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Mouse Genetic Background Influences the Dental Phenotype

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Abstract

Dental enamel covers the crown of the vertebrate tooth and is considered to be the hardest tissue in the body. Enamel develops during secretion of an extracellular matrix by ameloblast cells in the tooth germ, prior to eruption of the tooth into the oral cavity. Secreted enamel proteins direct mineralization patterns during the maturation stage of amelogenesis as the tooth prepares to erupt. The amelogenins are the most abundant enamel proteins, and are required for normal enamel development. Phenotypic differences were observed between incisors from individual *Amelx* (*Amelogenin*) null mice that had a mixed 129xC57BL/6J genetic background, and between inbred wld-type (WT) mice with different genetic backgrounds (C57BL/6J, C3H/HEJ, FVB/NJ). We hypothesized this could be due to modifier genes, as human patients with a mutation in an enamel protein gene causing the enamel defect amelogenesis imperfecta (AI) also can have varied appearance of dentitions within a kindred. Enamel density measurements varied for all WT inbred strains midway during incisor development. Enamel thickness varied between some WT strains and, unexpectedly, dentin density varied extensively between incisors and molars of all WT and *Amelx* null strains studied. WT FVB/NJ incisors were more similar to Amelx null than to the other WT strains in incisor height/weight ratio and pattern of enamel mineralization. Strain-specific differences led to the conclusion that modifier genes may be implicated in determining both normal development and severity of enamel appearance in AI mouse models and may in future studies be related to phenotypic heterogeneity within human AI kindreds reported in the literature.

Keywords

genetic background; enamel; dentin; amelogenesis imperfecta; amelogenin

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Introduction

Dental enamel is the highly mineralized tissue that is produced by epithelially-derived ameloblast cells, and covers the crowns of vertebrate teeth. Early in development, prior to eruption of the tooth into the oral cavity, a single layer of ameloblasts secretes an organic matrix during the secretory stage of amelogenesis (Slavkin et al.,1982; Bei, 2009). The organic matrix is assembled just after secretion, and is processed by proteases secreted by ameloblasts as the enamel rod structures grow and mature (Bartlett, 2013). Through this process, the ameloblasts determine the intricate organization of developing enamel into rod or prism shaped structures, which have a decussating or crosshatched organization especially visible in rodent teeth (Boyde, 1969). Most of the cleaved peptides are removed as mineral crystals grow, and finally as the tooth erupts into the oral cavity, only a minor amount of enamel protein remains, which is thought to contribute to toughness of the mature enamel layer (He and Swain, 2008). Mature enamel covering the crown of the erupted tooth is the hardest tissue in the human body.

While the organic matrix normally guides organization of the enamel mineralized structures, the process can be interrupted or altered by the presence of abnormal enamel proteins translated from genes with mutations that affect the coding regions (Witkop and Sauk, 1976). Inherited enamel defects termed amelogenesis imperfecta (AI) have been reported due to mutations in the *AMELX* (*Amelogenin*), *ENAM* (*Enamelin)*, *MMP20* (*Matrix Metalloproteinase-20*), and *KLK4* (*Kallikrein 4*) genes (Lagerstrom et al., 1991; Rajpar et al., 2001; Hart et al., 2004; Kim et al., 2005; Ozdemir et al., 2005), which encode secreted structural proteins or proteases, and these mutations lead to a deficit in enamel thickness (hypoplasia) or to enamel structural and compositional anomalies (hypocalcification or hypomaturation). Additional gene mutations in *FAMC83H* and *WDR72*, which encode ameloblast intracellular proteins, are associated with AI (Lee et al., 2008; El-Sayed et al., 2009). Recently C4orf26, which encodes a putative extracellular acidic phosphoprotein, and LAMB3 which encodes a protein previously linked to the syndrome junctional epidermolysis bullosa, have been shown when mutated to be causative for AI (Parry et al., 2012; Kim et al., 2013). In addition, enamel defects can be part of syndromes including junctional epidermolysis bullosa and TDO (tricho-dento-osseous) syndrome (Wright et al., 1993, 1997). Patients with AI can have unsatisfactory esthetics, dental sensitivity and caries due to the enamel structural defects (Markovic et al., 2010).

Numerous reports have described phenotypic differences in patients' enamel resulting from mutations in different enamel related genes (Darling, 1956), and several investigators have reported mutations localized in different regions of the amelogenin gene, encoding the most abundant enamel protein, which alter the respective phenotypes of the defective enamel (Aldred et al., 1992; Lench and Winter, 1995; Wright et al., 2003). However, differences have also been described between multigenerational family members that presumably have the identical mutation (Witkop and Rao, 1971; Backman and Holmgren, 1988; Lench et al., 1994; Lench and Winter. 1995; Nusier et al., 2004; Wright et al., 2009a), but could be related to behavior, nutrition or environment. Differences attributed to X-inactivation can explain only mutations in genes located on the X chromosome, such as *AMELX* (Witkop and Sauk, 1976; Lench et al.,1994). Varying phenotypes noted between teeth in individuals with

autosomal mutations within a single dentition, between primary and secondary teeth within an affected individual, or within individual teeth, as well as the variable presence of AI-associated open bite, are more difficult to explain, and have been reported within families with *ENAM*, *FAM83H* or other autosomal gene mutations (Witkop and Sauk, 1976; Nusier et al., 2004; Wright et al., 2009a). Mice with a heterozygous mutation in the *Enamelin* gene also have varying enamel phenotypes, with nearly normal incisors while molars were discolored and subject to rapid wear (Hu et al., 2008).

To explain this variable phenotype in other tissues, “modifier genes” had been proposed (Haldane, 1941; Witkop and Sauk, 1971; Genin et al., 2008), and in many cases identified (Nadeau, 2001; Buchner et al., 2003; Hamilton and Yu, 2012). Modifier genes may act by altering onset, range of symptoms or clinical severity and have a role in incomplete penetrance (Nadeau, 2003; Tang et al., 2005). In general, genetic modifiers can function by up- or down-regulating expression of genes in a particular pathway, alteration of mRNA stability, DNA methylation or chromatin structure (Linder, 2006).

To better understand whether expression of modifier genes may lead to phenotypic variability in murine teeth, the inbred mouse was chosen for a model as non-dental wild-type (WT) phenotypic and genetic differences between inbred commercially available strains have been documented through genomic DNA sequence determination and the PHENOME project (Wade and Daly, 2005). A major contribution to understanding phenotypic differences between murine teeth was a 2002 report that described levels of susceptibility to dietary fluoride leading to enamel fluorosis, which varied significantly between mouse strains (Everett et al., 2002). Murine bone has also been shown to be differentially susceptible to fluorosis according to strain (Mousney et al., 2006; Everett, 2011). In untreated WT mice, strain-specific differences between femur size, density and mechanical measures have been reported (Beamer et al., 2002; Wergedal et al., 2005).

Mouse strains have been developed to model the human AI phenotypes by generating null mutations in various enamel protein genes and the resulting null mice have dental phenotypes similar to human patients with AI (Wright et al, 2009b). However, we had observed phenotypic differences between individual *Amelx* null mice that were on a mixed 129/SvJxC57BL/6 genetic background, as some mice had nearly normal enamel but others had severe enamel defects, and we suspected modifier genes may be responsible. Interestingly, *Mmp20* null mice developed distinctly different enamel appearance when the mutation was transferred to congenic background strains. The C57BL/6 congenic *Mmp20* null mice have a more severe phenotype compared to REJ129. The prism pattern is less disrupted in the 129 mice and 129 enamel has approximately full thickness, rather than being hypoplastic as in the C57BL/6 background (J. Bartlett, personal communication).

We chose to examine three inbred strains for comparison of wild-type and AI-affected teeth to begin to search for phenotypic differences that could lead to uncovering genes whose expression influences the enamel phenotype. C57BL/6J mice are the most widely used mice for laboratory studies; they are sensitive to fluoride in drinking water and have relatively low bone mineral density (Sacca et al., 2013), C3H/HeJ mice are considered general purpose lab mice; they are also sensitive to fluoride but have high bone mineral density (Sultzer,

1968) and FVB/NJ mice, which have large pronuclei and large litter sizes so they are frequently used in generation of transgenic mice; they are resistant to fluoride and have relatively greater body weight than the other two strains (Taketo et al., 1991; Everett et al., 2002, 2011; Wergedal et al., 2005). Each of these inbred strains has been well characterized during development and in some cases gene mutations or insertions with corresponding pathologies have been identified within the "WT" strains. We in addition transferred the *Amelx* null mutation into these strains in order to examine consequences to the phenotypes as well as phenotypic differences between inbred and mixed strains.

MATERIALS AND METHODS

Murine models

Wild-type (WT) inbred strains were purchased from Jackson Labs (Bar Harbor, ME) and all procedures were done in accordance with regulations of the University of Pennsylvania Institutional Animal Care and Use Committee. *Amelx* null mice on a 129SvJxC57BL/6J genetic background (Gibson et al., 2001) were repeatedly crossed with WTC57BL/6J mice with selection by PCR using tail DNA as described (Li et al., 2008) of mice with the null mutation until greater than generation nine was attained (>99% congenic). *Amelx* null C57BL/6J mice were then mated with WTC3H/HeJ or WTFVB/NJ mice to generate F1 hybrids, that were mated to generate mixed background null mice according to the strategy described by Nadeau (2001) in order to examine the phenotypic effect of altered backgrounds . This strategy resulted in 1 or 2 *Amelx* null pups per litter (25-50% according to the gender of the null mouse in the first generation, as *Amelx* is on the X chromosome). PCR was used to establish WT, heterozygous (+/-) or null phenotypes and weights were determined daily or at the time of euthanasia. *Amelx* heterozygous female mice were not included in measurements but were initially included as a model of elevated phenotypic heterogeneity in incisor enamel.

Phenotypic Analysis

Weight: Mice (n=27-30) were weighed for 21 consecutive days and means determined.

Photographic images were recorded using a Power Shot SX10 IS digital camera (Canon, Inc., Toyko, Japan). Mandibular incisor heights were measured from the incisal edge to gingival margin on the right mandibular incisor using the line tool of ImageJ (Rasband, 2012). Width measurements were taken between the mandibular midline and right incisor's distal edge at the intersection of the middle and gingival thirds. Height/width ratios were calculated to standardize photographic images.

MicroCT analysis for enamel and dentin density

Mandibles were fixed overnight in 4% paraformaldehyde and prepared for analysis as described (Gibson et al., 2011) except that scans were performed on a microtomograph imaging system (μCT 40, Scanco Medical AG, Brüttisellen, Switzerland) with 16 μm resolution at 70 kVp (Pugach et al., 2013). The images were processed by three-dimensional reconstruction software (μCT Evaluation Program v6.0, Scanco Medical) and analyzed for

enamel and dentin density and volume. Hydroxyapatite standards were used for instrument calibration, as described (Pugach et al., 2010).

Incisor measurements were analyzed at two locations. The bone barrel is the incisor location as it exits the mandibular bone for eruption, and the molar barrel is the incisor location subjacent to the first mandibular molar as described (Gibson et al., 2011). The first mandibular molar was analyzed at the position over the mesial root apex.

SEM and enamel thickness

Mandibles were fractured using a razor blade, and SEM analysis of fractured internal enamel and dentin surfaces of incisors and molars was completed at 15 kV (FEI Quanta 200 FEG, FEI, Hillboro, OR, USA or using the JEOL JSM T330A scanning electron microscope as described (Pugach et al., 2010; Gibson et al., 2011).

Statistics

Statistical significance for multiple samples was determined by ANOVA with Bonferroni's Multiple Comparison Test, with significance determined as $P<0.05$ (GraphPad Prism 5; Graph Pad Software, San Diego, CA, USA). For analysis of inbred and mixed background mouse weights, one-sided Student's t test was used for each time point with significance at $P<0.05$.

RESULTS

The common observation that various murine inbred strains have different patterns of weight gain during early development was first confirmed for our mice. The mean weights during the first 3 postnatal weeks of the inbred WTC57BL/6J strain and a WT mixed background (129xC57BL/6J) strain are shown in Fig. 1, where a consistent difference was observed at each time point. 129xC57BL/6J is the original genetic background for *Amelogenin* null (*Amelx* KO) mice (Gibson et al., 2001).

INCISOR PHENOTYPE

The appearance of the incisors from *Amelx* null mice shown in Fig. 2A-D illustrates the heterogeneity in appearance that was observed in our colony. These null mice on a 129xC57BL/6J genetic background do not express any of the amelogenin alternatively spliced transcripts that encode amelogenin proteins (Gibson et al., 2001) yet have distinct variations in appearance, which include color and level of chalkiness, tooth shape and amount of attrition. Male and female *Amelx* null mice had similar levels of phenotypic heterogeneity in incisors. Because some null mice had incisors nearly similar to WT in appearance, we hypothesized that the variation in the mixed genetic background could be linked to this observation, and we therefore transferred the *Amelx* null mutation to a C57BL/6J background by repeated mating with WTC57BL/6J mice and confirmed the genotype by PCR at each cross until the F9 generation was achieved.

The appearance of WT incisor teeth from different inbred strains was then documented as phenotypic differences between strains had anecdotally been reported. We found that

C57BL/6J (Fig. 3A) and C3H/HeJ (Fig. 3B) strains were similar in appearance but the FVB/NJ (Fig. 3C) mice had more pigmented incisor teeth with somewhat different shape. Measurements shown in Fig. 3D are the height/width ratios of mandibular incisors of the three WT strains shown in A,B,C plus the ratios of *Amelx* null C57BL/6J and null C3H/HeJxC57BL/6J strains for comparison. WTC57BL/6J and WTC3H/HeJ are not statistically different ($P>0.05$), while WTFVB/NJ and KOC57BL/6J are also similar to each other but different from the other WT strains.

The *Amelx* null gene on the C57BL/6J background was subsequently transferred to C3H/HeJxC57BL/6J and FVB/NJxC57BL/6J backgrounds according to the strategy described by Nadeau (2001) so that effects of the various strains could be determined by more sensitive means such as microCT and scanning electron microscopy.

INCISOR ENAMEL DENSITY

To better understand the differences between strains, microCT measurements were generated to evaluate density of enamel and dentin at two locations. Fig. 4A shows microCT analysis of WT enamel density at the bone barrel region of incisors for each strain. The bone barrel is located where the incisor exits the mandibular bone at eruption. At this region the measurement for the 129xC57BL/6J mixed background mice varied significantly from the C3H/HeJ and the FVB/NJ strains, but was similar to C57BL/6J. Fig. 4B shows density measurements for the incisor molar barrel region, which is a less mature region of incisor enamel inferior to the first molar. The density readings here vary widely with large standard deviations (SD) and significant difference was observed between FVB/NJ and either C57BL/6J or C3H/HeJ incisor enamel. Examination of the individual measurements led to the realization that the number of zero measurements for enamel density varied between these WT strains, and percent of samples with zero measurement is plotted in Fig. 4C. While there were no zero measurements for C3H/HeJ incisors, the number of zero measures increased to 20% for C57BL/6J to 50% for mixed 129xC57BL/6J background and to nearly 100% for FVB/NJ mice. The *Amelx* null mice were used as a control as they generally do not have mineral at this location of the incisor during development. A comparison of Figs. 4A and 4B shows that delay in incisor enamel mineralization observed in WTFVB/NJ mice seems to recover by the time the tooth erupts.

For each strain, the mean density of incisor enamel was greater at the mandibular bone barrel location compared to the molar barrel site. This increased density at bone barrel would be expected as during normal development, the enamel increases in mineral content as the tooth moves toward eruption. Molar barrel enamel in the incisor was also invariably less dense compared to molar tooth enamel (data not shown).

ENAMEL THICKNESS

Because the enamel layer in *Amelx* null mice is too thin to accurately measure by microCT, SEM images were used to measure enamel thickness in the various WT and *Amelx* null murine strains. Significant differences in enamel thickness were noted between WTC57BL/6J and WTFVB/NJ or WT129xC57BL/6J for incisors and between C57BL/6J and FVB/NJ for molars (Fig. 5 A,B). Although there was variability, significant differences

were not observed between thickness of incisor and molar enamel in any of the strains of *Amelx* null mice, perhaps because the mixed genetic background strains all contained C56BL/6 (C,D). For each WT strain, incisor enamel had greater thickness than the thickest region of molar enamel ($P<0.05$).

DENTIN DENSITY

Using microCT, dentin density was also evaluated in the individual strains for WT and *Amelx* null mice. For WT strains, the dentin differed in density in the mixed 129xC57BL/6J background mice from any of the 3 inbred strains at the bone barrel location (Fig. 6A), as well as for molar dentin (Fig. 6C). At the molar barrel location of the incisor, dentin varied from mixed 129xC57BL/6J background mice for both C57BL/6J and C3H/HeJ (Fig. 6B). Significant differences between dentin density in the *Amelx* null mice were noted for B6 (Fig. 6D), while all strains varied in dentin density at molar barrel location in incisors and in molars (Fig. 6E,F).

DISCUSSION

The AI inherited enamel defect has a heterogeneous phenotype in human patients, which is due partly to mutations in different enamel protein genes, or mutations in different domains of a single enamel protein gene. X-linked mutations may also lead to heterogeneity due to potential lyonization of X-chromosomal genes during enamel development. Yet another level of phenotypic difference has been mentioned repeatedly in the literature in both human kindreds and in mice, which may be attributed to the effects of modifier genes active in ameloblasts during enamel formation.

WT inbred strains are known to differ from each other in various parameters during development and in the adult mice (Linder, 2006; Taft et al., 2006), and we therefore chose three commonly studied but somewhat dissimilar inbred mouse strains to evaluate in order to detect significant differences. In various studies, inbred mouse strains have been reported to vary significantly from each other in weight, developmental timing, size, bone density, etc, and in relation to the effects of genetic mutations. In this study, all mice were treated identically, including availability and type of chow, vivarium humidity, temperature, light and dark cycles and were similar ages when analyzed. Nevertheless, differences in appearance of *Amelx* null incisors in mice with mixed genetic background were obvious, leading us to predict a modifier gene or other source of genotypic difference. These differences were not due to X-chromosomal lyonization, as heterozygous females were not included for the comparison.

Transgenic or null mice generated with mixed genetic backgrounds may be expected to demonstrate greater phenotypic heterogeneity than inbred strains, and the Jackson Laboratory has recommended that these mutations be constructed on mice with well-defined strain background such as C57BL/6J or FVB/NJ (The Jackson Laboratory; Linder, 2006). We noted that the WTFVB/NJ strain has several features more consistent with an *Amelx* null phenotype, including incisor height/width ratio (compare to KOC57BL/6 or KOC3H/HeJ/C56BL/6) and incisor molar barrel density (all KO strains), while WTC3H/HeJ and WTC57BL/6J appearance and microCT analyses are more similar to each other. Differences

between incisor and molar dentin density for most of the WT and *Amelx* null strains was somewhat surprising; variability between WT dental phenotypes can complicate analysis of effects of null mutations or transgenic expression on these backgrounds.

Heterogeneity in human phenotypes due to mutations leading to the various forms of AI has frequently been commented upon, related to differences within and between individual teeth in a patient, between primary and secondary dentition and between affected members of a single kindred. Lyonization, the inactivation of one of the X chromosomes in females, can explain heterogeneity in females with X-linked disease (Nadeau, 2001) but mutations in autosomal genes cannot be explained in this way. The variability of the phenotypes in *Amelx* null mice on a mixed genetic background is associated with the genes of two parents which segregate between offspring randomly, when only male mice are compared to avoid the lyonization effect. Understanding the potential impact of genetic modifiers which vary by murine strain can shed light on genetic networks which have potential for manipulation for therapeutic intervention (Hamilton and Yu, 2012).

Phenotypic differences can result from regulation of timing or level of expression of proteins important to ameloblast differentiation or enamel development, from availability of precursors of the mineral phase, or from factors related to general growth characteristics. Susceptibility to environmental fluoride varies tremendously in the human populations as does mineral density of hard tissues. Discovery of genetic causes for phenotypic differences between WT strains or strains with identical mutations but different genetic backgrounds will eventually lead to a more complete picture of forces important for generation or regeneration of tissues such as enamel that for humans must last from eruption of the secondary teeth into the oral cavity until the end of life, and have the potential for leading to therapeutic intervention in order to save a tooth. This work can lead toward a future goal of identifying the modifier genes that contribute to these phenotypes through tweaking levels of expression of target genes (Bandiera et al., 2010), e.g. by altering expression of transcription factors, miRNA or alterations within physiologically important pathways that direct development of the mineralized tissues in the tooth. A fuller understanding may lead to new insights into mechanisms involved in tooth regeneration and to aid in maintaining the dentition throughout adulthood.

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Abbreviations

WT	wild-type
KO	null mutation in mice
AMELX* or *Amelx	human or murine *Amelogenin* gene on the X chromosome
MMP20* or *Mmp20	human or murine *Matrix Metalloproteinase-20* gene

μCT	micro computed tomography
PCR	polymerase chain reaction
BB	bone barrel μCT region of incisor at eruption site from mandibular bone
MB	molar barrel μCT region of incisor inferior to molar mesial root apex
AI	amelogenesis imperfecta

References

Aldred MJ, Crawford PJM, Roberts E, Gillespie CM, Thomas NST, Fenton I, Sandkuul LA, Harper PS. Genetic heterogeneity in X-linked amelogenesis imperfecta. Genomics. 1992; 14:567–573. [PubMed: 1358807]

Backman B, Holmgren G. Amelogenesis imperfecta: a genetic study. Hum Hered. 1988; 38:189–206. [PubMed: 3169793]

Bandiera S, Hatem E, Lyonnet MS, Henrion-Caude A. microRNAs in diseases: from candidate to modifier genes. Clin Genet. 2010; 77:306–313. [PubMed: 20132241]

Bartlett JD. Dental enamel development: proteinases and their enamel matrix substrates. ISRN Dentistry Article ID. 2013; 684607:1–23.

Beamer WG, Donahue LR, Rosen CJ. Genetics and bone. Using the mouse to understand man. J Musculoskelet Neuronal Interact. 2002; 2:225–231. [PubMed: 15758440]

Bei M. Molecular genetics of ameloblast cell lineage. J Exp Zool. 2009; 312B:437–444.

Boyde A. Electron microscopic observations relating to the nature and development of prism decussation in mammalian dental enamel. Bull Group Int Rech Sci Stomatol. 1969; 12:151–207. [PubMed: 5255602]

Buchner DA, Trudeau M, Meisler MH. SCNM1, a putative RNA splicing factor that modifies disease severity in mice. Science. 2003; 301:967–969. [PubMed: 12920299]

Darling AI. Some observations on amelogenesis imperfecta and calcification of the dental enamel. Proc Royal Soc Med. 1956; 49:39–45.

El-Sayed W, Parry DA, Shore RC, Ahmed M, Jafri H, Rashid Y, Al-Bahlani S, Al Harasi A, Kirkham J, Inglehearn CF, Mighell AJ. Mutations in the beta propeller WDR72 cause autosomal-recessive hypomaturation amelogenesis imperfecta. Am J Hum Genet. 2009; 85:699–705. [PubMed: 19853237]

Everett ET, McHenry MAK, Reynolds N, Eggertsson H, Sullivan J, Kantmann C, Martinez-Mier EA, Warrick JM, Stookey GK. Dental fluorosis: variability among different inbred mouse strains. J Dent Res. 2002; 81:794–798. [PubMed: 12407097]

Everett E. Fluoride's effects on the formation of teeth and bones, and the influence of genetics. J Dent Res. 2011; 90:552–560. [PubMed: 20929720]

Genin E, Feingold J, Clerget-Darpoux F. Identifying modifier genes of monogenic disease: strategies and difficulties. Hum Genet. 2008; 124:357–368. [PubMed: 18784943]

Gibson CW, Yuan ZA, Hall B, Longenecker G, Chen E, Thyagarajan T, Sreenath T, Wright JT, Decker S, Piddington R, Harrison G, Kulkarni AB. Amelogenin-deficient mice display an amelogenesis imperfecta phenotype. J Biol Chem. 2001; 276:31871–31875. [PubMed: 11406633]

Gibson CW, Li Y, Suggs C, Kuehl MA, Pugach MK, Kulkarni AB, Wright JT. Rescue of the murine amelogenin null phenotype with two amelogenin transgenes. Eur J Oral Sci. 2011; 119(Suppl 1): 70–74. [PubMed: 22243230]

Haldane JBS. The relative importance of principal and modifying genes in determining some human diseases. J Genetics. 1941; 41:149–157.

Hamilton BA, Yu BD. Modifier genes and the plasticity of genetic networks in mice. PLoS Genetics. 2012; 8:e1002644. [PubMed: 22511884]

Hart PS, Hart TC, Michalec MD, Ryu OH, Simmons D, Hong S, Wright JT. Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. J Med Genet. 2004; 41:545–549. [PubMed: 15235027]

He LH, Swain MV. Understanding the mechanical behavior of human enamel from its structural and compositional characteristics. J Mech Behav Biomed Mat. 2008; 1:18–29.

Hu JCC, Hu Y, Smith CE, McKee MD, Wright JT, Yamakoshi Y, Papagerakis P, Hunter GK, Feng JQ, Yamakoshi F, Simmer JP. Enamel defects and ameloblast-specific expression in Enam knock-out/lacZ knock-in mice. J Biol Chem. 2008; 283:10858–10871. [PubMed: 18252720]

Kim JW, Simmer JP, Hart TC, Hart PS, Ramaswami MD, Bartlett JD, Hu JCC. MMP-20 mutation in autosomal recessive pigmented hypomaturation amelogenesis imperfecta. J Med Genet. 2005; 42:271–275. [PubMed: 15744043]

Kim JW, Seymen F, Lee KE, Ko J, Yildirim M, Tuna EB, Gencay K, Shin TJ, Kyun HK, Simmer JP, Hu JCC. LAMB3 mutations causing autosomal-dominant amelogenesis imperfecta. J Dent Res. 2013; 92:899–904. [PubMed: 23958762]

Lagerstrom M, Dahl N, Nakahori Y, Nakagome Y, Backman B, Landegren U, Pettersson U. A deletion in the amelogenin gene (AMG) causes X-linked amelogenesis imperfecta (AIH1). Genomics. 1991; 10:971–975. [PubMed: 1916828]

Lee S-K, Hu JCC, Bartlett JD, Lee K-E, Lin BP-J, Simmer JP, Kim JW. Mutational spectrum of FAM83H: The C-terminal portion is required for tooth enamel calcification. Hum Mut. 2008; 29:E95–E99. [PubMed: 18484629]

Lench NJ, Brook AH, Winter GB. SSCP detection of a nonsense mutation in exon 5 of the amelogenin gene (AMG) causing X-linked amelogenesis imperfecta (AIH1). Hum Molec Genet. 1994; 3:827–828. [PubMed: 8081371]

Lench NJ, Winter GB. Characterisation of molecular defects in X-linked amelogenesis imperfecta (AIH1). Hum mutation. 1995; 5:251–259.

Li Y, Suggs C, Wright JT, Yuan ZA, Aragon M, Fong H, Simmons D, Daly B, Golub EE, Harrison G, Kulkarni AB, Gibson CW. Partial rescue of the amelogenin null dental enamel phenotype. J Biol Chem. 2008; 283:15056–15062. [PubMed: 18390542]

Linder CC. Genetic variables that influence phenotype. Instit Lab Animal Research Journal. 2006; 47:132–140.

Markovic D, Petrovic B, Peric T. Case series: clinical findings and oral rehabilitation of patients with amelogenesis imperfecta. Eur Arch Paediatr Dent. 2010; 11:201–208. [PubMed: 20840832]

Mousny M, Banse X, Wise L, Everett ET, Hancock R, Vieth R, Devogelaer JP, Grynpas MD. The genetic influence on bone susceptibility to fluoride. Bone. 2006; 39:1283–1289. [PubMed: 16920415]

Nadeau JH. Modifier genes in mice and humans. Nat Rev/Genetics. 2001; 2:165–174.

Nadeau JH. Modifier genes and protective alleles in humans and mice. Current Opinions Genet Devel. 2003; 13:290–295.

Nusier M, Yassin O, Hart TC, Samimi A, Wright JT. Phenotypic diversity and revision of the nomenclature for autosomal recessive amelogenesis imperfecta. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004; 97:220–230. [PubMed: 14970781]

Ozdemir D, Hart PS, Ryu OH, Choi SJ, Ozdemir-Karatas M, Firatli E, Piesco N, Hart TC. MMP20 active-site mutation in hypomaturation amelogenesis imperfecta. J Dent Res. 2005; 84:1031–1035. [PubMed: 16246936]

Parry DA, Brookes SJ, Logan CV, Poulter JA, El-Sayed W, Al-Bahlani S, Al Harasi S, Sayed J, Raif el M, Shore RC, Dashash M, Barron M, Morgan JE, Carr IM, Taylor GR, Johnson CA, Aldred MJ, Dixon MJ, Wright JT, Kirkham J, Inglehearn CF, Mighell AJ. Mutations in C4orf26, encoding a peptide with in vitro hydroxyapatite crystal nucleation and growth activity, cause amelogenesis imperfect. Am J Hum Genet. 2012; 91:565–571. [PubMed: 22901946]

Pugach MK, Li Y, Suggs C, Wright JT, Aragon MA, Yuan ZA, Simmons D, Kulkarni AB, Gibson CW. The amelogenin C-terminus is required for enamel development. J Dent Res. 2010; 89:165–169. [PubMed: 20042744]

Pugach MK, Suggs C, Li Y, Wright JT, Kulkarni AB, Bartlett JD, Gibson CW. M180 amelogenin processed by MMP20 is sufficient for decussating murine enamel. J Dent Res. 2013; 92:1118–1122. [PubMed: 24072097]

Rajpar MH, Harley K, Laing C, Davies RM, Dixon MJ. Mutation of the gene encoding the enamel-specific protein, enamelin, causes autosomal-dominant Amelogenesis imperfecta. Hum Molec Genet. 2001; 10:1673–1677. [PubMed: 11487571]

Rasband, WS. Image J. US National Institutes of Health; Bethesda, MD, USA: 1997-2012. http://imageJ.nih.gov/ij/

Sacca, R.; Elder, B.; Wasson, K. The C57BL/6 mouse. Charles River Laboratories International, Inc.; 2013. white paper. www.criver.com/files/pdfs/.../C57bl6/rm_rm_r_c57bl6_white_paper.aspx

Slavkin HC, Zeichner-David M, MacDougall M, Bessem C, Bringas P, Honig LS, Lussky J, Vides J. Enamel gene products during murine amelogenesis in vivo and in vitro. J Dent Res. 1982 Spec No: 1467-1471.

Sultzer BM. Genetic control of leukocyte responses to endotoxin. Nature. 1968; 219:1253–1254. [PubMed: 4877918]

Taft RA, Davisson M, Wiles MV. Know Thy Mouse. TRENDS in Genetics. 2006; 22:659–653.

Taketo M, Schroeder AC, Mobraaten LE, Gunning KB, Hanten G, Fox RR, Roderick TH, Stewart CL, Lilly F, Hansen CT, Overbeek PA. FVB/N: an inbred mouse strain preferable for transgenic analysis. Proc Natl Acad Sci USA. 1991; 88:2065–2069. [PubMed: 1848692]

Tang, Y.; Lee, KS.; Yang, H,T.; Logan, DW.; Wang, S.; McKinnon, ML.; Holt, LJ.; Condie, A.; Luu, MT.; Akhurst, RJ. Genomics. Vol. 85. The Jackson Laboratory; 2005. Epistatic interactions between modifier genes confer strain-specific redundancy for Tgfb1 in developmental angiogenesis.; p. 60-70.http://www.dls.ym.edu.tw/sa/dls/upload/2-3/leg/mouse/JAX%20manual_Genetic%20background.pdf

Wade C,M, Daly MJ. Genetic variation in laboratory mice. Nat Genet. 2005; 37:1175–1180. [PubMed: 16254563]

Wergedal JE, Sheng MH-C, Ackert-Bicknell CL, Beamer WG, Baylink DJ. Genetic variation in femur extrinsic strength in 29 different inbred strains of mice is dependent on variations in femur cross-sectional geometry and bone density. Bone. 2005; 36:111–122. [PubMed: 15664009]

Witkop, CJ.; Sauk, JJ. Dental and Oral Manifestations of Hereditary Disease. American Academy of Oral Pathology; 1971.

Witkop CJ, Rao S. Inherited defects in tooth structure. Birth defects: original article series. 1971; VII: 153–184. [PubMed: 4375507]

Witkop, CJ.; Sauk, JJ. Heritable defects of enamel, in Oral Facial Genetics. Stewart, RE.; Prescott, GH., editors. Mosby; St. Louis: 1976. p. 151-226.

Wright JT, Fine JD, Johnson LB. Developmental defects of enamel in humans with hereditary epidermolysis bullosa. Archs Oral Biol. 1993; 38:945–955.

Wright JT, Kula K, Hall K, Simmons JH, Hart TC. Analysis of the tricho dento-osseous syndrome genotype and phenotype. Am J Med Genet. 1997; 72:197–204. [PubMed: 9382143]

Wright JT, Hart PS, Aldred MJ, Seow K, Crawford PJ, Hong SP, Gibson CW, Hart TC. Relationship of phenotype and genotype in X-linked amelogenesis imperfecta. Conn Tiss Res. 2003; 44(Suppl 1):72–78.

Wright JT, Frazier-Bowers S, Simmons D, Alexander K, Crawford P, Han ST, Hart PS, Hart TC. Phenotypic variation in FAM83H-associated amelogenesis imperfecta. J Dent Res. 2009a; 88:356–360. [PubMed: 19407157]

Wright JT, Hart TC, Hart PS, Simmons D, Suggs C, Daley B, Simmer J, Hu J, Bartlett JD, Li Y, Yuan ZA, Seow WK, Gibson CW. Human and mouse enamel phenotypes resulting from mutation or altered expression of AMEL, ENAM, MMP20 and KLK4. Cells Tissues Organs. 2009b; 189:224–229. [PubMed: 18714142]

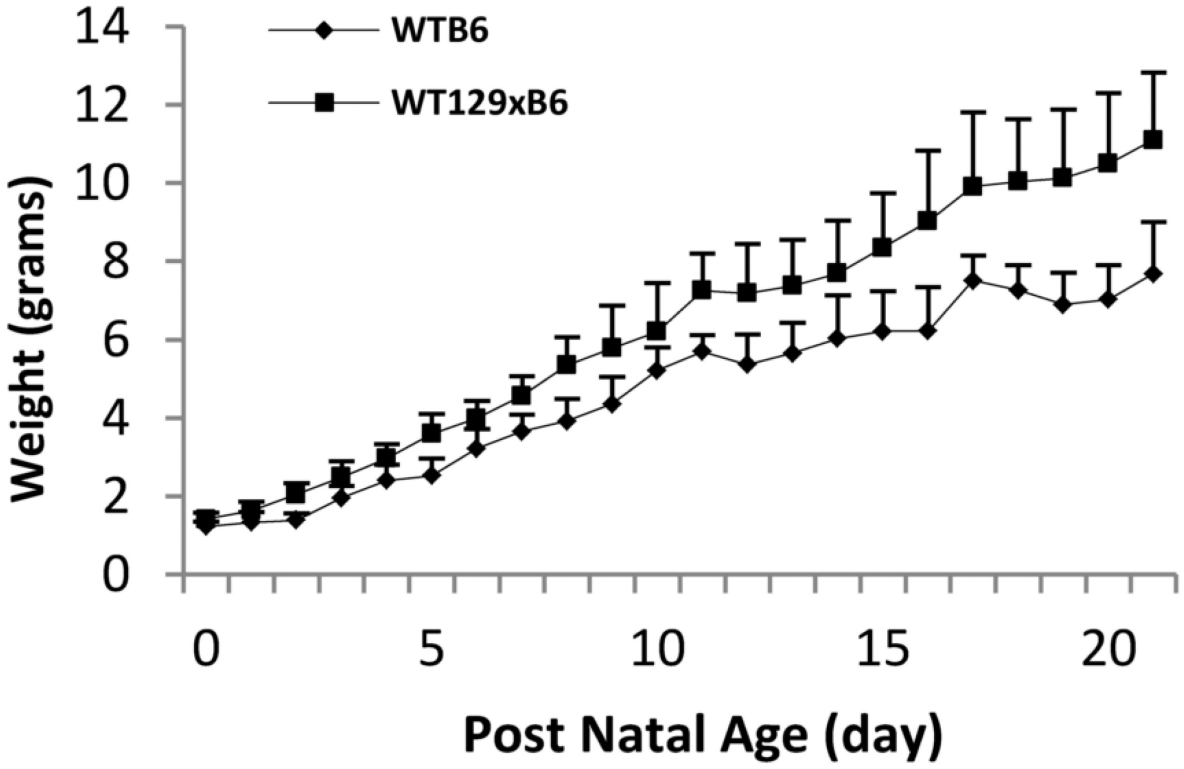

Fig. 1.

Mean weights of male and female mice with C57BL/6J and mixed 129xC57BL/6J genetic background during postnatal days 1-21 (n=mean of 27-30 for each day). For each day, inbred and mixed background mice differed ($P<0.05$).

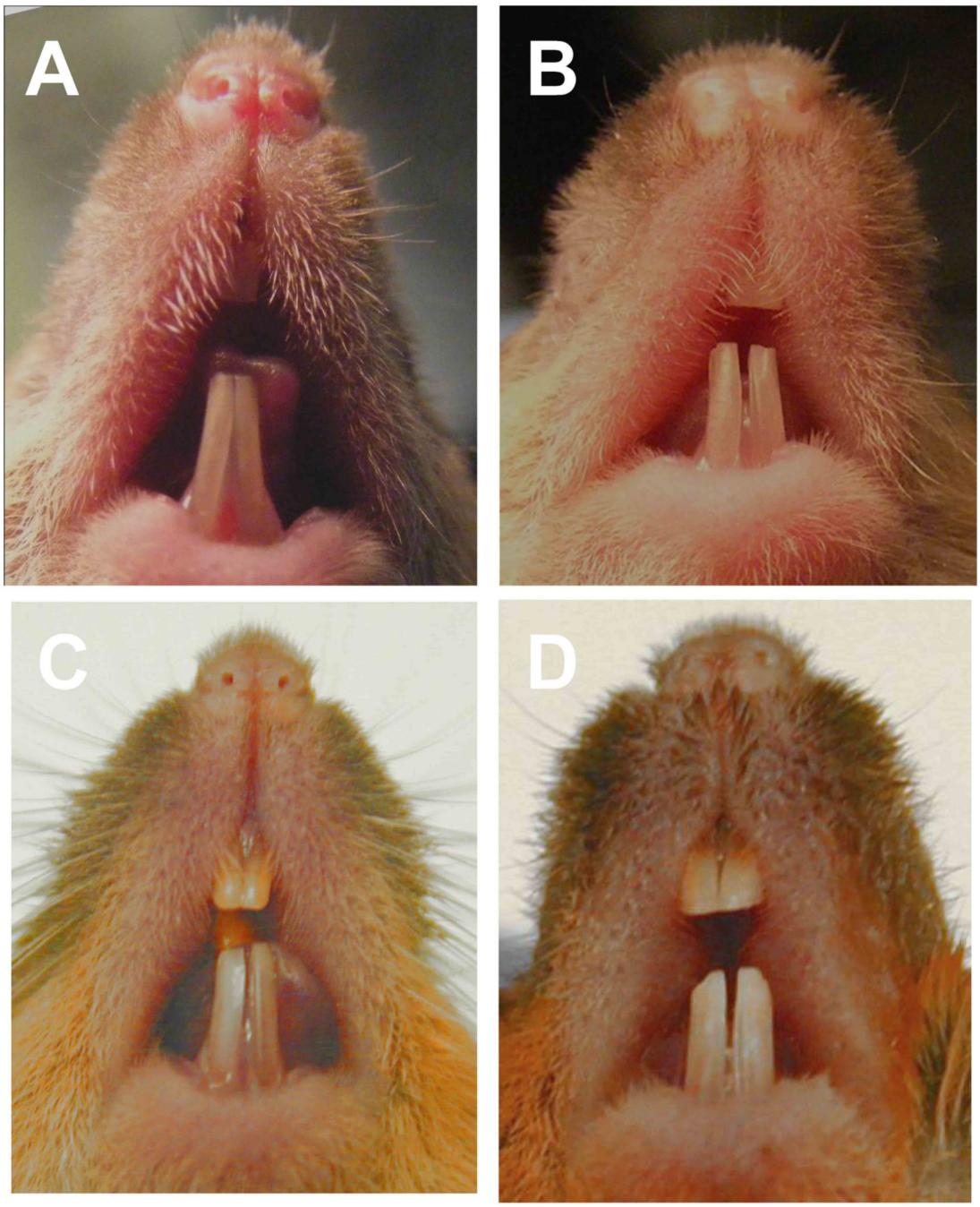

Fig. 2.

Images of murine incisors from *Amelx* null mice. Null mice with mixed genetic background and phenotypic variability in A (7 week male), B (8 week male), C (6 month male), D (6 month female).

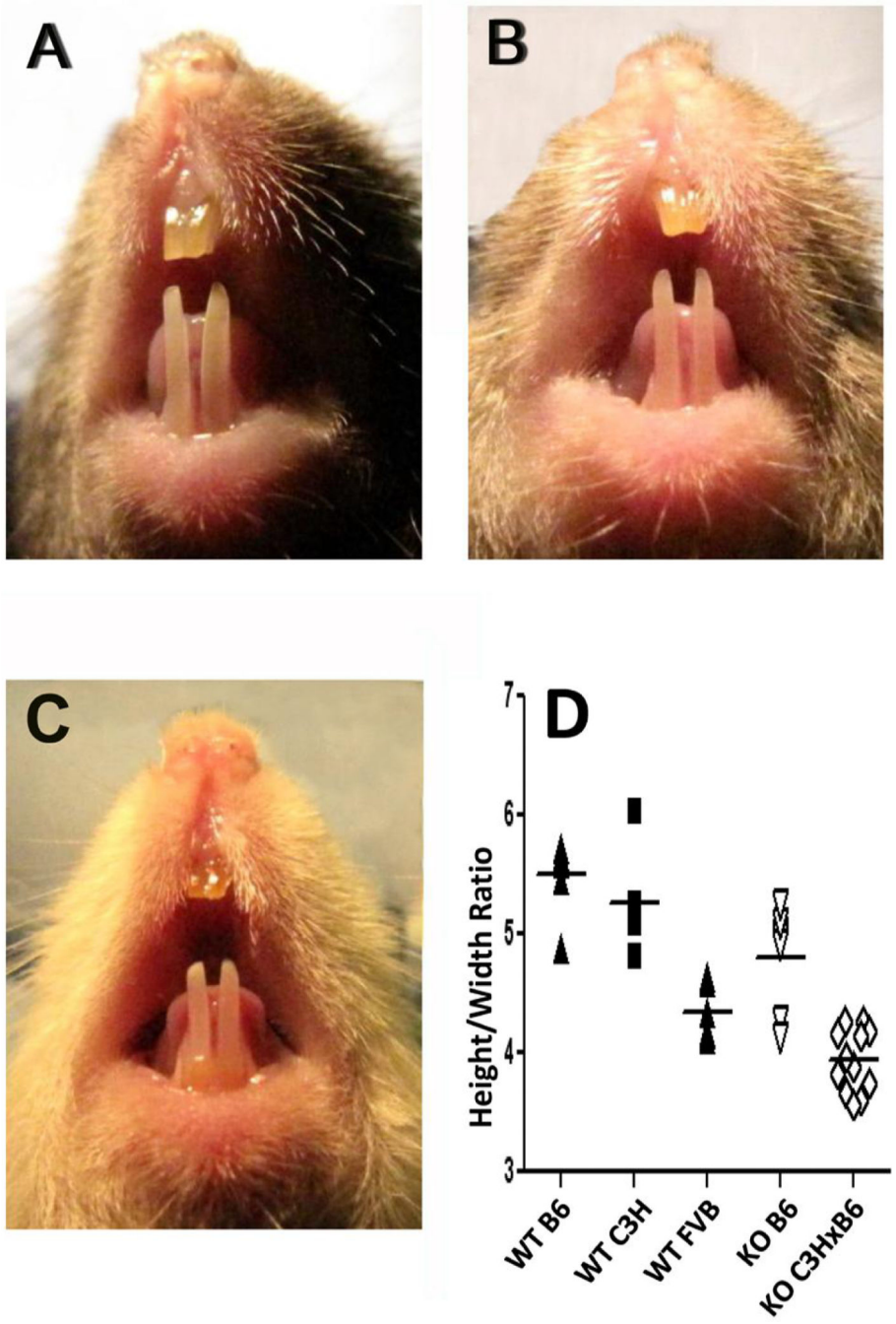

Fig. 3.

Images and dimensions of murine incisors from WT strains or *Amelx* null mice. A, WT mouse incisors C57BL/6; B, C3H/HeJ; C, FVB/NJ; D, ratio of height to width of incisor teeth for WT shown in A,B,C and KOC57BL/6 and KOC3H/HeJxC57BL/6 *Amelx* null mice (n=5-12 for each group). KO, *Amelx* null mice.

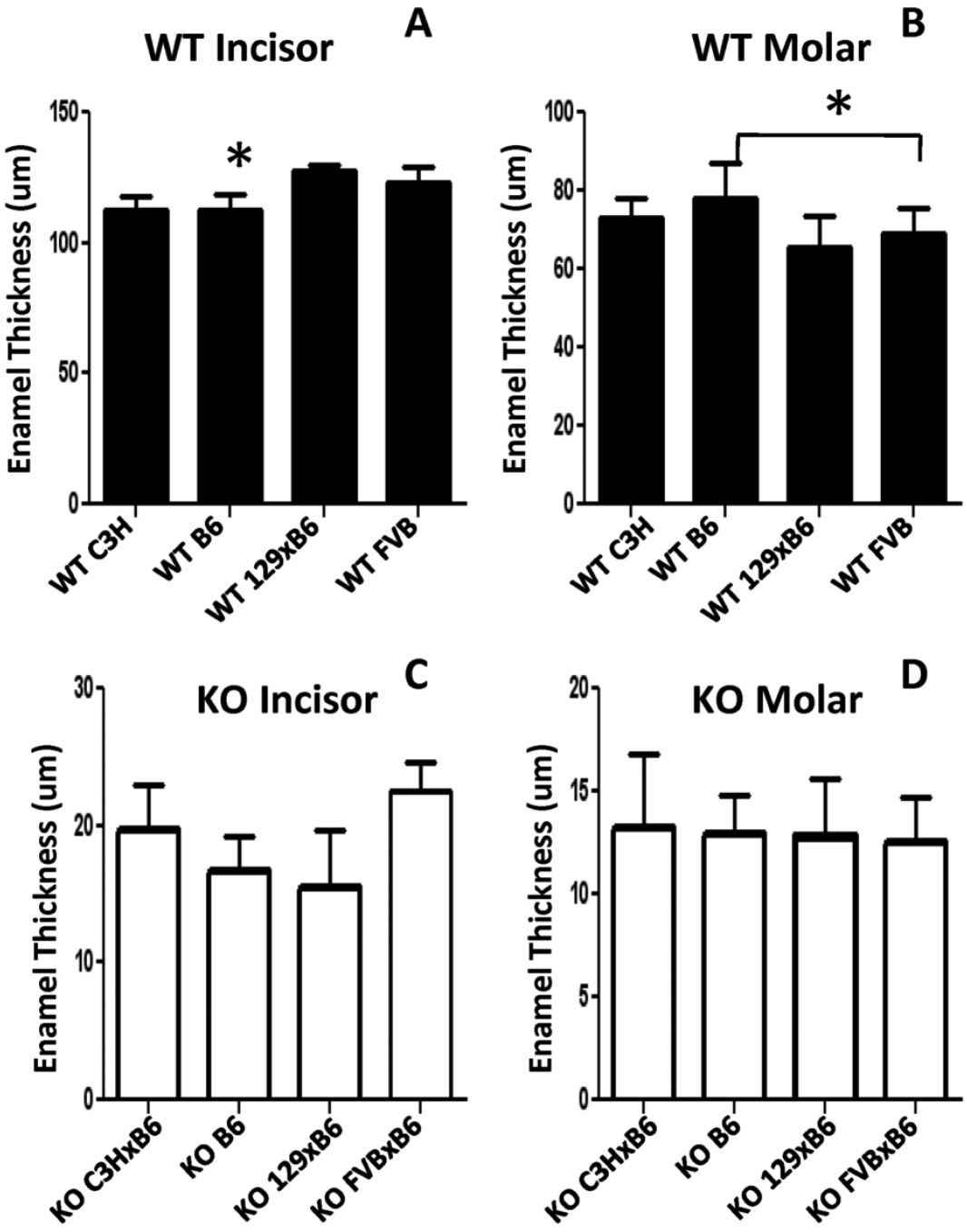

Fig. 4.

WT Incisor enamel density according to microCT analysis. A. Mandibular incisor bone barrel density (BB) *statistical difference from C3H/HeJ and FVB/NJ; B. Mandibular incisor molar barrel density (MB) *statistical difference from C3H/HeJ and C57BL/6; C. Percent of zero microCT enamel readings for each WT strain and *Amelx* null. Significant difference $P<0.05$. KO, *Amelx* null mice.

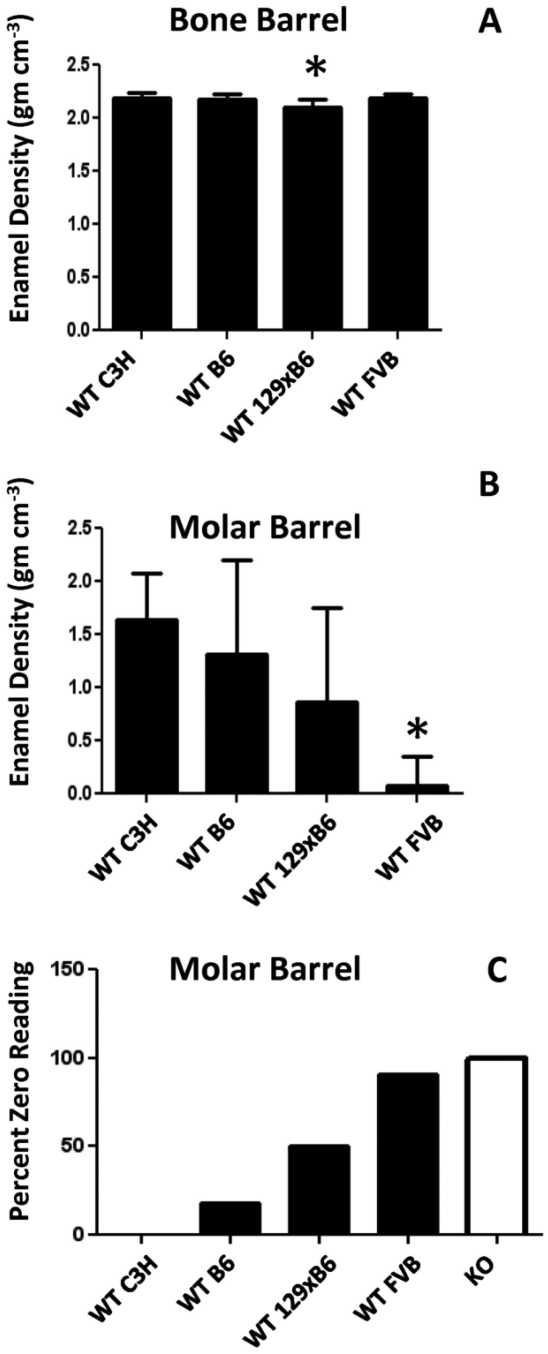

Fig. 5.

Enamel thickness by SEM. A, WT incisors *statistically different; B, WT molars *difference indicated by bar; C, *Amelx* null incisors; D, *Amelx* null molars C,D differences not noted. KO, *Amelx* null mice.

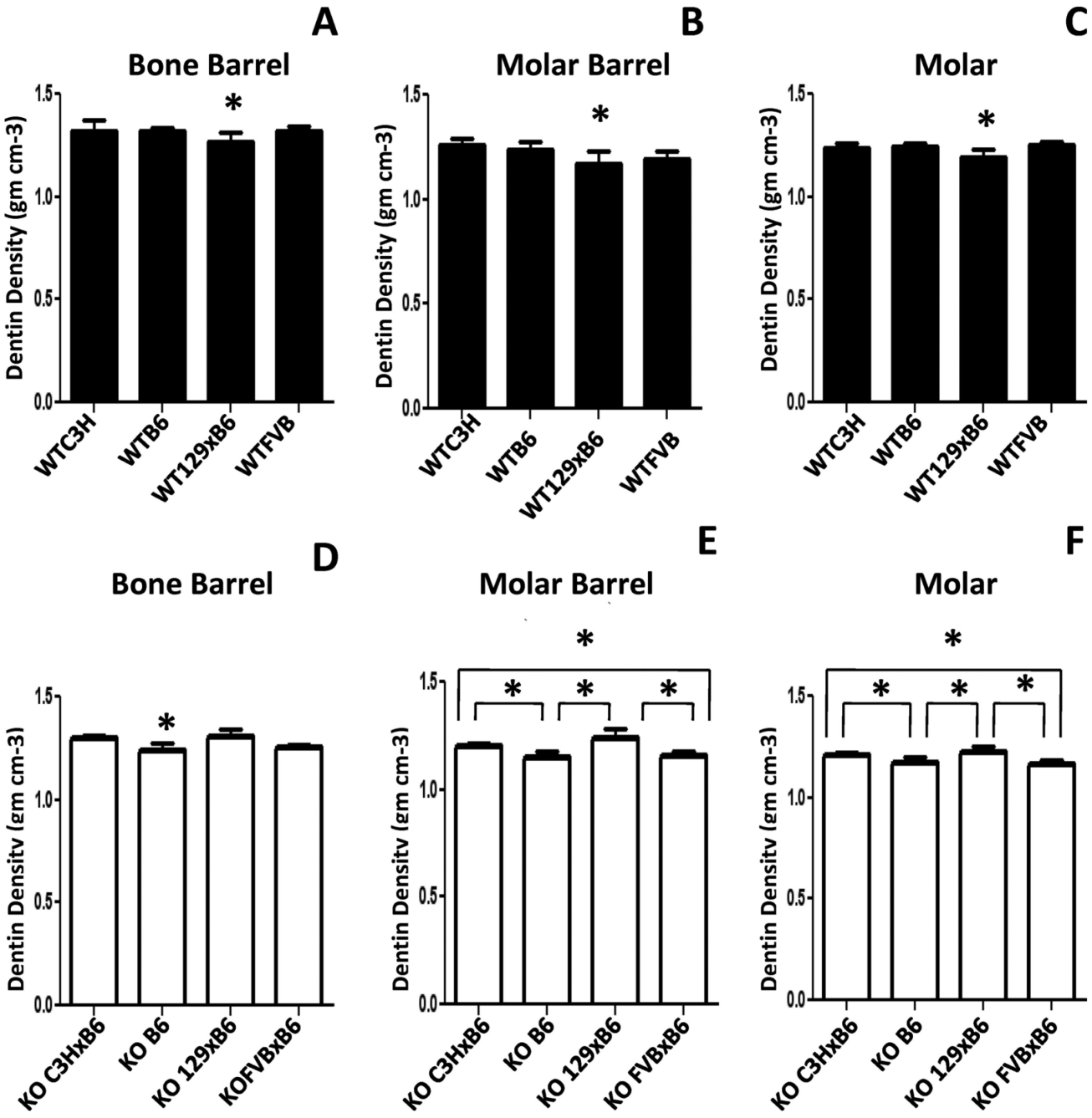

Fig. 6.

Dentin density measured by microCT. A, WT incisors at BB *statistically different from 3 inbred strains; B, WT incisors at MB *statistically different from C3H/HeJ and C57BL/6; C, WT molar *statistically different from 3 pure strains; D, *Amelx* null BB * statistically different from C3H/HeJ and C57BL/6x129; E. *Amelx* null MB; F, *Amelx* null molar E and F, differences are indicated with overlying bars. KO, *Amelx* null mice.