ENAMEL DEFECTS AND AMELOBLAST-SPECIFIC EXPRESSION IN *ENAMELIN* KNOCKOUT/LACZ KNOCKIN MICE

Jan C-C. Hu[1], Yuanyuan Hu[2], Charles E. Smith[3], Marc D. McKee[4], J. Timothy Wright[5], Yasuo Yamakoshi[2], Petros Papagerakis[1], Graeme K. Hunter[6], Jerry Q. Feng[7], Fumiko Yamakoshi[1], James P. Simmer[2]

From Department of Orthodontics and Pediatric Dentistry[1], Department of Biologic and Materials Sciences[2], University of Michigan School of Dentistry, Ann Arbor, MI 48108; Laboratory for the Study of Calcified Tissues and Biomaterials[3], Faculté de médecine dentaire, Université de Montréal, Montreal, QC CANADA H3C 3J7; Department of Anatomy and Cell Biology, Jamson T.N. Wong Laboratories for Calcified Tissue Research, McGill Centre for Bone and Peridodontal Research, and the FRSQ Network for Oral and Bone Health Research[4], McGill University, Faculty of Dentistry, Montreal, QC, Canada, H3A 2B2; Department of Pediatric Dentistry[5], University of North Carolina, Chapel Hill, NC 27599-7455; CIHR Group in Skeletal Development and Remodeling[6], Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada N6A 5C1; Department of Biomedical Sciences[7], Baylor College of Dentistry, Dallas, TX 75246.

Address correspondence to Jan C-C. Hu, BDS, PhD, U of M Dental Research Laboratory, 1210 Eisenhower Place, Ann Arbor, MI 48108; 734-975-9315; Fax: 734-975-9326; Email: janhu@umich.edu

Running Title: The Enamelin Knockout/LacZ Knockin Mouse

Supplementary Table. Statistical analyses of mineral and protein content of incisors at 7 weeks. The developing enamel covering continuously growing maxillary and mandibular incisors of wild type (+/+), heterozygous (+/-), and null (-/-) mice were transected into a series of sequential strips from apical towards the incisal ends. Strips associated with each of the three main stages of enamel development (S, secretory stage; EM, early maturation stage; NM, nearly mature enamel) were pooled from at least a dozen teeth per genotype to compute grand means for the total dry weight before ashing, mineral weight after ashing, mineral-to-protein ratio, and percent mineral by weight. For purposes of this study, the protein content of a strip was defined as the difference between its starting dry weight minus its final ashed weight. The percent mineral by weight in each strip was calculated by the equation (ashed weight/total dry weight) x 100. Grand means from pooled strips for each of the three stages of enamel development in each genotype were compared for significance using nonparametric tests available in Version 7 of Statistica for Windows (Statsoft Inc., Tulsa, OK). The statistical tests values are presented in the following table for one-way (items 1 and 2), two-way (item 3) and three-way (item 4) factor comparisons.

Supplemental Table: Statistical Tests[a] of Differences between Means

	P values[b]			
	Mineral wt	M:P Ratio[c]	Mineral (wt%)[d]	N[e]
1. Tooth location (right vs left jaw)	0.8203	0.8037	1.0000	250, 240
2. Jaw (maxillary vs mandibular incisor)	**0.0000**	**0.0004**	0.1432	158, 332
3. Jaw by Genotype (*Enam*)	**0.0000**	**0.0000**	ND	2, 490
Maxillary				2, 158
+/+ vs +/-	1.0000	1.0000	0.9311	
+/+ vs -/-	**0.0008**	1.0000	0.9203	
+/- vs -/-	**0.0001**	1.0000	0.8369	
Mandibular				2, 332
+/+ vs +/-	1.0000	0.3550	0.5059	
+/+ vs -/-	**0.0000**	**0.0131**	0.2957	
+/- vs -/-	**0.0000**	**0.0000**	**0.0196**	
4. Stage of Amelogenesis by Genotype (*Enam*) and by Jaw				
Maxillary				
S (Secretory Stage)				2, 44
+/+ vs +/-	1.0000	1.0000	0.9481	
+/+ vs -/-	1.0000	0.3779	0.7681	
+/- vs -/-	0.5187	1.0000	0.8176	
EM (Early Maturation)				2, 49
+/+ vs +/-	1.0000	0.8596	0.8026	
+/+ vs -/-	**0.0000**	**0.0221**	0.5963	
+/- vs -/-	**0.0000**	0.3907	0.8000	
NM (Nearly Mature)				2, 50
+/+ vs +/-	1.0000	1.0000	0.8133	
+/+ vs -/-	**0.0000**	**0.0000**	0.2497	
+/- vs -/-	**0.0000**	**0.0000**	0.3186	
Mandibular				
S (Secretory Stage)				2, 74
+/+ vs +/-	0.1834	1.0000	0.9137	
+/+ vs -/-	**0.0000**	**0.0018**	0.2418	
+/- vs -/-	**0.0118**	**0.0114**	0.2773	
EM (Early Maturation)				2, 75
+/+ vs +/-	**0.0031**	**0.0014**	0.1726	
+/+ vs -/-	**0.0000**	**0.0000**	0.0738	
+/- vs -/-	**0.0000**	0.2139	0.6756	
NM (Nearly Mature)				2, 133
+/+ vs +/-	**0.0055**	**0.0336**	0.5127	
+/+ vs -/-	**0.0000**	**0.0000**	0.1298	
+/- vs -/-	**0.0000**	**0.0000**	0.0774	

[a]Nonparametric

[b]Means were considered significantly different if P values were <0.05 (bold)

[c]M:P, mineral to protein ratio. A Mann-Whitney U test was used to compare means from two independent samples and the Kruskal-Wallis ANOVA with multiple comparisons of mean ranks was used to compare means by genotype (3 independent samples)

[d]A Fisher's Exact Test was used to compare two means based on percentages

[e]N, number of observations or degrees of freedom