RESEARCH REPORTS
Biological

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J Dent Res DOI: 10.1177/0022034513510944

Barrier Formation: Potential Molecular Mechanism of Enamel Fluorosis

APPENDIX

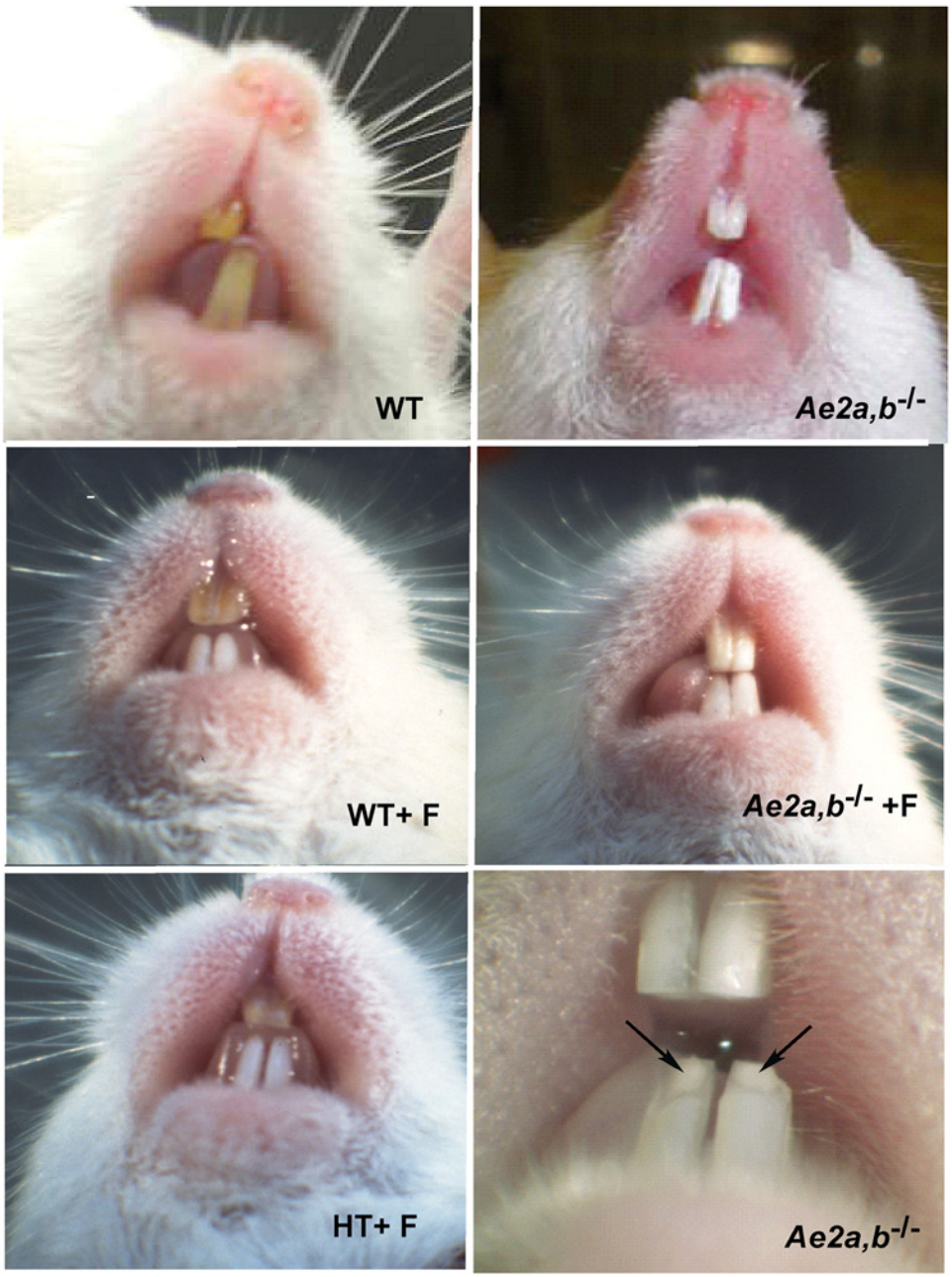
Appendix Table 1. Composition of Enamel from Fluorotic Homozygous Wild-Type Mice and Fluorotic Heterozygous *Ae2a,b* Mice Shows No Significant Differences between the Heterozygous Mutant and Homozygous Wild-Type Mice, Means ± SD

	Maturation Stage (% Weight)			
Element	Fluorotic Heterozygous *Ae2a,b* (n = 7)	Fluorotic Homozygous Wild Type (n = 3)	Nonfluorotic Homozygous Wild Type	p^*
CaO	45.8 ± 3.8	42.6 ± 4.2	50.2 ± 0.2	.95
P_2O_5	34.9 ± 2.3	34.2 ± 4.3	41.8 ± 0.2	.80
MgO	0.22 ± 0.10	0.22 ± 0.23	0.21 ± 0.05	.84
SO_3	0.18 ± 0.22	0.05 ± 0.05	0.01 ± 0.01	.36
Cl	0.24 ± 0.04	0.21 ± 0.04	0.34 ± 0.01	.28
F	0.11 ± 0.06	0.19 ± 0.15	0.04 ± 0.01	.27

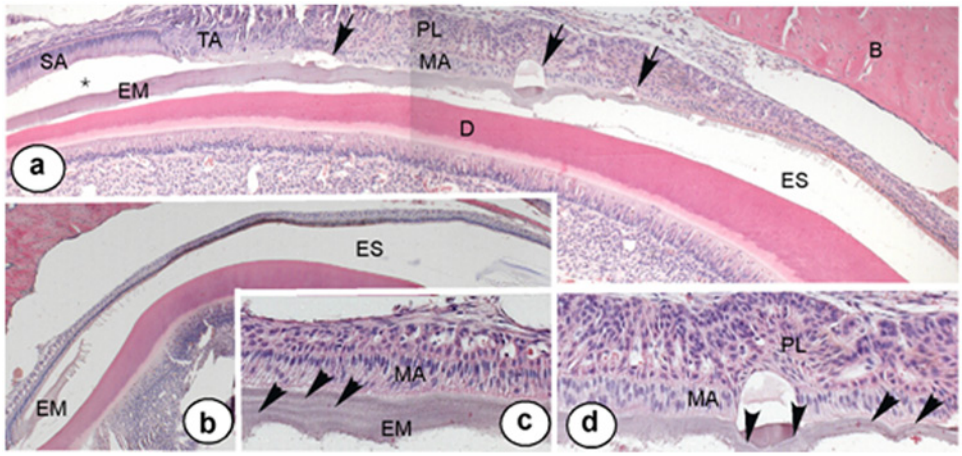
*Unpaired *t* test: fluorotic heterozygous *vs.* fluorotic homozygous mice. Collected data from various experiments. Fluorotic mice were exposed for 6 weeks to 100 mg/L of F in drinking water.

Appendix Table 2. Values Calculated by Analysis of Variance, *p* (Data to Table)

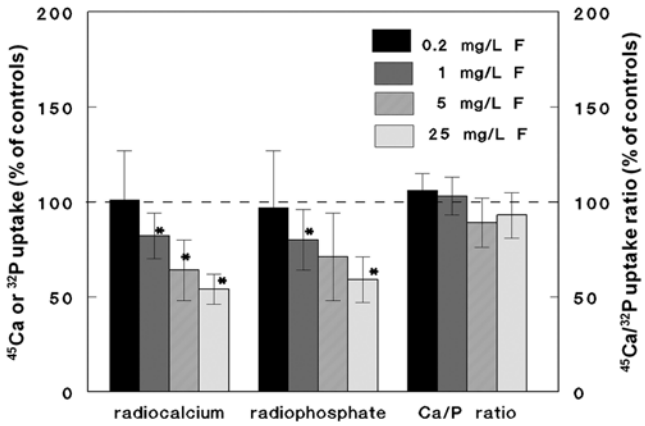
	Calcium		Magnesium	
	Secretion	Maturation	Secretion	Maturation
Among all 4 groups	.288	.0022	.064	.0024
WT *vs.* WT+F	—	—	—	—
WT *vs.* *Ae2a,b*$^{-/-}$	—	< .01	—	—
WT *vs.* *Ae2a,b*$^{-/-}$ +F	—	< .05	—	< .01
WT+F *vs.* *Ae2a,b*$^{-/-}$+F	—	—	—	< .01
WT+F *vs.* *Ae2a,b*$^{-/-}$	—	< .05	—	—
Ae2a,b$^{-/-}$ *vs.* *Ae2a,b*$^{-/-}$+F	—	—	—	—
	Phosphorus		Fluoride	
Among all 4 groups	.3299	.0008	.083	.004
WT *vs.* WT+F	—	—	—	—
WT *vs.* *Ae2a,b*$^{-/-}$	—	< .001	—	—
WT *vs.* *Ae2a,b*$^{-/-}$ +F	—	< .05	—	< .05
WT+F *vs.* *Ae2a,b*$^{-/-}$+F	—	—	—	< .05
WT+F *vs.* *Ae2a,b*$^{-/-}$	—	< .05	—	—
Ae2a,b$^{-/-}$ *vs.* *Ae2a,b*$^{-/-}$+F	—	—	—	< .01
	Sulphur		Chloride	
Among all 4 groups	.260	.245	.0323	.0001
WT *vs.* WT+F	—	—	—	< .001
WT *vs.* *Ae2a,b*$^{-/-}$	—	—	—	< .001
WT *vs.* *Ae2a,b*$^{-/-}$ +F	—	—	—	< .001
WT+F *vs.* *Ae2a,b*$^{-/-}$+F	—	—	—	< .001
WT+F *vs.* *Ae2a,b*$^{-/-}$	—	—	—	< .001
Ae2a,b$^{-/-}$ *vs.* *Ae2a,b*$^{-/-}$ +F	—	—	—	—
	Ca/P		Cl/Ca	
Among all 4 groups	.847	.204 (n = 18)	.0013	< .0001
WT *vs.* WT+F	—	—	< .01	—
WT *vs.* *Ae2a,b*$^{-/-}$	—	—	< .01	< .001
WT *vs.* *Ae2a,b*$^{-/-}$ +F	—	—	< .01	< .001
WT+F *vs.* *Ae2a,b*$^{-/-}$+F	—	—	—	< .001
WT+F *vs.* *Ae2a,b*$^{-/-}$	—	—	—	< .001
Ae2a,b$^{-/-}$ *vs.* *Ae2a,b*$^{-/-}$ +F	—	—	—	< .05



Appendix Figure 1. Changes in color of incisor enamel. Enamel of wild-type (WT) mice is orange (left, top). Incisors of fluorotic wild-type (WT+F) and heterozygous (HT+F) mice are more weakly stained. Enamel of *A2a,b*$^{-/-}$ and fluorotic *Ae2a,b*$^{-/-}$ (*Ae2a,b*$^{-/-}$ + F) mice are chalky white. Bottom right shows erosion of incisor enamel in an *Ae2a,b*$^{-/-}$ mouse.



Appendix Figure 2. Histology of nonfluorotic and fluorotic enamel from *Ae2a,b*$^{-/-}$ mice (decalcified sections). (a, c, d) Fluorotic enamel from *Ae2a,b*$^{-/-}$ mice with typical changes seen when wild-type rodents are exposed to a single high level of F but not in chronic exposure to fluoridated drinking water: cysts (a; arrows) and hypermineralized lines (c, d) in enamel matrix indicated by the presence of less intense hematoxylin-stained lines (arrow heads). (a, c, d) Retention and delayed matrix removal. After local detachment at transition–early maturation, the ameloblast layer reattaches in incisal direction (a) and forms a coherent layer. Ameloblasts and papillary layer shorten more than in wild-type controls (b: wild-type control). B, bone; de, dentin; em, enamel matrix; es, enamel space; ma, maturation ameloblasts; pl, papillary layer; sa, secretory ameloblasts; ta, transitional ameloblasts; *, artifact. (e-h) Hematoxylin and eosin.



Appendix Figure 3. Hamster molar tooth organ cultures with enamel containing a deep fluorotic hypermineralized line take up less mineral ions. Pairs of first upper molar tooth germ of hamster pups were grown in culture (see Bronckers *et al.*, 1984 [*Arch Oral Biol* 29:803-810]). At the second day of culture, explants in the early secretory stage were exposed overnight to 0.2, 1, 5, or 25 mg/L of F or equimolar NaCl (contralateral controls). Next day, the explants were transferred to fresh F-free medium and grown for another 5 days, with medium refreshed every other day. For the last 24 hrs of culture, explants were labeled with $^{45}Ca^{2+}$ and $^{32}PO_4^{3-}$ (1 μCi per mL), the radiolabels were extracted from the explants with 10% ice-cold trichloroacetic acid and counted for ^{45}Ca and ^{32}P. For the paired organ cultures, statistical significance was determined by Student *t* test for paired samples and, all other data, by the unpaired *t* test at $p < .05$. Uptake is expressed as percentage of control (paired *t* test; $^{*}p < .05$; means and standard deviation; n = 5).