RESEARCH REPORTS
Clinical

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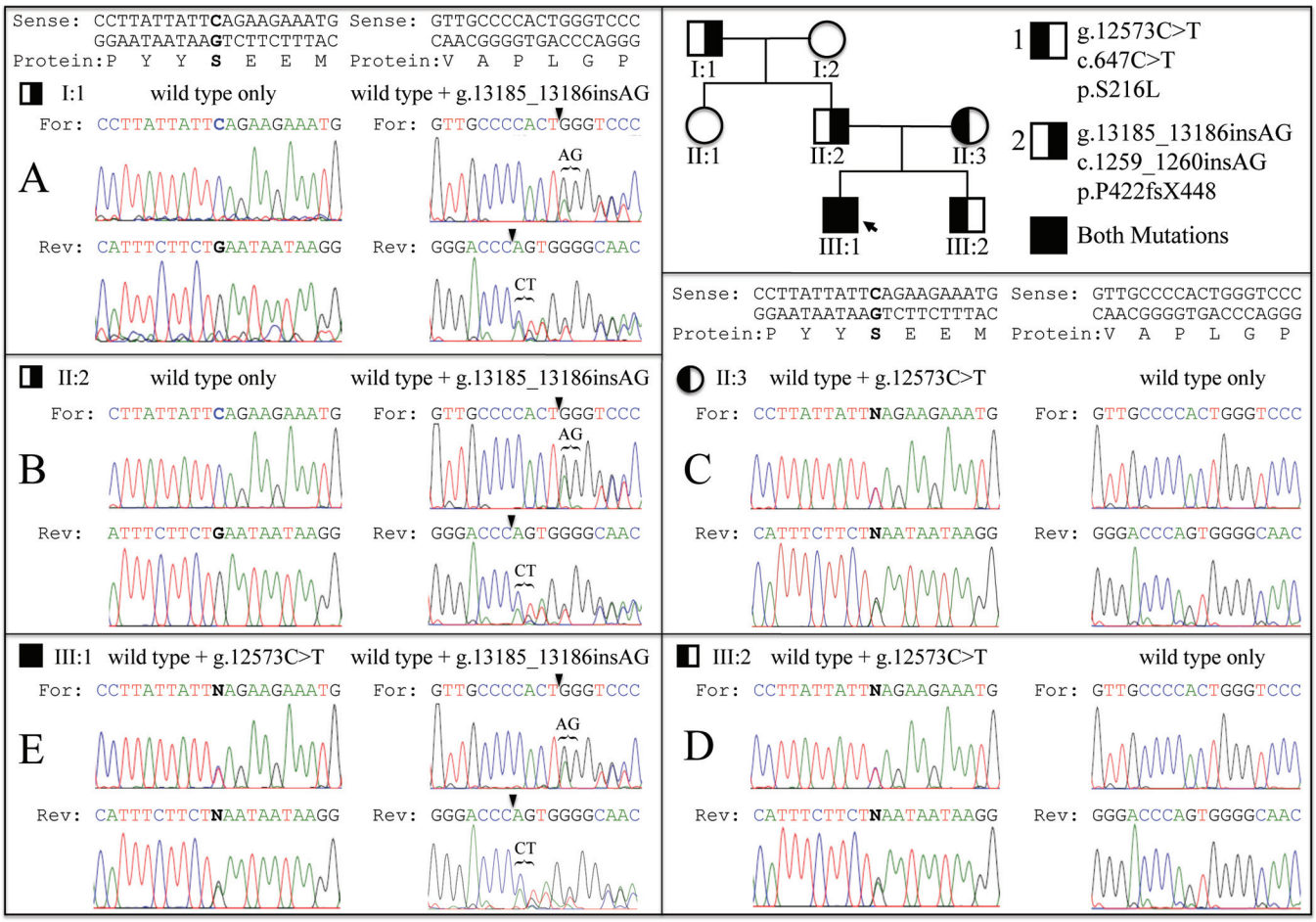
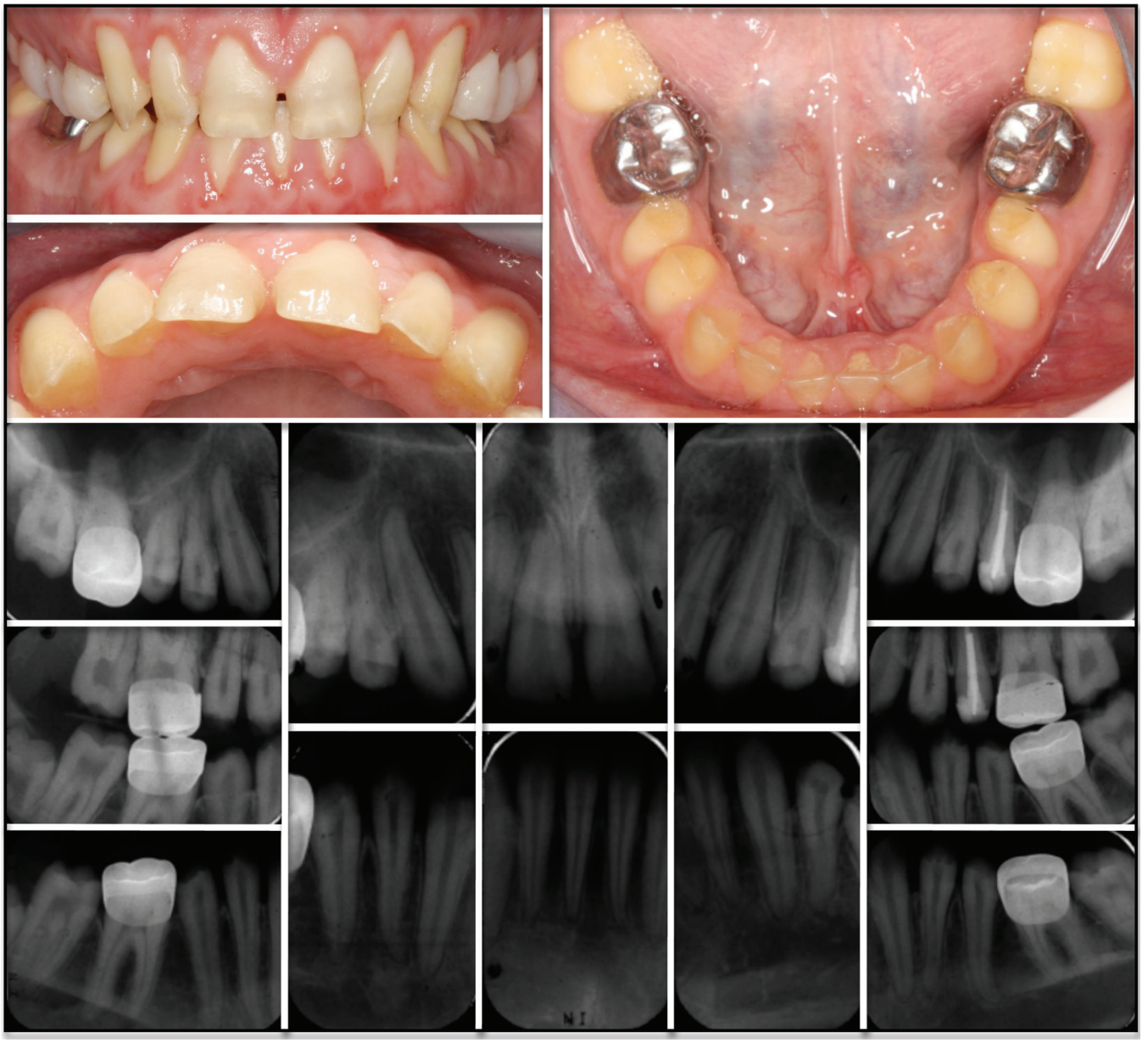
Altered Enamelin Phosphorylation Site Causes Amelogenesis Imperfecta

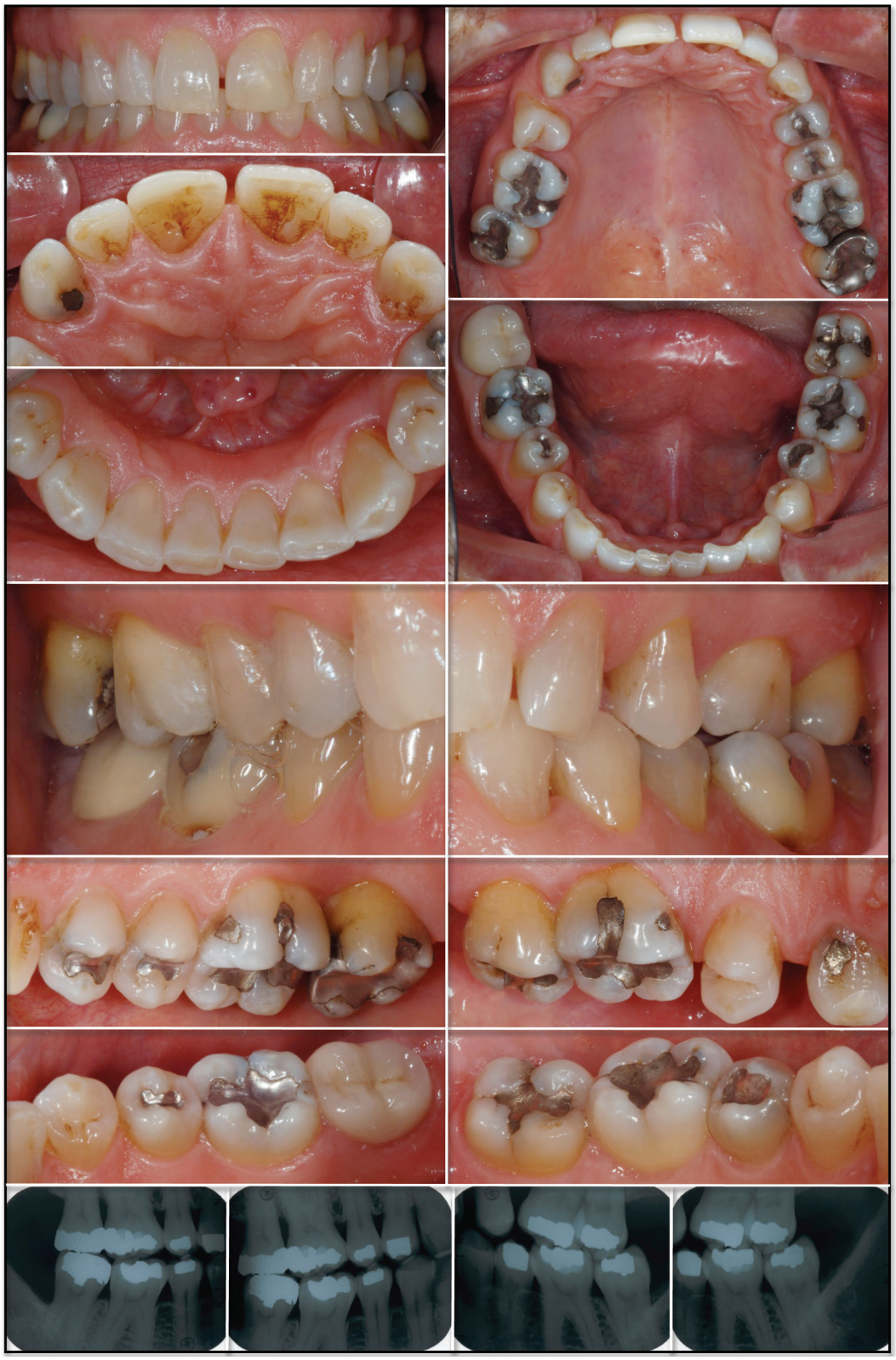
APPENDICES



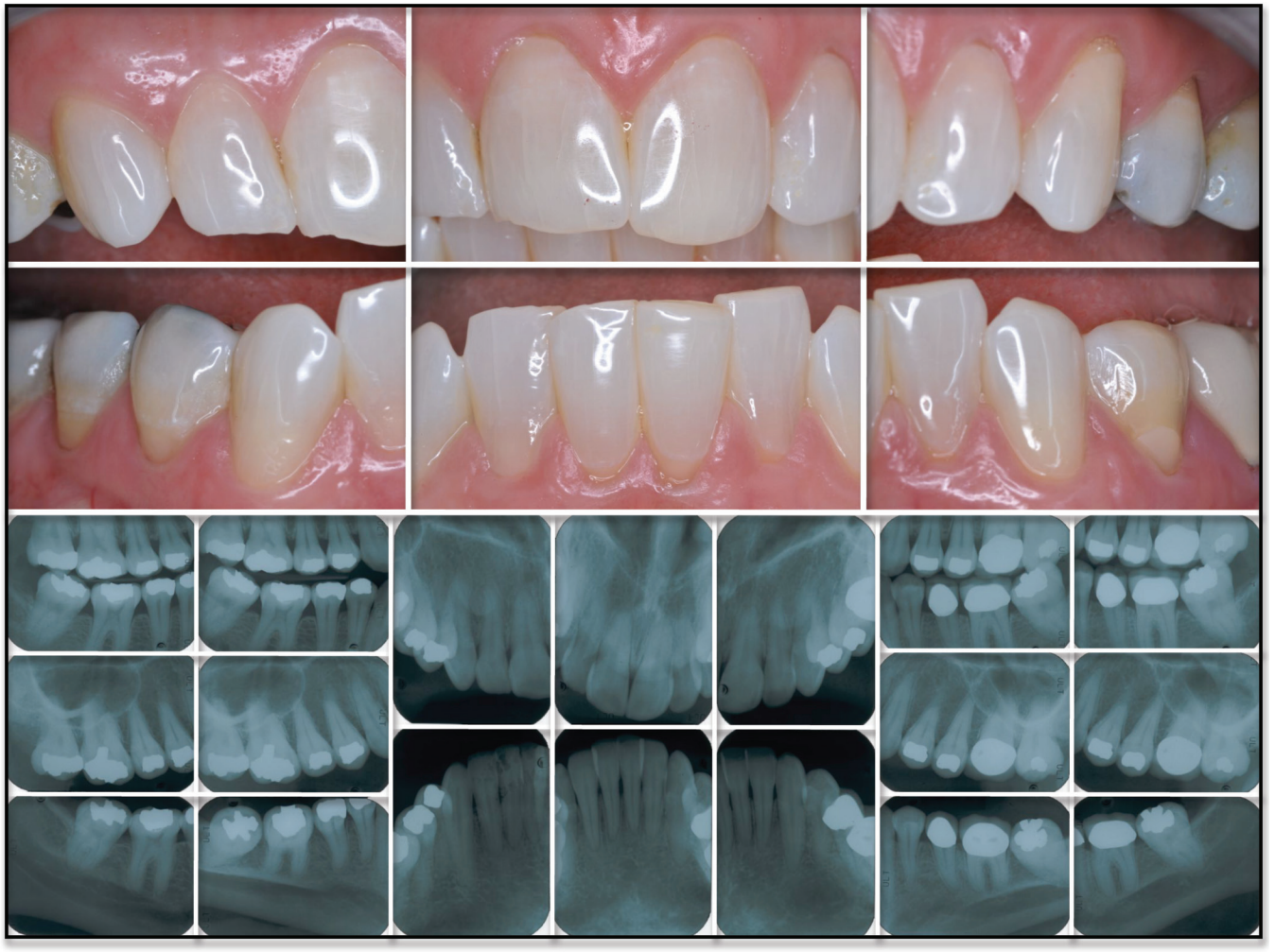
Appendix Figure 1. Distribution of the *ENAM* mutations in this kindred. **(Upper right)** Pedigree of a kindred with autosomal-dominant amelogenesis imperfecta. Two mutant *ENAM* alleles were identified in this family: g.12573C>T; c.647C>T; p.S216L and g.13185_13186insAG; c.1259_1260insAG; p.P422fsX448. **(A)** DNA sequencing chromatograms of the proband's grandfather (I:1) show a normal *ENAM* allele paired with a second *ENAM* allele having the g.13185_13186insAG mutation. After the AG insertion, the sequence from the mutant allele is frameshifted with respect to the normal allele, so the DNA sequencing chromatogram subsequently shows double peaks. **(B)** Chromatograms of the proband's father (II:2) show a normal *ENAM* allele paired with the *ENAM* allele that he inherited from the proband's grandfather carrying the g.13185_13186insAG mutation. **(C, D)** Chromatograms of the mother (II:3) and younger brother (III:2) show a normal *ENAM* allele paired with a defective *ENAM* allele carrying the 12573C>T mutation. The normal C and the mutant T peaks from the 2 alleles are superimposed on the sequencing chromatogram. **(E)** Chromatograms of the proband (III:I) show that both mutant *ENAM* alleles are present (12573C>T and g.13185_13186insAG).



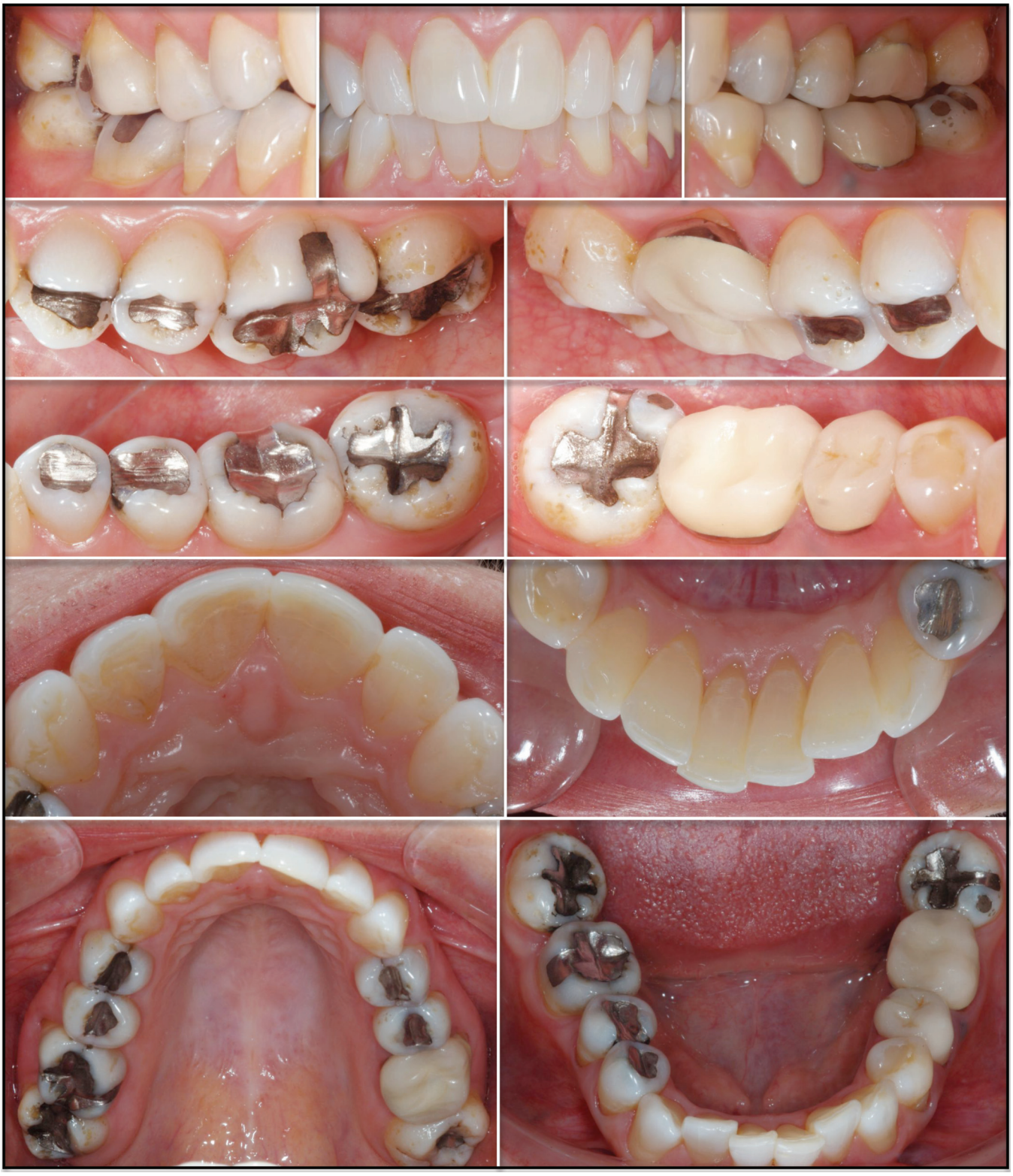
Appendix Figure 2. The proband (III:1) has severe enamel hypoplasia or aplasia resulting from 2 defective *ENAM* alleles (g.12573C>T; c.647C>T; p.S216L and g.13185_13186insAG; c.1259_1260insAG; p.P422fsX448). The exposed crowns are the color of dentin, with no evidence of enamel being present. Generalized gingival inflammation is observed. Radiographs showed no evidence of an enamel layer. The dentin and root morphologies were within normal limits.



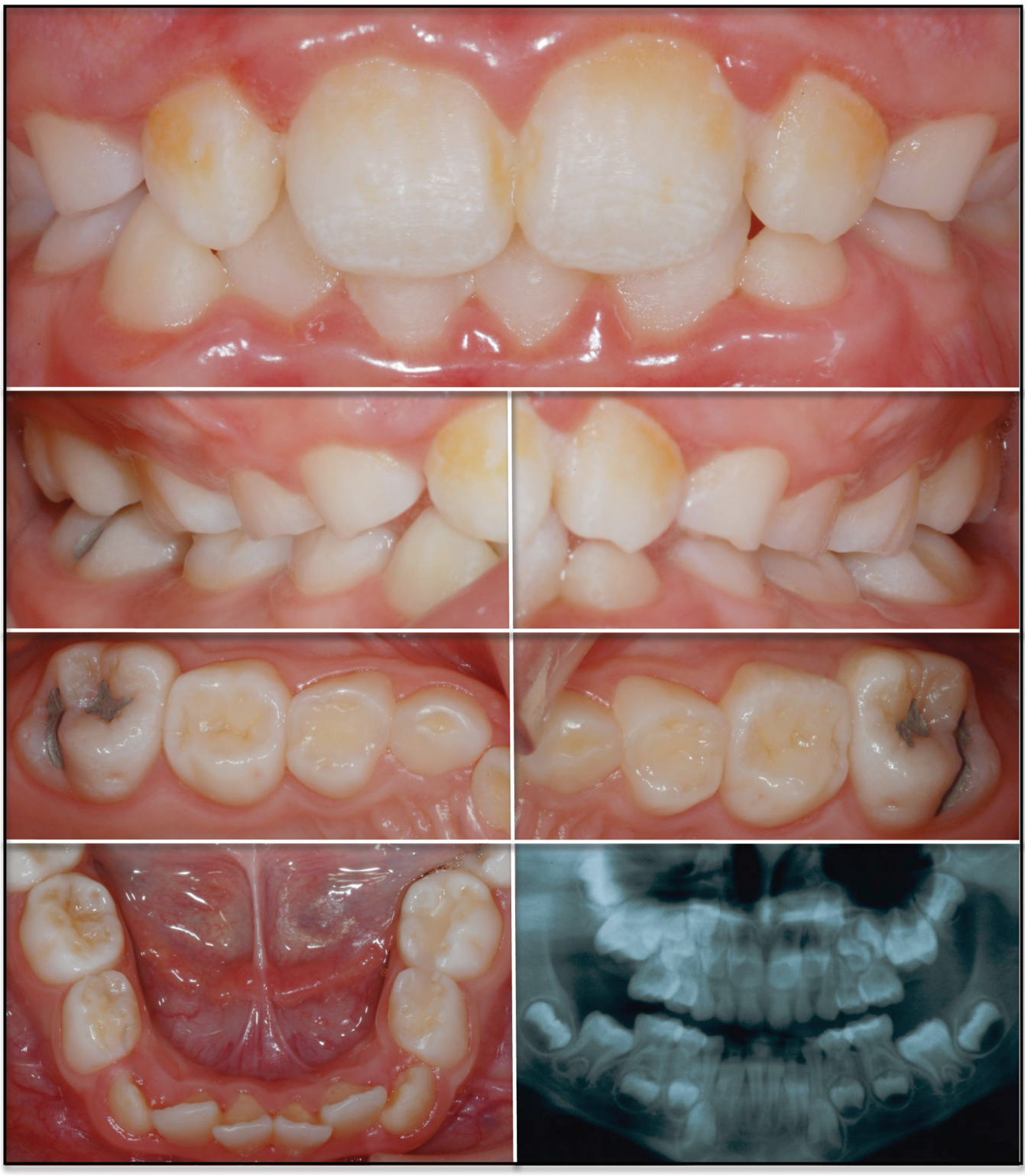
Appendix Figure 3. The father (II:2) has a defect in a single *ENAM* allele (g.13185_13186insAG; c.1259_1260insAG; p.P422fsX448). His dentition is dull gray but glossy, with most posterior teeth being restored with amalgam. The radiographs show no evidence of enamel hypoplasia, and the dentin and root morphologies are within normal limits. Local hypoplastic (pitted) areas are observed, but these are shallow, possibly due to the effects of tooth polishing.



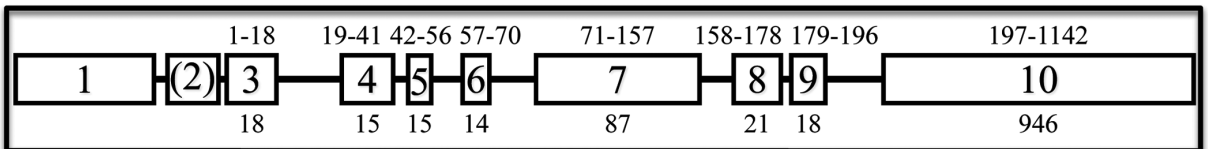
Appendix Figure 4. The mother of the proband (II:3) has localized enamel hypoplasia resulting from a C to T transition in a single *ENAM* allele (g.12573C>T; c.647C>T; p.S216L). The enamel is glossy-white, but translucent in the cervical third of the crowns, which transmits the color of the underlying dentin. Pitting is evident on the mesial-buccal surface of the right maxillary first bicuspid (#5). Radiographs show no evidence of enamel hypoplasia, and the opacity of enamel contrasts well with dentin. The dentin and root morphologies are within normal limits.



Appendix Figure 5. Oral photographs of the mother of the proband (II:3) show glossy anteriors, suggesting that the enamel might have become secondarily polished. All of the posterior teeth have been restored with amalgam, composite, or crowns. Localized enamel hypoplasia (pitting) is easiest to observe in areas that have not become polished, such as the lingual surfaces of the maxillary left bicuspids (#12, #13) and second molar (#15).



Appendix Figure 6. The younger brother of the proband (III:2) has a defect in a single allele of *ENAM*: the C to T transition that he inherited from his mother (g.12573C>T; c.647C>T; p.S216L). The enamel shows the same chalky-white color as the mother, but is not polished. The tooth surface is only slightly rough and retains plaque (colored orange). Enamel pits are observed, particularly at the cusp tips of the primary cuspids and posterior teeth. Radiographs show no evidence of enamel hypoplasia, and the enamel contrasts well with the underlying dentin. The dentin and root morphologies are within normal limits.



Ex/Int	Nucleotides (nt)	Amino Acids
Exon 1	221 nt, from 2-222	Non-coding
Intron 1	320 nt, from 223-542	
(Exon 2)	61 nt, from 543-603	Non-coding
Intron 2	95 nt, from 604-698	
Exon 3	114 nt, from 699-812	Met^{1}-Leu^{18}
Intron 3	3603 nt, from 813-2927	
Exon 4	69 nt, from 2928-2996	Val^{19}-Gln^{41}
Intron 4	110 nt, from 2997-3106	
Exon 5	45 nt, from 3107-3151	Met^{42}-Glu^{56}
Intron 5	757 nt, from 3152-3908	
Exon 6	42 nt, from 3909-3950	Met^{57}-His^{70}
Intron 6	1615, from 3951-5565	
Exon 7	261 nt, from 5566-5826	Met^{71}-Gln^{157}
Intron 7	1263 nt, from 5827-7089	
Exon 8	63 nt, from 7090-7152	Ala^{158}-Gln^{178}
Intron 8	135 nt, from 10555-10689	
Exon 9	54 nt, from 9048-9101	Arg^{179}-Gly^{196}
Intron 9	4171 nt, from 9102-13272	
Exon 10	4804 nt, from 13273-18076	Asn^{197}-Ala^{1142}

Ex/Int	Protein	Gene	References
Exon 5	p.K53X	g:2382A>T	Mårdh *et al*. (2002) *Hum Mol Genet* 11:1069-74 Kim *et al*. (2006) *Eur J Oral Sci* 114 Suppl 1:3-12
Intron 6	p.M71-Q157del	g.4806A>C	Kim *et al*. (2005) *J Dent Res* 84:278-82
Intron 8	p.A158-Q178del	g.6395G>A	Rajpar *et al*. (2001) *Hum Mol Genet* 10:1673-7
Exon 9	p.R179M	c.G817T	Gutierrez *et al*. (2007) *Arch Oral Biol* 52:503-6
Intron 9	p.N197fsX277	g.8344delG	Kida *et al*. (2002) *J Dent Res* 81:738-42 Hart *et al*. (2003) *Arch Oral Biol* 48:589-96 Kim *et al*. (2005) *J Dent Res* 84:278-82 Pavlic *et al*. (2007) *Arch Oral Biol* 52:209-17
Exon 10	p.S216L	g.12573C>T	Chan *et al*. (this paper) *J Dent Res*
Exon 10	p.S246X	g.12663C>A	Ozdemir *et al*. (2005) *J Dent Res* 84:1036-41
Exon 10	p.V340-M341insSQYQYCV		Ozdemir *et al*. (2005) *J Dent Res* 84:1036-41
Exon 10	p.P422fsX448	g.13185/6insAG	Hart *et al*. (2003) *J Med Genet* 40:900-6 Ozdemir *et al*. (2005) *J Dent Res* 84:1036-41 Pavlic *et al*. (2007) *Arch Oral Biol* 52:209-17 Kang *et al*. (2009) *J Dent Res* 88:266-9 Chan *et al*. (this paper) *J Dent Res*
Exon 10	p.P998fsX1062	g.14917delT	Kang *et al*. (2009) *J Dent Res* 88:266-9

Appendix Figure 7. *ENAM* gene structure based upon the human cDNA (NM_031889.2) and gene (NG_013024.1) reference sequences. The lower box shows the 10 known disease-associated mutations in *ENAM*. Some reports describe enamel malformations resulting from defects in both *ENAM* alleles. Persons homozygous for p.P422fsX448 have been described previously (Hart *et al.*, 2003). A compound heterozygote with p.P422fsX448 paired with p.V340_M341insSQYQYCV has been described (Ozdemir *et al.*, 2005). Here we describe a compound heterozygote with a p.S216L mutation in one *ENAM* allele paired with the p.P422fsX448 frameshift in the other.

APPENDIX REFERENCES

Hart TC, Hart PS, Gorry MC, Michalec MD, Ryu OH, Uygur C, *et al*. (2003). Novel ENAM mutation responsible for autosomal recessive amelogenesis imperfecta and localised enamel defects. *J Med Genet* 40:900-906.

Ozdemir D, Hart PS, Firatli E, Aren G, Ryu OH, Hart TC (2005). Phenotype of ENAM mutations is dosage-dependent. *J Dent Res* 84:1036-1041.