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Hypomaturation Amelogenesis Imperfecta due to *WDR72* Mutations: A Novel Mutation and Ultrastructural Analyses of Deciduous Teeth

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Key Words
Amelogenesis imperfecta · WDR72 · Enamel · Teeth · Scanning electron microscopy

Abstract
Background: Mutations in *WDR72* have been identified in autosomal recessive hypomaturation amelogenesis imperfecta (AI). ***Objective:*** to describe a novel *WDR72* mutation and report the ultrastructural enamel phenotype associated with a different WDR72 mutation. ***Methods:*** A family segregating autosomal recessive hypomaturation AI was recruited, genomic DNA obtained and *WDR72* sequenced. Four deciduous teeth from one individual with a previously published *WDR72* mutation, extracted as part of clinical care, were subjected to scanning electron microscopy, energy-dispersive X-ray analysis and transverse microradiography. ***Results:*** A novel homozygous nonsense mutation, R897X, was identified in *WDR72* in a family originating from Pakistan. Ultrastructural analysis of enamel from the deciduous teeth of an AI patient with the *WDR72* mutation S783X revealed energy-dispersive X-ray analysis spectra with normal carbon and nitrogen peaks, excluding retention of enamel matrix protein. However, transverse microradiography values were significantly lower for affected teeth when compared to normal teeth, consistent with reduced mineralisation. On scanning electron microscopy the enamel rod form observed was normal, yet with inter-rod enamel more prominent than in controls. This appearance was unaltered following incubation with either α-chymotrypsin or lipase. ***Conclusions:*** The novel *WDR72* mutation described brings the total reported *WDR72* mutations to four. Analyses of deciduous tooth enamel in an individual with a homozygous *WDR72* mutation identified changes consistent with a late failure of enamel maturation without retention of matrix proteins. The mechanisms by which intracellular WDR72 influences enamel maturation remain unknown.

Introduction

Enamel is unique among human biomineralised tissues and when formed normally can last a lifetime without any significant capacity for repair. It is approximately 95% mineral content by weight [Smith, 1998] and primarily consists of large hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$)

Abbreviations used in this paper

AI	amelogenesis imperfecta
EDX	energy dispersive X-ray analysis
KLK4	kallikrein 4
MMP20	matrix metalloproteinase 20
SEM	scanning electron microscopy
TMR	transverse microradiography

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crystals [Nylen et al., 1963; Daculsi and Kerebel, 1978]. A key to enamel strength is the highly ordered orientation of hydroxyapatite crystals within enamel rods. Each enamel rod reflects the pathway taken by an ameloblast [Skobe, 2006]. These epithelial-derived cells are responsible for secretion of the enamel protein matrix necessary for mineralisation and then its controlled removal as the enamel matures to its final form [Porto et al., 2009].

Amelogenesis, the process of enamel formation, can be considered to occur in three stages: secretory, transition and maturation [Warshawsky and Smith, 1974; Smith and Nanci, 1995]. As secretory stage ameloblasts retreat towards the eventual tooth surface they produce enamel matrix that includes amelogenin [Yang et al., 2010], enamelin [Al-Hashimi et al., 2009] and ameloblastin [Chun et al., 2010]. The matrix immediately begins to mineralise [Kirkham et al., 1988; Cuisinier et al., 1992; Smith, 1998] and this process is promoted further via ameloblast-mediated enamel matrix processing, in which matrix metalloproteinase 20 (MMP20) plays a critical role [Iwata et al., 2007]. Kallikrein 4 (KLK4), a serine protease enzyme, is functional later during the maturation stage as hydroxyapatite crystal growth is completed and virtually all organic matrix is removed [Simmer and Hu, 2002; Simmer et al., 2009]. Ameloblasts then undergo apoptosis at the external enamel surface prior to tooth eruption [Tsuchiya et al., 2009].

Amelogenesis imperfecta (AI) is a genetically and clinically heterogeneous group of inherited conditions typically characterised by generalised enamel defects of both primary and permanent dentitions [Witkop, 1988; Aldred et al., 2003]. The clinical phenotype is influenced by the stage of amelogenesis predominantly affected, although clinical classification can be difficult. Hypoplastic forms of AI are due to defects in the secretory stage leading to diminished volumes of enamel matrix protein, which in turn result in very thin enamel. By contrast, hypocalcified and hypomaturation forms of AI are characterised by near-normal volumes of enamel matrix that typically is not processed and removed appropriately, leading to failure of normal biomineralisation [Witkop, 1988; Ng and Messer, 2009]. In hypomaturation AI the opaque, discoloured enamel typically chips away from the supporting dentine, whereas in hypocalcified AI the enamel is soft enough to scrape away with a hand instrument [Witkop, 1988]. These two forms of AI are sometimes collectively referred to as hypomineralised AI, reflecting the difficulties in distinguishing between ill-defined phenotypes, especially where teeth have been subjected to post-eruption changes.

Mutations in 6 genes have been shown to cause AI to date. These include the enamel matrix protein encoding genes *AMELX* (MIM 301200) [Lagerström et al., 1991; Barron et al., 2010] and *ENAM* (MIM 608563) [Rajpar et al., 2001; Hart et al., 2003] as well as the enamel matrix-modifying protease genes *MMP20* (MIM 612529) [Kim et al., 2005; Lee et al., 2010] and *KLK4* (MIM 603767) [Hart et al., 2004]. In addition, mutations in *FAM83H* (MIM 130900), a gene of unknown function that encodes an intracellular protein, are a frequent cause of autosomal dominant hypocalcified AI [Kim et al., 2008; Lee et al., 2008; El-Sayed et al., 2010]. Finally, we reported three different *WDR72* mutations as a novel cause of autosomal recessive hypomaturation AI in families originating from either Pakistan or Oman [El-Sayed et al., 2009]. WDR72 is an intracellular protein with a predicted β-propeller structure expected to mediate reversible protein-protein interactions. Its functions remain unknown and its discovery has opened a new area of research in enamel biomineralisation.

In this study we report a novel homozygous mutation in exon 15 of *WDR72,* causing hypomaturation AI in a further consanguineous Pakistani family, underlining the importance of *WDR72* mutations as a cause of autosomal recessive hypomaturation AI. In addition we report the first ultrastructural analyses of deciduous teeth from a patient in a previously reported AI family with a homozygous mutation in *WDR72*.

Materials and Methods

Subjects

A consanguineous family (P6) of Pakistani origin was ascertained, in which 2 sisters had hypomaturation AI. Peripheral blood samples were obtained from 1 affected individual and genomic DNA was prepared by a conventional salting method. Four deciduous molar teeth affected by AI that had been extracted for clinical reasons were obtained from an individual in a previously reported family (P2) with homozygous c.2348C>G; p.S783X mutation [El-Sayed et al., 2010]. Samples were obtained with informed consent in a process approved by the Leeds (West) NHS Trust Ethics committee.

WDR72 Mutation Analysis

All 19 coding exons and exon/intron boundaries of the *WDR72* gene were PCR amplified using oligonucleotide primers described by El-Sayed et al. [2009]. PCR products were purified using EXOSAP enzyme. Purified products were sequenced using the Big Dye terminator Kit v.3.1 (Applied Biosystems, Forster City, Calif., USA) and size fractionated on an ABI 3130 XL DNA analyser. The sequence produced was analysed using the ABI Prism sequence Analyser and SeqScape software packages (Applied Biosystems).

Scanning Electron Microscopy, Energy-Dispersive X-Ray Spectroscopy and Transverse Microradiography

Standard methods were used for preparation of tooth sections (100 μm) from deciduous teeth. These were then investigated by scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and transverse microradiography (TMR) [Shore et al., 2002; Barron et al., 2008; El-Sayed et al., 2010]. Microstructural analysis was undertaken using a Jeol 35 SEM fitted with the Deben Genie upgrade (Deben Engineering, Debenham, UK). EDX elemental analysis was performed using a detector fitted with an ultrathin window and driven by WinEDX 3 software (Thomson Scientific, Carlton, Vic., Australia). TMR involved sampling across sections from 4 affected and 2 control teeth at a minimum of 10 different points each within the enamel. Analysis was done using Inspektor TMR software version 20.0.27.16, 2000 (Inspektor Research Systems, Amsterdam, The Netherlands). For enzyme digestion, scanned sections were re-polished and re-etched with 35% phosphoric acid for 15 s. Sections were incubated in either α-chymotrypsin (C5088-2MG; Sigma, Dorset, UK) or lipase (Sigma) as described previously [Shore et al., 2002].

Results

WDR72 Mutation and Clinical Phenotype

The clinical phenotype observed in the proband and her sister from the new AI family (P6) was consistent with AI with extensive post-eruptive enamel loss. No other health problems segregated with AI. The dentitions in both sisters were heavily restored with the remaining enamel pigmented and predominantly yellow/brown in colour (fig. 1a). Serial dental radiographs taken over many years were reviewed for both sisters and were characterised by features consistent with hypomaturation AI (fig. 1b–d). The pattern of inheritance was consistent with autosomal recessive inheritance (fig. 1e).

Sequencing all the exons and intron-exon boundaries of the *WDR72* gene identified a nonsense mutation (c.2728C>T; p.R897X) in exon 15 of *WDR72* (forward and reverse strands) in the affected proband (fig. 1e).

Ultrastructural Analyses of Deciduous Teeth

TMR sampling of teeth from the Pakistani family (P2) with a previously identified mutation in *WDR72* [El-Sayed et al., 2010] revealed a reduction in the mean mineral density percentage in affected enamel (37.9% ± 2.83%) compared to control enamel (76.9% ± 3.81%). This reduction is statistically significant ($p < 0.001$). Elemental analysis by EDX demonstrated that the ratio of carbon:oxygen and calcium:phosphorous were indistinguishable from control enamel with no nitrogen present (fig. 2a, b). This excludes the possibility that there is retained protein in WDR72 mutant teeth as has been shown in other forms of AI.

SEM examination of enamel from affected teeth identified obvious enamel rods across the enamel width available for examination (fig. 2c–e). However, in affected enamel the boundaries (inter-rod enamel) between individual enamel rods were more obvious and of a different electron density to those observed in normal enamel. There also appeared to be reduced decussation of the layers of enamel rods that give rise to the Hunter-Schreger bands observed in normal enamel. The SEM appearances of affected and control enamel were unaltered after incubation of the sections with the α-chymotrypsin or lipase (data not presented).

Discussion

The p.R897X mutation is the fourth *WDR72* mutation described in AI patients. Like the three previously observed mutations (p.S783X, p.W978X and p.S953V*fs*X20), it lies within exons 14–16 of a 19-exon gene, which may suggest a degree of clustering towards the carboxy-terminus, downstream of the WD40 domains. The mechanism by which *WDR72* nonsense mutations result in hypomaturation AI is unknown, but the lack of missense mutations suggests a null phenotype due to mRNA nonsense-mediated decay rather than production of stable truncated proteins.

EDX is a sensitive technique for identification of retained organic material in enamel [Shore et al., 2002; Barron et al., 2008; El-Sayed et al., 2010]. When organic material is retained, incubation with either α-chymotrypsin or lipase can be informative and reveal the enamel ultrastructure in greater detail [Shore et al., 2002]. In this study the absence of retained enamel matrix protein in affected enamel indicates that ameloblasts are able to direct degradation and removal of enamel matrix in a manner similar to that in normal teeth. MMP20 and KLK4, which are expressed predominantly by secretory and maturation-stage ameloblasts, respectively, are the two proteases critical to this process of protein degradation and removal. Mutations in their respective genes are recognised to result in an autosomal recessive hypomaturation AI clinical phenotype with reduced enamel mineralisation evident on dental radiography [Hart et al., 2004; Kim et al., 2005; Ozdemir et al., 2005; Papagerakis et al., 2008; Simmer et al., 2009]. Characterisation of mouse models null for either Mmp20 or Klk4 confirmed some inappropriate retention of enamel matrix proteins

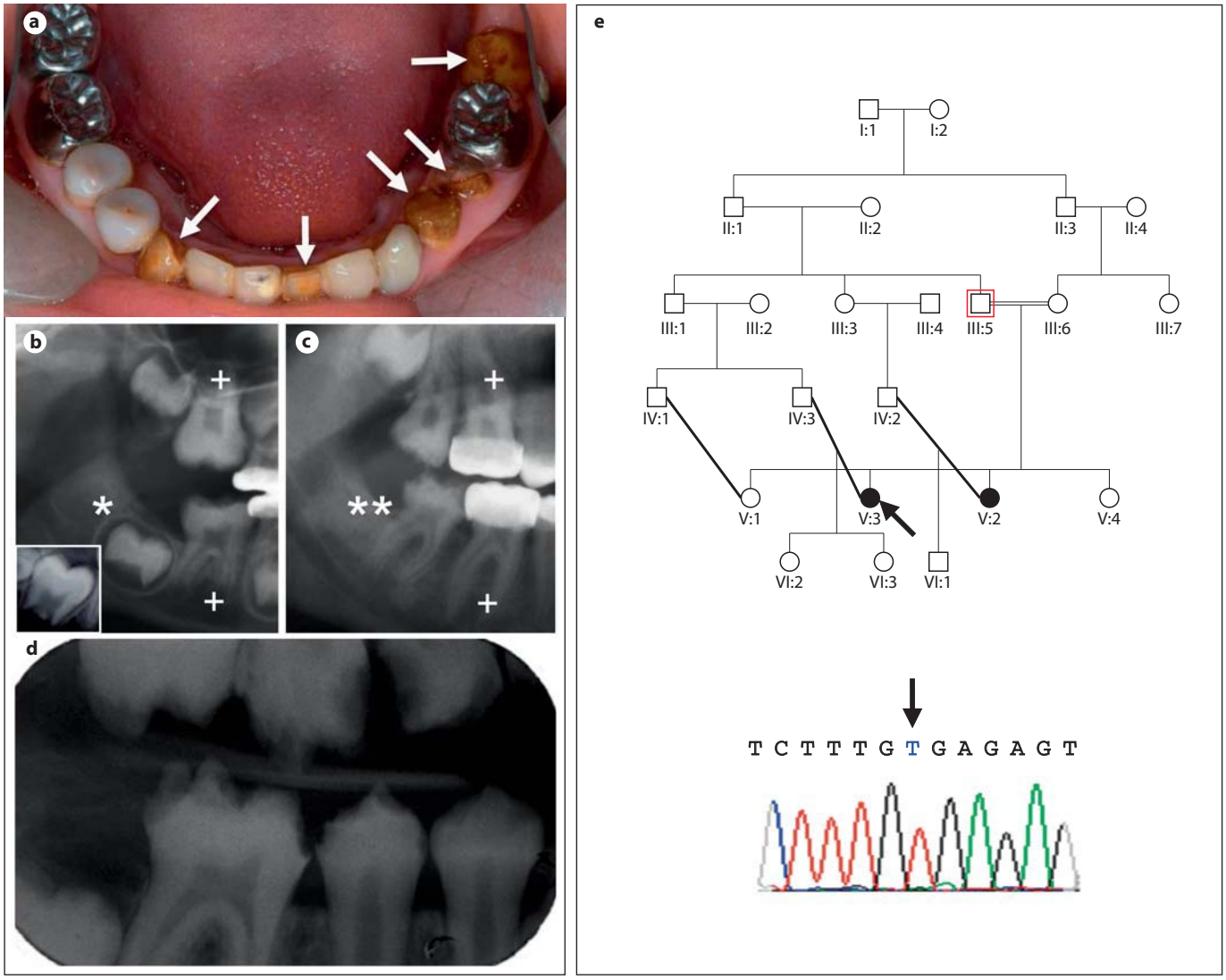

Fig. 1. Clinical and radiographic phenotype, pedigree and novel WDR72 mutation. **a** Heavily restored lower dentition of the proband's sister aged 18 years. The visible enamel of teeth, including where restorations have failed, has a yellow/brown discolouration (arrows). **b–d** Radiographs of two affected individuals. Details from panoramic radiographs taken of the proband's sister when aged 8 years (**b**) and 20 years (**c**). The developing lower right second molar tooth (*) has a normal crown morphology in **b**, but without the expected contrast in radiodensity observed in someone without AI (**inset**). The same tooth (**) approximately 8 years after eruption and restoration failure is characterised by considerable enamel loss consistent with early functional failure. Both the upper and lower first permanent molar teeth (+) have required restoration with metal crowns due to functional failure. **d** A dental bitewing radiograph of the proband taken aged 12 years illustrating the typical post-eruptive pattern of enamel loss that starts soon after tooth eruption into the mouth. **e** The pedigree confirmed consanguinity (proband marked with arrow) with confirmation of the c.2728C>T mutation.

[Simmer et al., 2009; Wright et al., 2009]. Absence of residual proteins in enamel in this study rules out WDR72 as a critical upstream intracellular regulator of MMP20 or KLK4 expression or secretion from ameloblasts.

In health enamel rods abut each other with a thin layer of inter-rod enamel that is characterised by different orientation of hydroxyapatite crystals rather than a different composition [Boyde, 1989]. The abnormal appearance of the inter-rod enamel observed in this study may represent disruption to this late stage of enamel maturation. Little is known about the control of inter-rod enamel formation. Variations in inter-rod enamel are recog-

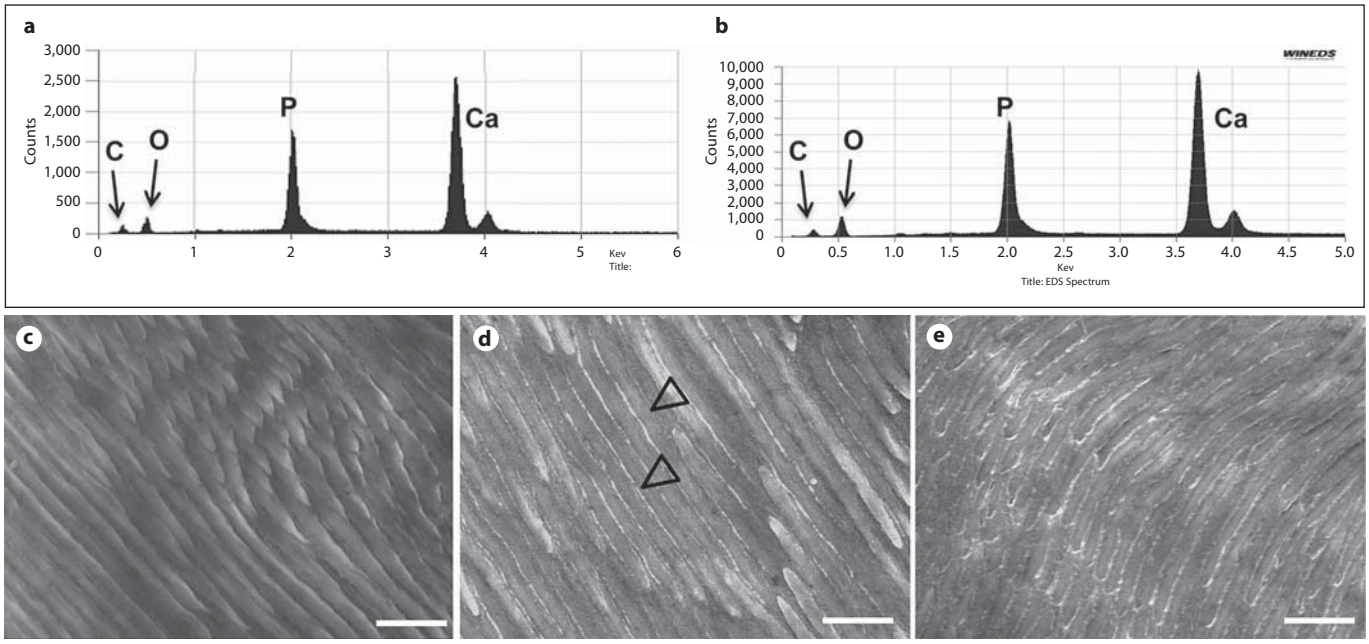

Fig. 2. Ultrastructural phenotyping of deciduous teeth. **a**, **b** EDX spectra: the C:O and Ca:P ratios were indistinguishable between control (**a**) and autosomal recessive hypomaturation AI (**b**) enamel with no N peak (between the C and O peaks) observed in either sample. **c–e** SEM of control (**c**) and AI-affected (**d**, **e**) enamel. The AI enamel rods are grossly normal but the inter-rod enamel is more obvious (pale lines marked with arrow heads) in the affected enamel compared to the controls. There is an apparent lack of enamel rod decussation in the affected enamel whereas in the control enamel there is different rod long axis orientation in the upper compared to the lower part of the image. Scale bars = 20 μm.

nised and there is a complete absence of inter-rod enamel in mice null for anion exchanger-2 [Lyaruu et al., 2008].

Data presented in this study are consistent with *WDR72* mutations affecting the late stages of enamel maturation. The putative β-propeller structure of WDR72 could potentially contribute to a number of different maturation ameloblast processes. Cyclical changes in pH in the enamel matrix are recognised to be critical to several aspects of enamel maturation including crystal growth [Lacruz et al., 2010]. Key proteins expressed in ameloblast cell membranes, including carbonic anhydrases and solute carriers are known to be critical to pH in amelogenesis [Lyaruu et al., 2008; Paine et al., 2008; Lacruz et al., 2010]. However, surprisingly little is understood about how ameloblasts control pH changes. Cyclical alterations in pH link to ameloblast apical morphological switching between smooth and ruffled appearances. Endocytosis occurs in the ruffled, but not smooth ends, which lose their apical tight junctions and potentially allow passage of molecules between ameloblasts [Sasaki et al., 1991; Smith, 1998; Lacruz et al., 2010]. The mechanism by which these changes are controlled remains unknown.

Mineralisation requires the delivery of relevant ions to the maturing crystals. In part this is mediated via active Ca^{2+} efflux/extrusion from ameloblast sodium-calcium exchangers [Okumura et al., 2010]. Maturation stage ameloblasts secrete proteins such as amelotin and odontogenic ameloblast-associated protein at the interface between ameloblasts and maturing enamel, and these are believed to play important roles in enamel maturation [Moffatt et al., 2008; Gao et al., 2010]. Control of the secretory functions of maturation stage ameloblasts remains unknown. WDR7, the closest homologue of WDR72, is involved in Ca^{2+}-dependent vesicle exocytosis, raising the possibility that WDR72 may have a similar role in amelogenesis [Nagano et al., 2002; Coleman and Bykhovskaia, 2009].

The data presented are primarily consistent with disruption to late amelogenesis. However, the apparent loss of enamel rod decussation raises the possibility of a fundamental alteration to the way that adjacent ameloblasts interact with each other during secretion. Any lack of prism decussation would be likely to have a detrimental effect on the physical properties of the final enamel, but this observation requires confirmation in further samples.

In conclusion, *WDR72* mutations are a significant cause of autosomal recessive hypomaturation AI with a detrimental impact on the late stages of enamel maturation in amelogenesis. Elucidating the functions of WDR72 can be expected to provide novel and important insight into biomineralisation.

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References

1 Al-Hashimi, N., J.Y. Sire, S. Delgado (2009) Evolutionary analysis of mammalian enamelin, the largest enamel protein, supports a crucial role for the 32-kDa peptide and reveals selective adaptation in rodents and primates. J Mol Evol *69:* 635–656.

2 Aldred, M.J., R. Savarirayan, P.J. Crawford (2003) Amelogenesis imperfecta: a classification and catalogue for the 21st century. Oral Dis *9:* 19–23.

3 Barron, M.J., S.J. Brookes, C.E. Draper, D. Garrod, J. Kirkham, R.C. Shore, M.J. Dixon (2008) The cell adhesion molecule nectin-1 is critical for normal enamel formation in mice. Hum Mol Genet *17:* 3509–3520.

4 Barron, M.J., S.J. Brookes, J. Kirkham, R.C. Shore, C. Hunt, A. Mironov, N.J. Kingswell, J. Maycock, C.A. Shuttleworth, M.J. Dixon (2010) A mutation in the mouse Amelx tri-tyrosyl domain results in impaired secretion of amelogenin and phenocopies human X-linked amelogenesis imperfecta. Hum Mol Genet *19:* 1230–1247.

5 Boyde, A. (1989) Enamel; in Oksche A, Vollrath L (eds): Handbook of Microscopic Anatomy. Berlin, Springer-Verlag, pp 309–473.

6 Chun, Y.P., Y. Yamakoshi, F. Yamakoshi, M. Fukae, J.C. Hu, J.D. Bartlett, J.P. Simmer (2010) Cleavage site specificity of MMP-20 for secretory-stage ameloblastin. J Dent Res *89:* 785–790.

7 Coleman, W.L., M. Bykhovskaia (2009) Rab3a-mediated vesicle recruitment regulates short-term plasticity at the mouse diaphragm synapse. Mol Cell Neurosci *41:* 286–296.

8 Cuisinier, F.J., P. Steuer, B. Senger, J.C. Voegel, R.M. Frank (1992) Human amelogenesis. I: high resolution electron microscopy study of ribbon-like crystals. Calcif Tissue Int *51:* 259–268.

9 Daculsi, G., B. Kerebel (1978) High-resolution electron microscope study of human enamel crystallites: size, shape, and growth. J Ultrastruct Res *65:* 163–172.

10 El-Sayed, W., D.A. Parry, R.C. Shore, M. Ahmed, H. Jafri, Y. Rashid, S. Al-Bahlani, S. Al Harasi, J. Kirkham, C.F. Inglehearn, A.J. Mighell (2009) Mutations in the β propeller WDR72 cause autosomal-recessive hypomaturation amelogenesis imperfecta. Am J Hum Genet *85:* 699–705.

11 El-Sayed, W., R.C. Shore, D.A. Parry, C.F. Inglehearn, A.J. Mighell (2010) Ultrastructural analyses of deciduous teeth affected by hypocalcified amelogenesis imperfecta from a family with a novel Y458X FAM83H nonsense mutation. Cells Tissues Organs *191:* 235–239.

12 Gao, Y., W. Wang, Y. Sun, J. Zhang, D. Li, Y. Wei, T. Han (2010) Distribution of amelotin in mouse tooth development. Anat Rec *293:* 135–140.

13 Hart, P.S., T.C. Hart, M.D. Michalec, O.H. Ryu, D. Simmons, S. Hong, J.T. Wright (2004) Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. J Med Genet *41:* 545–549.

14 Iwata, T., Y. Yamakoshi, J.C. Hu, I. Ishikawa, J.D. Bartlett, P.H. Krebsbach, J.P. Simmer (2007) Processing of ameloblastin by MMP-20. J Dent Res *86:* 153–157.

15 Kim, J.W., S.K. Lee, Z.H. Lee, J.C. Park, K.E. Lee, M.H. Lee, J.T. Park, B.M. Seo, J.C. Hu, J.P. Simmer (2008) FAM83H mutations in families with autosomal-dominant hypocalcified amelogenesis imperfecta. Am J Hum Genet *82:* 489–494.

16 Kim, J.W., J.P. Simmer, T.C. Hart, P.S. Hart, M.D. Ramaswami, J.D. Bartlett, J.C. Hu (2005) MMP-20 mutation in autosomal recessive pigmented hypomaturation amelogenesis imperfecta. J Med Genet *42:* 271–275.

17 Kirkham, J., C. Robinson, J.A. Weatherell, A. Richards, O. Fejerskov, K. Josephsen (1988) Maturation in developing permanent porcine enamel. J Dent Res *67:* 1156–1160.

18 Lacruz, R.S., A. Nanci, I. Kurtz, J.T. Wright, M.L. Paine (2010) Regulation of pH During Amelogenesis. Calcif Tissue Int *86:* 91–103.

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

20 Lee, S.K., J.C. Hu, J.D. Bartlett, K.E. Lee, B.P. Lin, J.P. Simmer, J.W. Kim (2008) Mutational spectrum of FAM83H: the C-terminal portion is required for tooth enamel calcification. Hum Mutat *29:* E95–E99.

21 Lee, S.K., F. Seymen, H.Y. Kang, K.E. Lee, K. Gencay, B. Tuna, J.W. Kim (2010) MMP20 hemopexin domain mutation in amelogenesis imperfecta. J Dent Res *89:* 46–50.

22 Lyaruu, D.M., A.L. Bronckers, L. Mulder, P. Mardones, J.F. Medina, S. Kellokumpu, R.P. Oude Elferink, V. Everts (2008) The anion exchanger Ae2 is required for enamel maturation in mouse teeth. Matrix Biol *27:* 119–127.

23 Moffatt, P., C.E. Smith, R. St-Arnaud, A. Nanci (2008) Characterization of Apin, a secreted protein highly expressed in tooth-associated epithelia. J Cell Biochem *103:* 941–956.

24 Nagano, F., H. Kawabe, H. Nakanishi, M. Shinohara, M. Deguchi-Tawarada, M. Takeuchi, T. Sasaki, Y. Takai (2002) Rabconnectin-3, a novel protein that binds both GDP/GTP exchange protein and GTPase-activating protein for Rab3 small G protein family. The J Biol Chem *277:* 9629–9632.

25 Ng, F.K., L.B. Messer (2009) Dental management of amelogenesis imperfecta patients: a primer on genotype-phenotype correlations. Pediatr Dent *31:* 20–30.

26 Nylen, M.U., E.D. Eanes, K.A. Omnell (1963) Crystal growth in rat enamel. J Cell Biol *18:* 109–123.

27 Okumura, R., Y. Shibukawa, T. Muramatsu, S. Hashimoto, K. Nakagawa, M. Tazaki, M. Shimono (2010) Sodium-calcium exchangers in rat ameloblasts. J Pharmacol Sci *112:* 223–230.

28 Ozdemir, D., P.S. Hart, O.H. Ryu, S.J. Choi, M. Ozdemir-Karatas, E. Firatli, N. Piesco, T.C. Hart (2005) MMP20 active-site mutation in hypomaturation amelogenesis imperfecta. J Dent Res *84:* 1031–1035.

29 Paine, M.L., M.L. Snead, H.J. Wang, N. Abuladze, A. Pushkin, W. Liu, L.Y. Kao, S.M. Wall, Y.H. Kim, I. Kurtz (2008) Role of NBCe1 and AE2 in secretory ameloblasts. J Dent Res *87:* 391–395.

30 Papagerakis, P., H.K. Lin, K.Y. Lee, Y. Hu, J.P. Simmer, J.D. Bartlett, J.C. Hu (2008) Premature stop codon in MMP20 causing amelogenesis imperfecta. J Dent Res *87:* 56–59.

31 Porto, I.M., J. Merzel, F.B. de Sousa, L. Bachmann, J.A. Cury, S.R. Line, R.F. Gerlach (2009) Enamel mineralization in the absence of maturation stage ameloblasts. Arch Oral Biol *54:* 313–321.

32 Rajpar, M.H., K. Harley, C. Laing, R.M. Davies, M.J. Dixon (2001) Mutation of the gene encoding the enamel-specific protein, enamelin, causes autosomal-dominant amelogenesis imperfecta. Hum Mol Genet *10:* 1673–1677.
33 Sasaki, S., T. Takagi, M. Suzuki (1991) Cyclical changes in pH in bovine developing enamel as sequential bands. Arch Oral Biol *36:* 227–231.
34 Shore, R.C., B. Backman, S.J. Brookes, J. Kirkham, S.R. Wood, C. Robinson (2002) Inheritance pattern and elemental composition of enamel affected by hypomaturation amelogenesis imperfecta. Connect Tissue Res *43:* 466–471.
35 Simmer, J.P., J.C. Hu (2002) Expression, structure, and function of enamel proteinases. Connect Tissue Res *43:* 441–449.
36 Simmer, J.P., Y. Hu, R. Lertlam, Y. Yamakoshi, J.C. Hu (2009) Hypomaturation enamel defects in Klk4 knockout/LacZ knockin mice. J Biol Chem *284:* 19110–19121.
37 Skobe, Z. (2006) SEM evidence that one ameloblast secretes one keyhole-shaped enamel rod in monkey teeth. Eur J Oral Sci *114(suppl 1):* 338–342.
38 Smith, C.E. (1998) Cellular and chemical events during enamel maturation. Crit Rev Oral Biol Med *9:* 128–161.
39 Smith, C.E., A. Nanci (1995) Overview of morphological changes in enamel organ cells associated with major events in amelogenesis. Int J Dev Biol *39:* 153–161.
40 Tsuchiya, M., R. Sharma, C.E. Tye, T. Sugiyama, J.D. Bartlett (2009) Transforming growth factor-β1 expression is up-regulated in maturation-stage enamel organ and may induce ameloblast apoptosis. Eur J Oral Sci *117:* 105–112.
41 Warshawsky, H., C.E. Smith (1974) Morphological classification of rat incisor ameloblasts. Anat Rec *179:* 423–446.
42 Witkop, C.J., Jr. (1988) Amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia revisited: problems in classification. J Oral Pathol *17:* 547–553.
43 Wright, J.T., T.C. Hart, P.S. Hart, D. Simmons, C. Suggs, B. Daley, J. Simmer, J. Hu, J.D. Bartlett, Y. Li, Z.A. Yuan, W.K. Seow, C.W. Gibson (2009) Human and mouse enamel phenotypes resulting from mutation or altered expression of AMEL, ENAM, MMP20 and KLK4. Cells Tissues Organs *189:* 224–229.
44 Yang, X., L. Wang, Y. Qin, Z. Sun, Z.J. Henneman, J. Moradian-Oldak, G.H. Nancollas (2010) How amelogenin orchestrates the organization of hierarchical elongated microstructures of apatite. J Phys Chem B *114:* 2293–2300.