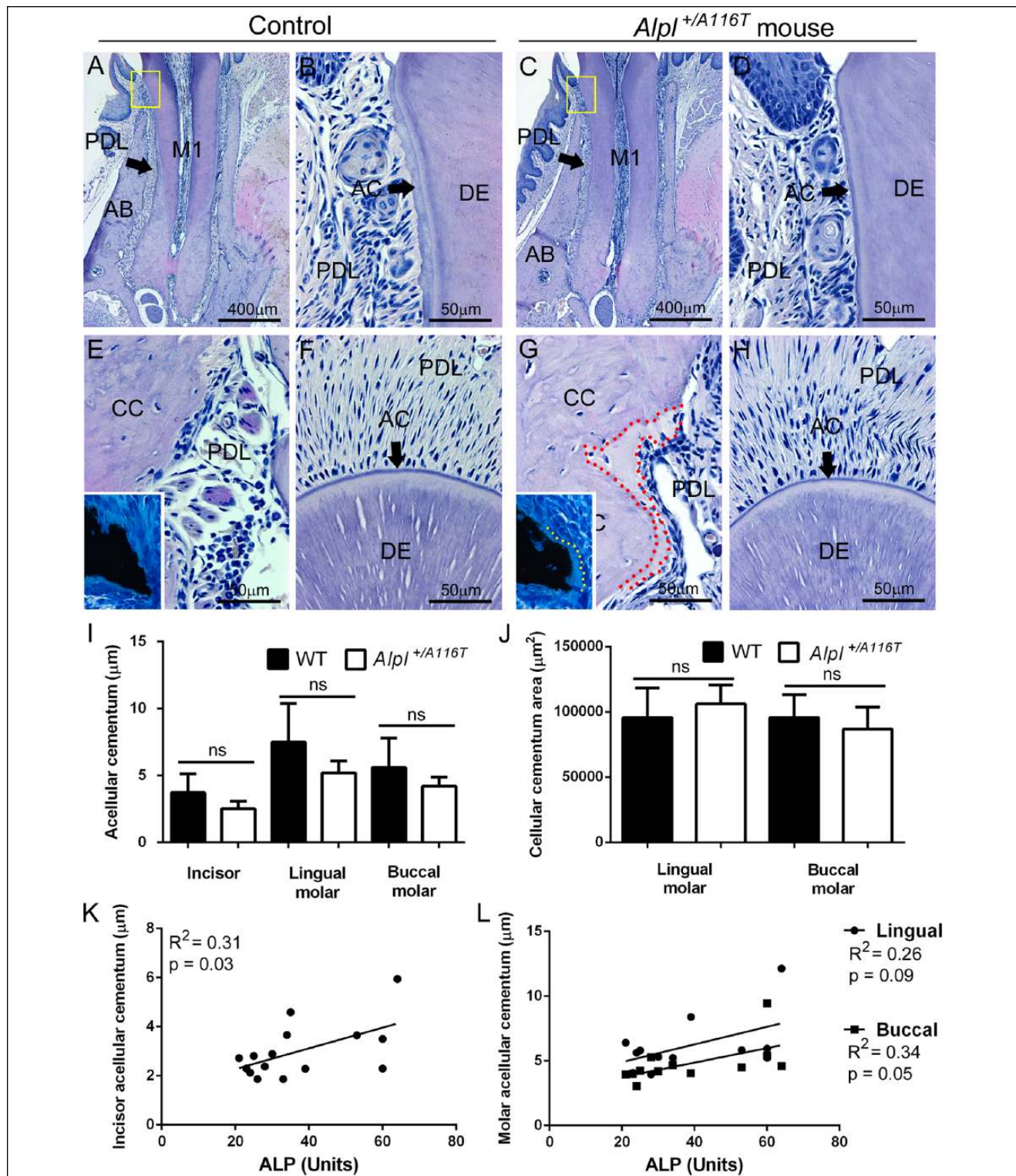
Figure 4. Acellular cementum thickness is correlated with serum alkaline phosphatase (ALP). (**A–D**) Histologic sections of mandibular first molar teeth (M1) from 120 d postnatal *Alpl*$^{+/A116T}$ mice suggest thin acellular cementum (AC; arrows where notated) compared with controls. Yellow boxes in A and C represent regions shown in B and D, respectively. (**E**, **G**) Compared with well-mineralized wild-type (WT) cellular cementum (CC), *Alpl*$^{+/A116T}$ mice feature an accumulated cementoid layer (area outlined in dotted red line in G). Insets in E and G show von Kossa staining, where *Alpl*$^{+/A116T}$ mouse molars feature a thick layer of unmineralized cementoid not observed in WT mice. (**F**, **H**) The acellular cementum of the *Alpl*$^{+/A116T}$ mandibular incisor appears thin compared with WT. (**I**) Histomorphometry of acellular cementum width in first molars (bucal and lingual aspects) and incisors reveals no statistically significant differences in *Alpl*$^{+/A116T}$ versus WT mice ($P > 0.05$; not significant [ns]). (**J**) Histomorphometry of cellular cementum area in first molars (buccal and lingual aspects) reveals no differences in *Alpl*$^{+/A116T}$ versus WT mice. (**K**) Serum ALP is significantly positively correlated with incisor acellular cementum width ($P = 0.03$), with nonsignificant positive relationships with (**L**) molar acellular cementum on lingual ($P = 0.09$) and buccal ($P = 0.05$) aspects. DE, dentin; PDL, periodontal ligament.

Dental Disease in HPP

The severity of human HPP is remarkably broad-ranging and spans life-threatening forms (infantile and perinatal) to milder forms (prenatal benign, childhood, adult, and odontohypophosphatasia; Whyte 2012). Skeletal defects include rickets during growth or osteomalacia in adult life, fractures, and bone pain. Dental defects commonly manifest in HPP, affecting cementum, dentin, enamel, and periodontal bone (Foster, Nociti, et al. 2014; Foster, Ramnitz, et al. 2014). We hypothesized that murine dental hard tissues are exceptionally sensitive in HPP because odontohypophosphatasia affects only the dentition, despite characteristic serum biochemical findings (Reibel et al. 2009). At present, 280 *ALPL* mutations have been identified for HPP (www.sesep.uvsq.fr), with often unclear genotype-phenotype relationships, including that for odontohypophosphatasia, where 21 *ALPL* mutations have been recorded within 8 of the 12 exons. Mild HPP can result from heterozygosity for missense mutations with a dominant-negative effect (i.e., inhibiting the enzymatic activity of the heterodimer), and these mutations sometimes localize to domains of TNAP affecting dimerization or allosteric properties (Fauvert et al. 2009).

The $Alpl^{-/-}$ mouse, featuring loss of TNAP, has been a useful model for studying the skeletal manifestations of the severe infantile form of HPP (Narisawa et al. 1997; Fedde et al. 1999). We have analyzed the dental pathologies in the $Alpl^{-/-}$ mouse, demonstrating inhibition of acellular cementum formation, delayed alveolar bone mineralization, disruption of odontoblast function and dentin mineralization, and enamel defects (McKee et al. 2011; Foster et al. 2012; Yadav et al. 2012; Foster, Nagatomo, et al. 2013; Zweifler et al. 2015). However, the severity of disease and shortened life span of these mice prevent long-term studies of the HPP skeleton and dentition, including investigations to rescue dental tissues.

A116T Mouse Model for Odontohypophosphatasia

To generate a mouse model of mildly decreased ALP and odontohypophosphatasia, we selected the *Alpl* c.346G>A point mutation, corresponding to an A116T substitution in a highly conserved residue adjacent to the enzyme active site (Silvent et al. 2014). Previous work by in vitro analysis reported a dominant-negative effect of this mutation (Lia-Baldini et al. 2001; Fauvert et al. 2009; Ishida et al. 2011), and our in vitro expression assays confirmed those findings. Plasma ALP of $Alpl^{+/A116T}$ heterozygotes ranged from 34% to 68% of the WT, with a mean reduction of 50%, consistent with the variation observed in the original HPP kindred (Hu et al. 2000). Broad-ranging ALP is likely related to instances of observed lack of penetrance in human subjects with HPP, and the same applies to mouse models. Still, the discovery of an observable dental phenotype in $Alpl^{+/A116T}$ where ALP is mildly reduced has prompted us to develop a full study on heterozygous $Alpl^{+/-}$ mice, as it is possible that carriers of HPP mutations may not be entirely asymptomatic.

Based on the presentation in this family, we predicted primarily dental manifestations in $Alpl^{+/A116T}$ mice, including defective formation of cementum, dentin, and enamel. Analysis of these $Alpl^{+/A116T}$ mice revealed no detectable defects in the skeleton except for alterations in cranial and alveolar bone and teeth. These findings validate this *Alpl* mutation as primarily affecting the murine craniofacial region. However, the $Alpl^{+/A116T}$ mouse presented a milder-than-expected dental phenotype respective to the clinical presentation in the human kindred. The $Alpl^{+/A116T}$ phenotype was primarily observed as hypomineralization of alveolar bone and cellular cementum, with minor changes in acellular cementum and no significant dentin or enamel defects, or premature loss of teeth. The importance of TNAP to cementogenesis was further supported by a significant positive correlation of incisor acellular cementum to plasma ALP and nonsignificant trends for molars. We previously identified mouse incisors as being more sensitive to disturbance in *Alpl*/TNAP, possibly due to their rapid and continuous formation, and/or an altered P_i:PP_i ratio (i.e., inorganic phosphate to inorganic pyrophosphate; Foster, Nagatomo, et al. 2013; Zweifler et al. 2015).

Two limitations of this model should be acknowledged—namely, the mildly affected periodontal structure and function and the lack of tooth loss. However, the model does provide a viable mouse with dentoalveolar effects of HPP, and several important insights were gained: (1) Teeth and periodontia developed and functioned despite a moderate reduction in ALP; (2) alterations in cementum and correlation of incisor cementum to residual plasma ALP activity supported cumulative data indicating that this tissue is highly dependent on TNAP activity; and (3) alveolar bone was identified as a target tissue in HPP, where emphasis is more often placed on cementum and dentin defects. Observed mineralization defects in alveolar bone in $Alpl^{+/A116T}$ mice may be related to the continuous rapid turnover rate of this tissue compared with other bones (Sodek and McKee 2000), prompting a higher requirement for local TNAP function. The cause for the apparent increase in resorption of alveolar and interproximal bone in $Alpl^{+/A116T}$ mice is not clear, although it may be related to altered tissue properties, constant mechanical loads from chewing, and/or subtle changes in periodontal attachment or function. The fact that resorptive lesions were observed only with advanced age supports the hypothesis that this is a functional response that gradually arises after teeth enter occlusion.

Overall, the $Alpl^{+/A116T}$ mouse model provides valuable insights into the effects of a relatively mild reduction of TNAP activity on the dentoalveolar complex, highlighting changes in alveolar bone and providing a new model for testing potential therapies.

Author Contributions

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