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Hypomaturation Amelogenesis Imperfecta Caused By A Novel *SLC24A4* Mutation

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Abstract

In this case report of autosomal recessive pigmented hypomaturation amelogenesis imperfecta (AI), we identify a novel homozygous missense mutation (g.165151T>G; c.1317T>G; p.Leu436Arg) in *SLC24A4*, a gene encoding a potassium-dependent sodium-calcium exchanger that is critical for hardening dental enamel during tooth development.

Keywords

Inherited Diseases; Mutations; Enamel; Tooth; Calcium

“Amelogenesis Imperfecta” or AI has been used as a diagnosis for inherited enamel malformations that occur in the absence of defects outside of the dentition,^{1, 2} and to refer to the presence of enamel defects in syndromes (e.g. Platyspondyly with amelogenesis imperfecta; OMIM, 601216).³ AI is a diverse collection of inherited diseases featuring varied forms of enamel malformations (hypoplasia, hypocalcification, hypomaturation, pigmented hypomaturation, and local hypoplasia), and multiple patterns of inheritance (autosomal dominant; autosomal recessive, X-linked). Many AI diseases exhibit only enamel defects (isolated AI), while others have systemic problems (syndromic AI). In the United States isolated AI affects about 1/13,000 persons.⁴ A clinical diagnosis of isolated AI is based upon the appearance, thickness, and hardness of the patients’ enamel and analyses of oral radiographs and pattern of inheritance.²

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The results of genetic studies have changed our perspective of AI. Inherited enamel defects can be caused by mutations in many genes and some distinctions between isolated and syndromic types have blurred. Junctional Epidermolysis Bullosa (JEB) is a recessive form of syndromic AI that features enamel malformations and skin fragility.⁵ Mutations in both alleles of *LAMA3*, *LAMB3*, *LAMC2*, and *COL17A1* cause JEB, while defects in a single allele can cause isolated AI. Isolated enamel defects have been associated with defects in *AMELX*,⁶ *ENAM*,⁷ *AMBN*,⁸ *MMP20*,⁹ *FAM83H*,¹⁰ *ITGB6*,^{11, 12} *C4orf26*,¹³ *KLK4*,¹⁴ *WDR72*,¹⁵ and *SLC24A4*,¹⁶ *COL17A1*,¹⁷ and *LAMB3*.^{18, 19} As enamel malformations are often the only clinical manifestation evident at the time of diagnosis, clinicians are frequently uncertain as to whether the presenting case is isolated or syndromic. Syndromic AI and their genetic causes include Cone-Rod Dystrophy and AI (*CNNM4*),²⁰ Enamel Renal Syndrome (*FAM20A*),^{21, 22} Tricho-Dento-Osseous syndrome (*DLX3*),²³ Immunodeficiency 9 (*ORAI*),²⁴ Immunodeficiency 10 (*STIM1*),^{25, 26} Oculodentodigital Dysplasia (*GJA1*),^{27, 28} Kohlschütter-Tönz Syndrome (*ROGDI*),²⁹ and Nance Horan Syndrome (NHS).^{11, 30} Although some syndromic AI diseases have distinctive clinical features, identifying the gene/mutation that causes the disease provides a specific diagnosis, discerns between isolated and syndromic AI, and improves assessment of the patients' prognosis.

CASE REPORT

A seven-year-old girl in the mixed dentition stage of dental development presented at the Pediatric Dentistry Clinic at Istanbul University. The chief complaint concerned the enamel layer of her dentition, which showed milky brown staining even as the teeth erupted (Fig. 1). The medical history was non-contributory, and no malformations outside of the enamel were evident clinically. Later, after finding that *SLC24A4* defects caused the disease, we specifically inquired about organs that might express NCKX4 (the protein product of *SLC24A4*),³¹ such as lungs, but abnormalities outside of the dentition were not observed and problems with the lungs were specifically denied.

Oral examination of the mixed dentition revealed that the teeth were of normal size and contour, but mostly brown in color. There was significant attrition, particularly of the posterior teeth, suggesting that the enamel lacked hardness. The enamel surfaces were rough and pitted, largely due to chipping. The coarse areas were a deeper brown than surrounding parts of the crowns, suggesting a susceptibility to extrinsic staining. The enamel appeared most normal in the cervical regions, where attrition had not yet roughened the surface, and the enamel hadn't stained. The hardening, or maturation, of enamel is accomplished by modulating (smooth-ended and ruffle-ended) ameloblasts and occurs more readily at the cervical margins where the enamel is thinner.³²

The enamel did not contrast well with dentin on the panoramic radiograph, although the images of most unerupted teeth showed a distinct white line at the dentino-enamel junction (DEJ) (Fig. 2). The thickness of enamel layer on unerupted teeth was within normal limits. A pedigree was drawn up based upon information provided by the mother of the proband (Fig. 3). The proband (IV-2) was the product of a consanguineous union of first cousins and was the only person affected. A clinical diagnosis was made of isolated autosomal recessive pigmented, hypomaturational amelogenesis imperfecta.

Oral and radiographic examinations of the parents (III-4, III-5) and sibling (IV-1) were within normal limits (Fig. 4). Poor oral hygiene and a high prevalence of dental decay is routinely observed in this population, so the finding of dental caries in other family members does not support a conclusion of increased caries susceptibility in the unaffected heterozygotes.

The nuclear family (III-4, III-5, IV-1, IV-2) was recruited into our study. The human study protocol and consents were reviewed and approved by the Ethics Committee at the University of Istanbul, Turkey and Institutional Review Board at the University of Michigan. Study participants signed appropriate written consents after an explanation of their contents and their questions about the study had been answered. Genomic DNA was extracted from blood samples obtained from the four recruited members and used for mutation analyses. The five *Kallikrein 4* (*KLK4*) coding exons and adjacent intron borders were amplified and characterized by DNA sequencing as previously described,¹⁴ but no sequence variations were identified (data not shown). The 17 *SLC24A4* coding exons and intron borders were sequenced as described previously³³ and a sequence variation in the 13th coding exon was identified (g.165151T>G; c.1317T>G; p.Leu436Arg). The parents and sibling were heterozygous, while the proband was homozygous for this novel *SLC24A4* mutation (Fig. 5), which is not listed in the 1000 Genomes database.³⁴ PolyPhen-2³⁵ predicted the leucine to arginine substitution to be probably damaging and gave a score of 0.994.

This is fifth *SLC24A4* mutation reported to cause AI (Fig. 6).^{16, 26, 33} The enamel phenotype of the proband closely matches those of previously reported AI-causing *SLC24A4* defects.

DISCUSSION

Much has been learned in the last decade about the genetic etiologies of inherited enamel defects, although we probably are only about half way to knowing the full spectrum of genes that cause AI.^{36, 37} More AI-causing genes have been discovered than anyone originally thought possible. Because so many known and unknown genes can cause AI, a genetic diagnosis is not easy to make and is currently accomplished only in the context of a genetics study. Familiarity with genotype-phenotype correlations (how the enamel looks when a specific gene is mutated) can help narrow down the possibilities.³⁸ With a little luck, mutation analyses of a short list of prioritized candidate genes can discover the AI-causing mutation, as was done in this study. Increasingly, whole-exome sequencing (WES) is used to identify sequence variations in the most critical 2% of the genome (associated with protein coding regions). These variations are then analyzed to pinpoint which one causes the disease.³⁹ This approach has greatly advanced our knowledge of the genes and mutations that cause rare inherited diseases.⁴⁰ When most of the genes that cause AI are known, genetic testing will be used routinely to diagnose AI in patients.

One predictable consequence of genetic testing that identifies the genes/mutations that cause and define the AI disease will be expansion of the recognized clinical phenotype. Clinicians will start to notice and report subtle phenotypes that were not previously associated with AI. In mouse studies, *Slc24a4* transcripts were detected in abundance in the brain, lungs, aorta,

and thymus.³¹ In contrast only 26 *SLC24A4* expressed sequence tags (ESTs) have been identified among over 3.3 million ESTs for normal tissues in the human EST database (Hs. 385530), which did not sample developing teeth. There may be significant differences between mice and humans in the expression of *SLC24A4*. *Slc24a4* null mice showed a reduced ability to locate an odorous source.⁴¹ As genetic testing to identify the genetic cause of AI in each patient becomes the standard of care, clinicians will likely notice additional phenotypes that go along with specific AI diseases and give us a better understanding of how these inherited conditions affect our patients.

Given the complexity of the AI diagnosis and the potential for hidden systemic conditions, it is recommended that clinicians request a genetic consultation or mediate enrollment of their AI patients into a genetics study.

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Figure 1.
Oral photographs of the female proband at age 6.

Figure 2.
Panorex radiograph of the female proband at age 6.

Figure 3.
Pedigree of the AI kindred.

Figure 4.
Photos and panoramic radiographs of unaffected heterozygotes.

Figure 5.

SLC24A4 chromatograms. The parents and sibling were heterozygous for *SLC24A4* mutation g.165151T>G; c.1317T>G; p.Leu436Arg (top), while the proband showed the mutation on both *SLC24A4* alleles. The gene and cDNA and mutation descriptions are based upon *SLC24A4* reference sequences NG_023408.1 and NM_153646.3, respectively.

Figure 6.

SLC24A4 gene structure and disease-causing mutations. **A:** The *SLC24A4* gene structure is for *SLC24A4* transcript variant 1 (TV1; NM_153646.3). The 17 numbered boxes are exons. Introns are indicated by a black line. The intron and exon lengths are not drawn to scale. The numbers below each exon indicate the range of amino acids encoded by each exon. Darkened areas are noncoding. **B:** *SLC24A4* mutations reported to cause AI. The gene and cDNA and mutation descriptions are based upon *SLC24A4* reference sequences NG_023408.1 and NM_153646.3, respectively.