Complex morphological and molecular genetic examination of amelogenesis imperfecta: A case presentation of two Czech siblings with a non-syndrome form of the disease

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CASE REPORT

Abstract

Amelogenesis imperfecta (AI) is an overarching term for a group of rare inherited disorders of hard tooth tissues. It is characterized by various defects in proper enamel formation. AI is a severe disorder that affects both the aesthetics and function of the dentition, with affected teeth increasingly suffering from dental caries. Therefore, early diagnosis and lifelong stomatological interventions are important. Due to the complex nature of AI family history, stomatological, radiographic, and molecular genetic examinations should be part of the diagnostic portfolio. Additionally, we utilized new visualization methods for the assessment of teeth demineralization. We present a case report of two affected Czech sisters (6 and 8 years old) with clinically defined AI. These are the first Czech cases in which comprehensive clinical and genetic analysis had been carried out and reflect the complex clinical nature, positive treatment options, and limitations of candidate-gene molecular genetic testing.

INTRODUCTION

Amelogenesis imperfecta (AI; MIM# 104500, MIM# 104510, MIM# 104530, MIM# 130900, MIM# 204650, MIM# 204700, MIM# 301200, MIM# 301201, MIM# 612529, MIM# 613211 and MIM# 614832) was defined by Crawford *et al.* as, "a group of conditions of genetic origin that affect the structure and clinical appearance of enamel of all or almost all of the dentition, and that may be associated with morphological or biochemical changes in other parts of the body". *(Crawford et al.* 2007). AI is a developmental disorder of dental enamel, characterized by hypoplasia and/or hypomineralization, which can be inherited through virtually all modes of inheritance (i.e. autosomal dominant-, autosomal recessive-, dominant-, and recessive X-linked inheritance; Bailleul-Forestier

et al 2008, Crawford *et al.* 2007, Hu *et al.* 2007, Lagerstrom et al 1991, Pemberton *et al.* 2007). In addition, sporadic cases have been reported (Urzúa *et al.* 2011), and may be due to *de novo* mutations, or they may be illustrative of variable expression with or without incomplete penetrance. The prevalence of AI, depending on the population tested, ranges from 1:718 (Northern Sweden) to 1:14,000 (Michigan, USA)(Chaudhary *et al.* 2009, Crawford *et al.* 2007; Urzúa *et al.* 2011; Wright *et al.* 2011).

AI is an "overarching term" for a clinically and genetically heterogeneous group of disorders, which unfortunately means that there are inherent difficulties related to its classification. Most authors favor the clinical classification and divide AI into three major forms, i.e. hypoplastic, hypomaturation, and hypocalcified AI. Depending on the timing of the disruption of enamel, the first type arises during the secretory stage of amelogenesis when the defect in the enamel matrix is caused by interference with the proper function of the ameloblast. Cells do not grow sufficiently and result in the enamel layer being pathologically thin, i.e. hypoplastic. The most severe form of hypoplastic AI is enamel agenesis, where there is almost no clinical or radiographic evidence of enamel (Hu *et al.* 2007; Urzúa *et al.* 2011). In the hypomaturation form of AI, the underlying defect is in the growth of crystals during the maturation phase of enamel formation, which is caused by the supporting proteins not being completely removed. The enamel layer is pathologically softened, yellow and its radiologic density approaches that of dentin. Finally, in hypocalcified type of AI, mineralization failure is extreme. The enamel is of normal thickness, but there is a complete absence of matrix mineralization making the enamel extremely soft. The enamel can be easily removed with an instrument during inspection or it may come slough off spontaneously right after tooth eruption. Patients with hypocalcified enamel rapidly form a calculus and develop (acute- and chronic) periodontitis (Hu *et al.* 2007).

AI comes in two forms, i.e. a non-syndrome form (isolated) and a syndrome form, which can include characteristic features such as those associated with senso-neurological symptomatology. In the latter instance most notably there are three rare syndromes, with prevalence of less than <1 in 1,000,000 in the general population, comprising Heimler – (MIM# 234580 – sensorineural hearing loss, and nail defects), Kohlschütter-Tonz – (MIM# 226750, epilepsy, dementia), and Jalili (MIM# 217080 cone rod dystrophy) autosomal recessive syndromes.

A differential diagnosis of AI comprises not only other inherited disorders such as Dentinogenesis imperfecta (MIM# 125490), but also extends to generalized defects of the teeth caused by exogenous factors, e.g. fluorosis, tetracycline staining, MIH syndrome, and defects in calcium and phosphate metabolism (Crawford *et al.* 2007).

In terms of the molecular pathogenesis, enamel consists of 96% inorganic and 4% organic matrix, which includes enamel proteins (e.g. dominant amelogenins and minority non-amelogenins – enamelin, tuftelin, and ameloblastin) and enzymes (e.g. metalloproteinases, proteinases, and phosphatases). The amelogenin protein is coded by the AMELX *gene* (located on Y chromosome) and the enamelin is coded by the ENAM gene (also, located on the Y chromosome). Apart from tooth enamel, amelogenin is also found in bones, bone marrowmarrow, and brain cells. The AMELX gene plays a major role in enamel formation, whereas the AMELY gene (located on Y-chromosome) is redundant.

In addition, mutations in the AMELX, ENAM, Kallikrein-4 (*KLK-4*), and *MMP-20* (metalloproteinase) genes have also been repeatedly implicated in the development of AI. Recently, mutations in the *C4ORF26* gene (http://www.ncbi.nlm.nih.gov/pubmed/21127961) encoding a peptide with linked to *in vitro* hydroxyapatite crystal nucleation and growth activity, including alterations in the *DLX3, FAM83H, WDR72* genes) were found to be pathogenic, as reviewed elsewhere (http://www.ncbi.nlm.nih.gov/pubmed/21127961). Nonetheless, mutations in these genes explain less than one-half of all AI cases. Therefore, in the future, we can expect that genome analysis will likely sub-stratify AI according to its molecular pathogenesis.

The aim of this case presentation is to demonstrate the a) complex nature of AI in two affected siblings of Czech origin, b) the necessity of comprehensive morphological, c) genetic analysis, and including the d) limitations of the candidate-gene testing approach.

CASE PRESENTATION

Two healthy parents from Pilsen, together with two affected daughters (born 2005 and 2007) and an unaffected son (born 2010) (Figures 1–2), were referred to our clinic by their general dentist. Genealogic analysis was insignificant, including the presence of AI within their kinship (Figure 3). From an epidemiological perspective, the drinking water used by the family complied with all hygienic standards and radon levels are normal.

The younger of the affected girls was generally healthy, without any allergies, and had only taken antibiotics only once, for treatment of a complicated rotavirus infection. Her psychomotor development had been progressing normally. The girl had opaque yellow teeth partially covered with soft enamel and all deciduous dentition were affected. The first signs of enamel damage were visible from the age of six months, when her first teeth erupted and presented as yellow, tiny, and soft. While therapeutically replacing the dentition, one of the extracted incisors was split open and examined with an electron-scanning microscope. The typical "pitting" of the enamel was revealed, confirming a morphological diagnosis of AI (Figure 4). This examination
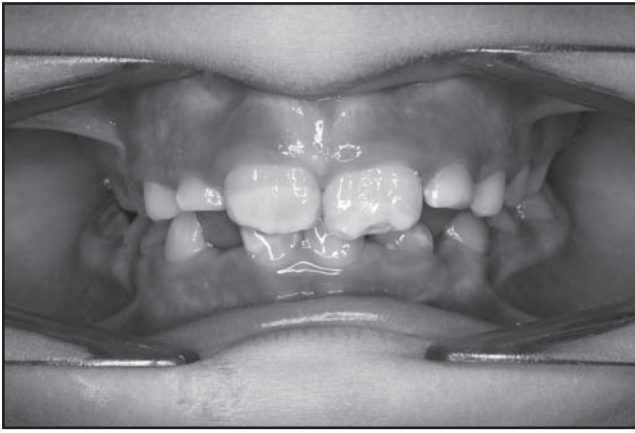
Fig. 1. AI in older of the patients (born 2005) at the first visit (4/2012) - defects and change of color and texture of permanent incisors can be seen.

Fig. 2. Younger patient (born 2007) with AI at the first visit to the clinic (4/2012) - all teeth are without enamel, with yellow-brown color and rough surface.
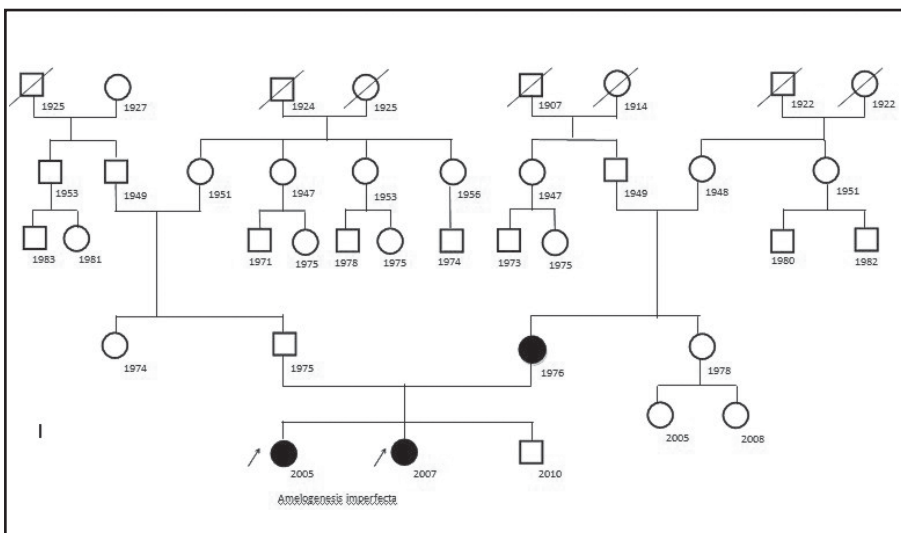
Fig. 3. Genealogical chart of the family.

also showed that enamel was thinner and, in places, even absent. At higher magnifications, there were visible cracks and lacunae where the enamel matrix had fallen off and/or was missing. In accordance with a diagnosis of AI, the dentine was observed to be unaffected (Figure 5).

Like her younger sister, the older girl was also generally healthy, with normal psychomotor development, however, she had not experienced any allergies, serious illnesses or taken any antibiotics. Using the same diagnostic approach, the identical morphological form of AI was confirmed as in her younger sibling. However, she had a milder presentation, with changes in the hard tissues being less pronounced. Her primary teeth were without clinical findings, and only after eruption of her first permanent molars and all incisors did the yellow lesions with white strips began to appear. Both patients were normal from a clinical syndrome perspective. They also underwent examinations of their retina, hearing, including other neurological examinations, however, no disturbances were found (data not shown).

Therapy for the upper central incisors of the older patient was initiated. Radiologic analysis proved that these were unaffected by caries. Subsequently, we used a DIAGNO-cam (KaVo Dental Excellence, Germany) analyser to directly inspect changes of the enamel, both on the incisors and the first permanent molars, which were also found to be affected (Figure 6), i.e. presence of decay, change in opacity, and consistency of enamel. Subsequently, the appearance of the upper central incisors was modified: after performing slight preparation of the incisal halves of the two upper incisors, the teeth were etched with 37% phosphoric acid, priming and bonding was done and then we covered the defects with composite G-aenial (Company GC), colour A2. This dental work was articulated and duly polished.

This treatment will continue for the remaining teeth in order to avoid abrasion and reduction of the overall height of the bite, as well as prevent caries. Both female patients were referred for orthodontic treatment and the parents were informed about other procedures, including possible prosthetic treatment when they reached adulthood.

Assuming the existence of an autosomal recessive inheritance pattern, Sanger DNA sequencing of the entire coding region of the ENAM, KLK4, AMELX, C4ORF26, DLX3, FAM83H, MMP20 and WDR72 genes was carried out (ATG GenMed GmbH, Germany) for both affected siblings and their parents. Single nucleotide coding sequence alterations were found in the AMELX, MMP20, WDR72 and C4ORF26 genes causing missense and splice site mutations in both

affected sisters. Nonsense, frameshift, and indel mutations were not detected (data available upon request).

The pathogenic potential of obtained genetic alterations was assessed using Alamut verze 2.4 software
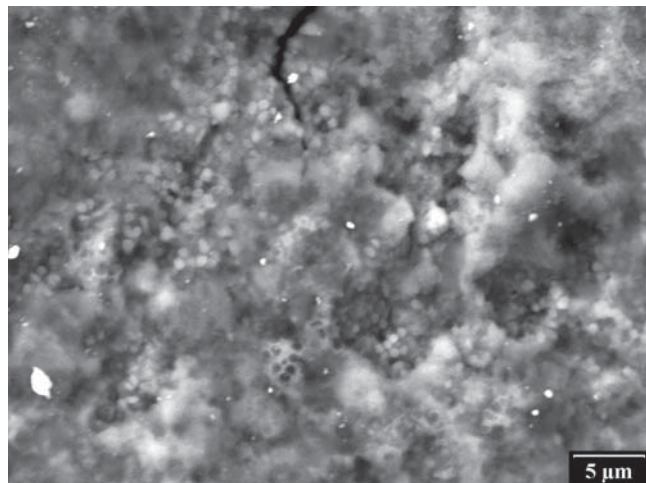

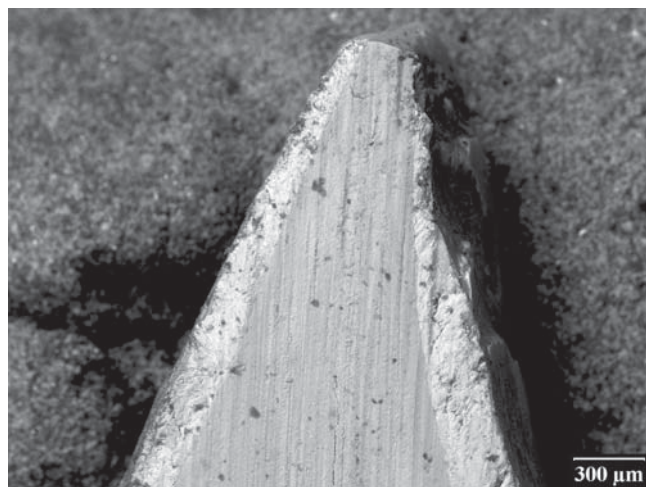**Fig. 4.** View of the enamel in a scannin electron microscope.

Fig. 5. Eliminated primary incisor of younger patient with significantly impaired layer of enamel.
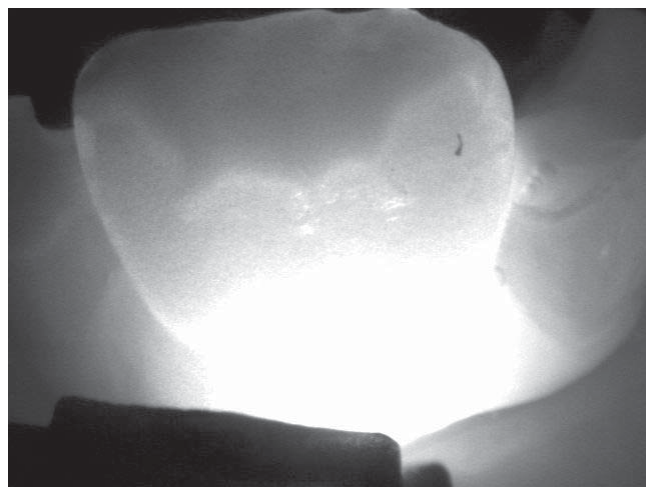

Fig. 6. Views of tooth 41 using DIAGNOcam – less damage of the tooth 41 is shown by change in the density of enamel (see clinical case Fig. 1).

(Integrated Biosciences, France). All variants were validated against dbSNP entries; their MAF scores were established, including their entry in the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/). Splice site variants were also assessed using Human Splice Finder software (http://www.umd.be/HSF/). Finally, all variants were cross-referenced against the Exome Variant Server database (http://evs.gs.washington.edu/EVS/). However, the aforementioned software predictions, discordant presence of variants between the affected sisters, including the presence of some variants in the unaffected parents, disproved an unambiguous pathogenic nature.

DISCUSSION

AI is represented by a clinically and genetically heterogeneous group of disorders that affect the dental enamel, occasionally in association with other dental, oraloral, and other tissues. In order to study the complex nature of this condition we performed a complex stomatologic, radiologic and a molecular genetic analysis of a Czech family with two female siblings affected by AI. The medical examinations provided clear evidence that the two sisters have a non-syndrome form of the disease, which is very likely to be recessive since the index cases, i.e. the brother and parents were unaffected. A subsequent, *de novo* origin for these alterations in both sisters would be highly improbable, and an X-linked inheritance pattern was excluded since the girls' brother was unaffected and consanguinity was excluded using pedigree analysis.

This otherwise debilitating disease can be successfully managed by early therapy (both preventive and restorative), with lifelong treatment from childhood. It is important to initiate therapy during infancy in an effort to protect the primary dentition, which is later followed by application of crowns or adhesive plastic restorations. Children diagnosed with AI also have malocclusions that require a multidisciplinary team involving a restorative dentist and an orthodontist. The pediatric dentist's should work closely with the family, offering support and motivation that promotes good oral care for the patient and the patient's family. In this case, the family of the affect girls is very involved and cooperating well; the outlook for positive treatment outcomes is good.

A differential diagnosis needs to include other conditions that have AI-like components. These include, for example; Jalili syndrome (or oculo-dental syndrome; MIM# 217080), which was first reported by Jalili and Smith in 1988, in 29 members of one family (Jalili & Smith 1988). This syndrome is a combination of cone-rod dystrophy and amelogenesis imperfecta. Cone-rod dystrophy is a rare retinal disorder that leads to an initial loss of central vision, color vision, and photophobia in childhood or early adulthood (usually during the first 2 decades of life) with subsequent night

blindness and visual field restriction (Michaelides *et al.* 2004; Parry *et al.* 2009). In the majority of cases, a mutation in a single gene (*CNNM4* gene, in the 2q11 region) has been found (Jalili 2010). Kohlschütter-Tonz syndrome (or amelo-cerebro-hypohidrotic syndrome; MIM# 226750) is a rare neurodegenerative disorder characterized by progressive dementia preceded by spasticity and epilepsy and with an Al-like component. It is associated with a symmetrical, generalized developmental defect of tooth enamel affecting both primary and permanent dentition (Christodoulou *et al.* 1988). Finally, Heimler syndrome (MIM# 234580), which was described in 1991 by Heimler *et al.* in a pair of siblings, also need to be considered based on its Al-like component. To date, 8 cases have been reported (Lima *et al.* 2011). The syndrome is characterized by sensorineural hearing loss, generalized enamel hypoplasia of the permanent dentition with normal primary dentition, and nail defects (Beau's lines and leukonychia (Lima *et al.* 2011; Tischkowitz *et al.* 1999).

In the two affected siblings presented here, neurological, ophthalmological, dermatological, hearing loss examinations were normal; therefore, these syndromes could be excluded.

Naturally, negative findings from the DNA sequencing of a set of candidate genes do not rule out an Al diagnosis. We did not performed an analysis of large deletions and duplications in the tested genes since the respective assays were not available. Therefore, we have enlisted these patients in the next generation sequencing pipeline for whole exome analysis.

In summary, the presented cases are the first Czech cases in which comprehensive clinical and genetic analysis has been carried. The results from the analysis show the complex clinical nature, viable treatment options, potential for positive outcomes, as well as limitations related to the candidate-gene approach to molecular genetic testing.

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Conflict of interest declaration
The authors have no conflict of interest that could influence the content or processing of this manuscript.

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