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Amelogenesis Imperfecta: Genotype-Phenotype Studies in 71 Families

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Abstract
Amelogenesis imperfecta (AI) represents hereditary conditions affecting the quality and quantity of enamel. Six genes are known to cause AI (AMELX, ENAM, MMP20, KLK4, FAM83H, and WDR72). Our aim was to determine the distribution of different gene mutations in a large AI population and evaluate phenotype-genotype relationships. Affected and unaffected family members were evaluated clinically and radiographically by one examiner. Genotyping was completed using genomic DNA obtained from blood or saliva. A total of 494 individuals were enrolled, with 430 (224 affected, 202 unaffected, and 4 not definitive) belonging to 71 families with conditions consistent with the diagnosis of AI. Diverse clinical phenotypes were observed (i.e. hypoplastic, hypocalcified, and hypomaturation). Genotyping revealed mutations in all 6 candidate genes. A molecular diagnosis was made in 132 affected individuals (59%) and in 26 of the families (37%). Mutations involved 12 families with FAM83H (46%), 6 families with AMELX (23%), 3 families with ENAM (11%), 2 families with KLK4 and MMP20 (8% for each gene), and 1 family with a WDR72 mutation (4%). Phenotypic variants were associated with allelic FAM83H and AMELX mutations. Two seemingly unrelated families had the same KLK4 mutation. Families affected with AI where candidate gene mutations were not identified could have mutations not identifiable by traditional gene sequencing (e.g. exon deletion) or they could have promoter sequence mutations not evaluated in this study. However, the results suggest that there remain new AI causative genes to be identified.

Introduction

While there are about 85 hereditary conditions that affect enamel formation found in OMIM 2010 [OMIM, 2010], amelogenesis imperfecta (AI) represents hereditary conditions that predominantly affect the quantity

Abbreviation used in this paper	
AI	amelogenesis imperfecta

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and quality of enamel in the absence of other developmental traits [Witkop, 1989]. These conditions are clinically variable and genetically heterogeneous. Prior to the identification of any genes known to cause AI, a variety of studies described the prevalence and phenotype of AI [Witkop et al., 1973; Witkop and Sauk, 1976; Rowley et al., 1982; Crawford and Aldred, 1988]. Population studies were conducted in the USA, Israel [Witkop, 1957; Chosack et al., 1979], and Scandinavian countries [Witkop, 1957; Chosack et al., 1979; Backman and Holm, 1986; Sundell, 1986; Backman, 1988] where 2 large groups affected by AI were described. Since the identification of the first gene known to cause AI, i.e. the *AMELX* gene, only 1 large study has examined the different candidate AI genes in multiple families segregating for different forms of AI [Kim et al., 2006]. The study by Kim et al. [2006] evaluated 24 AI families and found disease-causing mutations in 6 of the families. They sequenced all of the AI disease-causing genes known at the time *(AMELX, ENAM, KLK4,* and *MMP20)* and 3 other candidate genes *(AMBN, DLX3,* and *TUFT1).* Mutations in *AMELX, ENAM,* and *MMP20* were identified.

Since then, numerous other genes associated with enamel defects that also occur with systemic conditions have been identified (e.g. *CNNM4* – Jalili syndrome, OMIM No. 217080 [Parry et al., 2009]; *GJA1* – oculodentodigital dysplasia, OMIM No. 164200 [Vitiello et al., 2005], and *SLC4A4* – renal tubular acidosis, with ocular abnormalities and mental retardation, OMIM No. 604278 [Igarashi et al., 1999] to name just a few). Two new AI disease-causing genes have been identified including *FAM83H* and *WDR72* [Kim et al., 2008; El-Sayed et al., 2009]. Multiple allelic mutations in the *FAM83H* gene have been identified as causative of autosomal dominant hypocalcified AI (OMIM No. 130900). Depending on the mutation the phenotype can be either a generalized hypocalcification of the entire dentition or a more localized hypocalcified defect that localizes predominantly in the cervical areas of teeth [Wright et al., 2009]. Mutations in the *WDR72* gene are associated with hypomaturation AI (OMIM No. 613211). Thus, there are now 6 genes known to cause AI. It is unknown how many families or cases of AI in the general population are associated with these known genes. Answering this question would provide some insight into the potential for further heterogeneity of the AI conditions. Therefore, the purpose of this study was to establish the mutations in genes known to be associated with AI and other candidate genes (e.g. *DLX3* and *AMBN*).

Methods

Individuals and families were recruited into a prospective study to identify AI-causative genes and to identify phenotype-genotype associations. Individuals and families were referred by clinicians globally, self-referred by contacting our study website, or identified through direct clinical contact. All individuals had hypoplastic and/or hypomineralized enamel defects that occurred in the absence of systemic manifestations or known environmental exposures that would explain the enamel malformation. The defects tended to affect both primary and permanent dentitions and were present on most or all of the teeth. Conditions such as molar-incisor hypomineralization, fluorosis, or other complex or environmental conditions were excluded. Each participant's medical, dental, and family history was evaluated and pedigrees were constructed to help establish a potential mode of inheritance for the dental trait. Additional family members, including unaffected members, were evaluated whenever possible. Individuals were examined by the PI when possible, or by referring clinicians, to establish the enamel phenotype. Further characterization of the enamel phenotype involved radiograph evaluation whenever possible.

As described in previous publications [Wright et al., 2009], genomic DNA was isolated from peripheral blood leucocytes or saliva from the participants using a QIAamp blood kit (Qiagen, Santa Clara, Calif., USA) or an Oragene saliva kit (DNA Genotek, Kanata, Ont., Canada). PCR was typically performed in a volume of 50 μl containing 0.6 μM each forward and reverse primers, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 1× PCR buffer, 200 ng DNA, and 1 unit of *Taq* DNA polymerase. Amplicons were extracted from gels and sequenced using ABI Big Dye terminator chemistry and an ABI 3730 DNA Analyzer. PCR products were sequenced in both directions to minimize sequencing artifacts. The genes evaluated included *AMELX, ENAM, KLK4, MMP20, FAM83H, WDR72, AMBN, DLX3,* and *AMTN*.

Results

There were 91 families and 494 individuals evaluated in this study (table 1). Of the 91 families enrolled, a specific AI inheritance and phenotypic diagnosis corresponding to Witkop's nosology and clinical classification [Witkop, 1989] or recently modified classifications [Nussier et al., 2004] could be made in 71 families. Of the 20 families where a specific diagnosis could not be made, 9 families showed transmission of the enamel trait in an autosomal dominant or recessive fashion while in 11 families transmission appeared to be autosomal recessive or sporadic. Of the unclassified cases 10 had a hypoplastic enamel phenotype and 10 had hypomineralized phenotypes.

In the 71 diagnosed families there were 224 affected individuals that had a clearly delineable AI type, and 202 of their unaffected family members were evaluated. Affected individuals represented a racially diverse population involving families of Caucasian, African American, Hispanic, and Asian descent. AI causative mutations were

identified in 26 families and 132 individuals involving 19 different mutations that included all 6 known candidate AI genes. The majority of families and individual study participants with identifiable mutations were affected, with autosomal dominant hypocalcified AI (*FAM83H* mutations) accounting for 12 of the kindreds and 85 of the affected individuals (table 2). This corresponded to approximately 17% of the total AI kindreds identified as having a definitive diagnosis. Two families having a novel autosomal dominant localized hypocalcified AI phenotype were also identified as having *FAM83H* mutations. The second most prevalent gene, i.e. *AMELX*, involved 6 kindreds and 22 affected individuals with mutations. The least frequent mutations involved the autosomal recessive hypomaturation associated genes *MMP20*, *KLK4*, and *WDR72*. While multiple mutations in *MMP20* and *WDR72* have been reported, only 1 *KLK4* mutation has previously been reported. A second kindred, that is apparently unrelated to one previously sequelae for protein expression are shown in table 3. No mutations were identified in the families evaluated for *AMBN, DLX3,* or *AMTN*.

The clinical phenotypes corresponding to these different mutations were extremely diverse. The primary clinical manifestations involved either a localized or generalized deficiency of enamel thickness that manifest variously as pitting, groves, and furrows or generalized thin enamel through to localized or generalized hypomineralized enamel. The range of severity in both the hypoplastic and hypomineralized AI subtypes was marked as illustrated in figure 1. Several of the allelic mutations produced markedly different phenotypes that ranged from localized to generalized as observed with allelic *AMELX, ENAM,* and *FAM83H* mutations.

Discussion

In seeking to make sense of the pathology of inherited enamel conditions, the role of molecular genetics has been repeatedly emphasized [Aldred and Crawford, 1995] since the identification of the first AI-causative gene mutation [Lagerstrom et al., 1991]. Previous work has clearly shown the association between molecular change and phenotype [Wright et al., 2003; Kim et al., 2005a], revealing much about the process of enamel formation. The present clinical and genetic AI study spanning 15 years adds to this body of knowledge and helps define the diverse phenotypes resulting from multiple allelic and non-allelic genetic mutations. An important finding of this large AI population study is that evaluation of all 6 known

Table 1. AI total study population

	Total families	Total individuals	Total affected	Total unaffected	Unknown affected status
	91	494	260	226	8
Known diagnosis	71 (78)	430 (87)	224	202	4
Unknown diagnosis	20 (22)	64 (13)	36	24	4

Figures in parentheses are percentages.

Table 2. AI mutations

Mutations identified	Total families, 26[a]	Total individuals, 132[a]
FAM83H	12 (46)	85 (64)
AMELX	6 (23)	22 (17)
ENAM	3 (11)	16 (12)
KLK4	2 (8)	4 (3)
MMP20	2 (8)	2 (2)
WDR72	1 (4)	3 (2)

Figures in parentheses are percentages.
[a] Of the 71 families and 224 individuals with known phenotypes, 26 families (37%) and 132 affected individuals (59%) had identified mutations.

Table 3. AI-causative mutations identified in 71 families

AI mutations		Protein	Families
FAM83H	c.860C>A	p.S287X	1
FAM83H	c.1408C>T	p.Q470X	2
FAM83H	c.1289C>A, Indel in set 4 of ex-5	p.S430X	1
FAM83H	c.923_924delTC	p.L308fsX323	1
	FAM83Hc.1379G>A	p.W460X	1
FAM83H	c.1192C>T	p.Q398X	2
FAM83H	c.1872_1873delCC	p.L625fsX703	1
FAM83H	c.2080G>T	p.E694X	1
FAM83H	c.2029C>T	p.Q677X	2
AMELX	c.208C>A	p.P70T	2
AMELX	c.473delC	p.P158fsX187	2
AMELX	c.155delC	p.P52fsX53	2
ENAM	IVS9+1delG;c.588+1delG	p.N197fsX277	1
ENAM	c.1258_1259insAG	p.P422fsX448	1
ENAM	c.816-2A>G		1
KLK4	c.458G>A	p.W153X	2
MMP20	c.954-2A>T	p.I319Fs338X	1
MMP20	c.678T>A	p.H226Q	1
WDR72	c.1467_1468delAT	p.S489fsX498	1

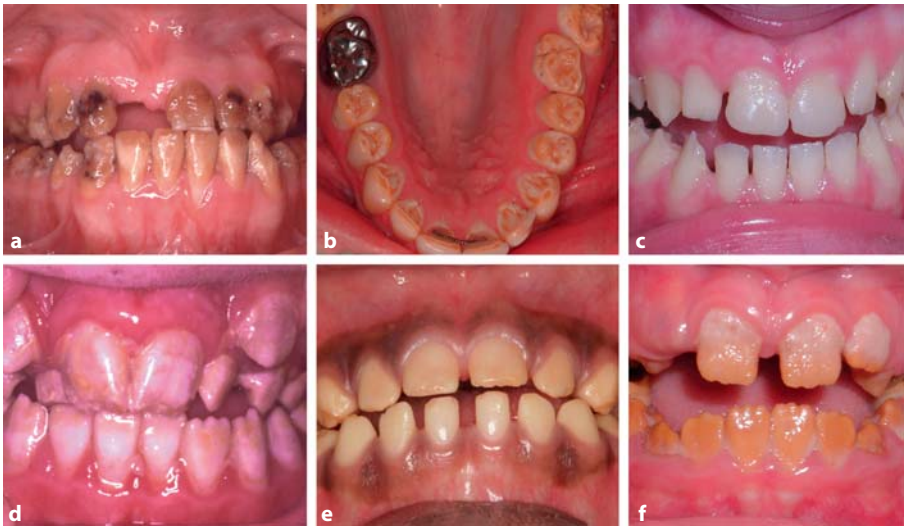

Fig. 1. Diverse phenotypes resulted from the different mutations as illustrated by the dentition of individuals affected by the 6 currently known AI-causative genes. **a** Permanent dentition affected by the *FAM83H* c.1192C>T mutation. **b** Permanent dentition affected by the *AMELX* c.208C>A mutation. **c** Permanent dentition affected by the *ENAM* c.816-2A>G mutation. **d** Permanent dentition affected by the *MMP20* c.954-2A>T mutation. **e** Primary dentition affected by the *KLK4* c.458G>A mutation. **f** Mixed dentition affected by the *WDR72* c.1467_1468delAT mutation.

disease-causing AI genes and several other AI candidate genes resulted in identification of the molecular etiology in approximately 60% of affected individuals and 40% of the families. This suggests that either there are numerous mutations in the noncoding regions of the genes tested or, and this is the more likely explanation, there are additional AI-causative genes yet to be identified. Given the diverse modes of inheritance (both autosomal dominant and recessive) and the heterogeneous phenotypes in those cases where genes were not identified (hypoplastic and hypomineralized), it is probable that multiple AI genes remain to be discovered. The previous multifamily evaluation of all known AI candidate genes reported by Kim et al. [2006] identified the molecular etiology for AI in only 25% of the families tested. Clearly, identification of the *FAM83H* as the cause of autosomal dominant hypocalcified AI [Kim et al., 2008] added significantly to the marked increase in diagnostic ability seen in the current study. Nine different *FAM83H* mutations were identified in 12 families and accounted for 64% (n = 85) of the total AI-affected individuals with a known mutation and 38% of the total AI-affected population.

The present study indicates that the autosomal recessive forms of AI are not uncommon, representing a substantial portion of the total number of families (n = 35 or about 50%), but accounted for only 55 affected individuals (about 25%). Mutations were identified in only 9 of the autosomal recessive families and included all of the known AI genes known previously associated with hypomaturation AI [Hart et al., 2004; Kim et al., 2005b; El-Sayed et al., 2009]. Only 1 family had been previously reported with a *KLK4* mutation [Hart et al., 2004] and in this series we reported a second family with the same p.W153X. Interestingly, this second family was African American and apparently unrelated to the African American family we had reported previously.

Although no mutations were found in *AMBN* or *AMTN,* both of these genes remain viable AI candidates due to their strong expression by ameloblasts [Krebsbach et al., 1996; Iwasaki et al., 2005]. A lack of *AMBN* in the knockout mouse is associated with marked enamel hypoplasia [Fukumoto et al., 2004]. Other new candidates continue to emerge, such as ODAM which also is strongly expressed by ameloblasts [Kestler et al., 2008]. Genetic approaches to identify new AI genes have identified new AI-causing genes and proteins not previously known to be important in enamel formation [Kim et al., 2008; El-Sayed et al., 2009]. The present study strongly supports that yet-to-be-discovered new AI genes exist and that multiple allelic mutations have been demonstrated in all but 1 of the current 6 known AI-causative genes. These allelic and nonallelic gene mutations are associated with rather specific phenotype-genotype relationships. These phenotype-genotype relationships can be extremely useful in determining which genes should be evaluated when seeking a molecular diagnosis in the diverse AI conditions.

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References

Aldred, M.J., P.J.M. Crawford (1995) Amelogenesis imperfecta – towards a new classification. Oral Dis *1:* 2–5.

Backman, B. (1988) Amelogenesis imperfecta – clinical manifestations in 51 families in a northern Swedish country. Scand J Dent Res *96:* 505–516.

Backman, B., A.K. Holm (1986) Amelogenesis imperfecta: prevalence and incidence in a northern Swedish county. Community Dent Oral Epidemiol *14:* 43–47.

Chosack, A., E. Eidelman, I. Wisotski, T. Cohen (1979) Amelogenesis imperfecta among Israeli Jews and the description of a new type of local hypoplastic autosomal recessive amelogenesis imperfecta. Oral Surg Oral Med Oral Pathol *47:* 148–156.

Crawford, P., M. Aldred (1988) Amelogenesis imperfecta: autosomal dominant hypomaturation-hypoplasia type with taurodontism. Br Dent J *164:* 71–73.

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 beta propeller WDR72 cause autosomal-recessive hypomaturation amelogenesis imperfecta. Am J Hum Genet *85:* 699–705.

Fukumoto, S., T. Kiba, B. Hall, N. Iehara, T. Nakamura, G. Longenecker, P.H. Krebsbach, A. Nanci, A.B. Kulkarni, Y. Yamada (2004) Ameloblastin is a cell adhesion molecule required for maintaining the differentiation state of ameloblasts. J Cell Biol *167:* 973–983.

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

Igarashi, T., J. Inatomi, T. Sekine, S.H. Cha, Y. Kanai, M. Kunimi, K. Tsukamoto, H. Satoh, M. Shimadzu, F. Tozawa, T. Mori, M. Shiobara, G. Seki, H. Endou (1999) Mutations in SLC4A4 cause permanent isolated proximal renal tubular acidosis with ocular abnormalities. Nat Genet *23:* 264–266.

Iwasaki, K., E. Bajenova, E. Somogyi-Ganss, M. Miller, V. Nguyen, H. Nourkeyhani, Y. Gao, M. Wendel, B. Ganss (2005) Amelotin – a novel secreted, ameloblast-specific protein. J Dent Res *84:* 1127–1132.

Kestler, D.P., J.S. Foster, S.D. Macy, C.L. Murphy, D.T. Weiss, A. Solomon (2008) Expression of odontogenic ameloblast-associated protein (ODAM) in dental and other epithelial neoplasms. Mol Med *14:* 318–326.

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.

Kim, J.W., F. Seymen, B.P. Lin, B. Kiziltan, K. Gencay, J.P. Simmer, J.C. Hu (2005a) ENAM Mutations in autosomal-dominant amelogenesis imperfecta. J Dent Res *84:* 278–282.

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

Kim, J.W., J.P. Simmer, B.P. Lin, F. Seymen, J.D. Bartlett, J.C. Hu (2006) Mutational analysis of candidate genes in 24 amelogenesis imperfecta families. Eur J Oral Sci *114(suppl 1):* 3–12, discussion 39–41, 379.

Krebsbach, P.H., S.K. Lee, Y. Matsuki, C.A. Kozak, K.M. Yamada, Y. Yamada (1996) Full-length sequence, localization, and chromosomal mapping of ameloblastin. J Biol Chem *271:* 4431–4435.

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.

Nussier, M., O. Yassin, T.C. Hart, A. Samimi, J.T. Wright (2004) Phenotypic diversity and revision of the nomenclature for autosomal recessive amelogenesis imperfecta. Oral Surg Oral Med Oral Pathol *97:* 220–230.

OMIM (2010) Online Mendelian Inheritance in Man. http://www.ncbi.nlm.nih.gov/omim.

Parry, D.A., A.J. Mighell, W. El-Sayed, R.C. Shore, I.K. Jalili, H. Dollfus, A. Bloch-Zupan, R. Carlos, I.M. Carr, L.M. Downey, K.M. Blain, D.C. Mansfield, M. Shahrabi, M. Heidari, P. Aref, M. Abbasi, M. Michaelides, A.T. Moore, J. Kirkham, C.F. Inglehearn (2009) Mutations in CNNM4 cause Jalili syndrome, consisting of autosomal-recessive cone-rod dystrophy and amelogenesis imperfecta. Am J Hum Genet *84:* 266–273.

Rowley, R., F.J. Hill, G.B. Winter (1982) An investigation of the association between anterior open-bite and amelogenesis imperfecta. Am J Orthod *81:* 229–235.

Sundell, S. (1986) Hereditary amelogenesis impertecta: an epidemiological, genetic and clinical study in a Swedish child population. Swed Dent J *31(suppl):* 1–38.

Vitiello, C., P. D'Adamo, F. Gentile, E.M. Vingolo, P. Gasparini, S. Banfi (2005) A novel GJA1 mutation causes oculodentodigital dysplasia without syndactyly. Am J Med Genet A *133A:* 58–60.

Witkop, C.J. (1957) Hereditary defects in enamel and dentin. Acta Genet Stat Med *7:* 236–239.

Witkop, C.J., Jr. (1989) Amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia revisited, problems in classification. J Oral Pathol *17:* 547–553.

Witkop, C.J., Jr., W. Kuhlmann, J. Sauk (1973) Autosomal recessive pigmented hypomaturation amelogenesis imperfecta: report of a kindred. Oral Surg Oral Med Oral Pathol *36:* 367–382.

Witkop, C.J., J.J. Sauk (1976) Heritable defects of enamel; in Stewart, R., G. Prescott (eds): Oral Facial Genetics. St. Louis, C.V. Mosby, pp 151–226.

Wright, J.T., S. Frazier-Bowers, D. Simmons, K. Alexander, P. Crawford, S.T. Han, P.S. Hart, T.C. Hart (2009) Phenotypic variation in FAM83H-associated amelogenesis imperfecta. J Dent Res *88:* 356–360.

Wright, J.T., P.S. Hart, M.J. Aldred, W.K. Seow, P.J.M. Crawford, S.P. Hong, C. Gibson, T.C. Hart (2003) Relationship of phenotype and genotype in X-linked amelogenesis imperfecta. Connect Tissue Res *44(suppl):* 72–78.