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Effects of *Fam83h* overexpression on enamel and dentine formation

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ABSTRACT

Objective: The aim of this study was to determine if FAM83H over-expression causes dentine or enamel malformations.

Materials and methods: The full-length mouse *Fam83h* cDNA was inserted into the pCAGIG vector between a β-actin promoter and β-globin enhancer for ubiquitous expression in transgenic mice. Recombinant mouse FAM83H was expressed and used to generate polyclonal antibodies. Western blots showed enhanced expression of the *Fam83h* transgene. The effects of transgene expression on tooth development were assessed by microhardness measurements of enamel and dentine. Total thickness of incisor enamel at the level of the alveolar crest was measured and decussating rod patterns were visualized by scanning electron microscopy (SEM).

Results: Three transgenic mouse lines were selected based upon their transgene expression levels. There was no statistically significant difference in the Vickers microhardness values of enamel or dentine between the transgenic lines or between the transgenic lines and wild type mice. No statistically significant differences in enamel thickness were observed between the transgenic lines and the wild type mice. SEM analysis revealed no apparent differences in the enamel crystal and rod morphologies.

Conclusion: Our findings demonstrate that over-expression of FAM83H in mice does not produce a phenotype in dentine or enamel.

1. Introduction

Family with sequence similarity 83, member h (*FAM83H*) is an obscure gene and protein that became especially interesting to dentists and scientists studying tooth development when it was discovered that two nonsense mutations (p.Arg325* and p.Gln398*) in *FAM83H* on chromosome 8q24.3 caused autosomal-dominant hypocalcified amelogenesis imperfecta (ADHCAI, OMIM #130900) in humans.[1] Additional reports of

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Table 1 – Sites of *FAM83H* mutations causing ADHCAI. FAM83H primary sequence showing the positions of the 20 novel AI-causing *FAM83H* nonsense (bold) or frameshift (bold/italic) mutations. The cDNA numbers in the mutation list start from the translation initiation site of *FAM83H* reference sequence NM_198488.3.

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MARRSQSSSQGDNPLAPGYLPPHYKEYYRLAVDALAEGGSEAYSRFLATEGAPDFLCPEELEHVSRHLRP   70
PQYVTREPPEGSLLDVDMDGSSGTYWPVNSDQAVPELDLGWPLTFGFQGTEVTTLVQPPPPDSPSIKDEA  140
RRMIRSAQQVVAVVMDMFTDVDLLSEVLEAAARRVPVYILLDEMNAQHFLDMADKCRVNLHHVDFLRVRT  210
VAGPTYYCRTGKSFKGHVKEKFLLVDCAVVMSGSYSFMWSFEKIHRSLAHVFQGELVSSFDEEFRILFAQ  280
      1          2     3      5 6                7
SEPLVPSAAALARMDAYALAPYAGAGPLVGVPGVGAPTPFSFPKRAHLLFPPPREEGLGFPSFLDPDRHF  350
                                                  8                 9
LSAFRREEPPRMPGGALEPHAGLRPLSRRLEAEAGPAGELAGARGFFQARHLEMDAFKRHSFATEGAGAV  420
         10               11        12   13 14 15         16
ENFAAARQVSRQTFLSHGDDFRFQTSHFHRDQLYQQQYQWDPQLTPARPQGLFEKLRGGRAGFADPDDFT  490
LGAGPRFPELGPDGHQRLDYVPSSASREVRHGSDPAFAPGPRGLEPSGAPRPNLTQRFPCQAAARPGPDP  560
                                                                 17
APEAEPERRGGPEGRAGLRRWRLASYLSGCHGEDGGDDGLPAPMEAEAYEDDVLAPGGRAPAGDLLPSAF  630
                                  18              19                20
RVPAAFPTKVPVPGPGSGGNGPEREGPEEPGLAKQDSFRSRLNPLVQRSSRLRSSLIFSTSQAEGAAGAA  700
AATEKVQLLHKEQTVSETLGPGGEAVRSAASTKVAELLEKYKGPARDPGGGAGAITVASHSKAVVSQAWR  770
EEVAAPGAVGGERRSLESCLLDLRDSFAQQLHQEAERQPGAASLTAAQLLDTLGRSGSDRLPSRFLSAQS  840
HSTSPQGLDSPLPLEGSGAHQVLHNESKGSPTSAYPERKGSPTPGFSTRRGSPTTGFIEQKGSPTSAYPE  910
RRGSPVPPVPERRSSPVPPVPERRGSLTLTISGESPKAGPAEEGPSGPMEVLRKGSLRLRQLLSPKGERR  980
MEDEGGFPVPQENGQPESPRRLSLGQGDSTEAATEERGPRARLSSATANALYSSNLRDDTKAILEQISAH 1050
GQKHRAVPAPSPGPTHNSPELGRPPAAGVLAPDMSDKDKCSAIFRSDSLGTQGRLSRTLPASAEERDRLL 1120
RRMESMRKEKRVYSRFEVFCKKEEASSPGAGEGPAEEGTRDSKVGKFVPKILGTFKSKK*           1179
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#	cDNA	Protein	References
1	c.860C>A	p.S287*	23
2	c.891T>A	p.Y297*	24
3	c.906T>G	p.Y302*	18,25
4	c.923_924delTC	p.L308fs*323	23
5	c.924dupT	p.V309Rfs*324	18
6	c.973C>T	p.R325*	1,18
7	c.1192C>T	p.Q398*	1,12,26,27
8	c.1243G>T	p.E415*	24
9	c.1289C>A	p.S430*	27
10	c.1330C>T	p.Q444*	12,26
11	c.1354C>T	pQ452*	9,18,25,28
12	c.1366C>T	p.Q456*	26
13	c.1374C>A	p.Y458*	29
14	c.1379G>A	p.W460*	23
15	c.1380G>A	p.W460*	24
16	c.1408C>T	p.Q470*	23
17	c.1872_1873delCC	p.L625fs*703	23
18	c.1993 C>T	p.Q665*	14
19	c.2029 C>T	p.Q677*	9,14,18,24,27
20	c.2080G>T	p.E694*	23

FAM83H mutations causing ADHCAI quickly followed. As of this writing 20 novel *FAM83H* mutations causing ADHCAI have been reported in 33 AI kindreds (Table 1). Six of the *FAM83H* mutations have been identified independently in multiple kindreds.

Amelogenesis imperfecta (AI) is a collection of inherited diseases that exhibit enamel malformations without symptoms outside of the dentition.[2] Defects in other genes besides *FAM83H* that have been shown to cause non-syndromic amelogenesis imperfecta include *AMELX*,[3] *ENAM*,[4] *MMP20*,[5] *KLK4*,[6] *WDR72*,[7] and *C4orf26*.[8] Combined, these genes account for about half of all non-syndromic AI cases.[9] The other causative genes are unknown.

Defects in *FAM83H* account for more AI cases than any other single gene. *FAM83H* has 20 reported novel disease-causing mutations. The number of novel mutations in the other AI-associated genes are: 18 in *AMELX*,[10]12 *ENAM*,[11] 5 *C4orf26*,[8] 5 *WDR72*, 4 *MMP20*, and 1 *KLK4*. *FAM83H* mutations are also highest on a percentage basis. Among the 19 AI-causing defects identified in 39 families, 9 were in *FAM83H*, 4 in *ENAM*, 4 in *AMELX*, 2 in *MMP20*, and one in *WDR72*.[9]

Despite the relatively high prevalence of AI cases caused by *FAM83H* defects, the FAM83H gene and protein, and the types of mutations that cause disease are both unusual. Unlike most of the genes that cause isolated AI, *FAM83H* does not encode a