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Limited phenotypic variation of hypocalcified amelogenesis imperfecta in a Danish five-generation family with a novel *FAM83H* nonsense mutation

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Background. Autosomal dominant hypocalcified amelogenesis imperfecta (ADHCAI) is a disease with severe dental manifestations.

Objectives. The aims were by means of a genome-wide linkage scan to search for the gene underlying the ADHCAI phenotype in a Danish five-generation family and to study the phenotypic variation of the enamel in affected family members.

Results. Significant linkage was found to a locus at chromosome 8q24.3 comprising the gene *FAM83H* identified to be responsible for ADHCAI in other families. Subsequent sequencing of *FAM83H* in affected family members revealed a novel nonsense mutation, p.Y302X. Limited phenotypic variation was found among affected family members with loss of translucency and discoloration of the enamel. Extensive posteruptive loss of enamel was found in all teeth of affected subjects. The tip of the cusps on the premolars and molars and a zone along the gingival margin seemed resistant to posteruptive loss of enamel. We have screened *FAM83H* in another five unrelated Danish patients with a phenotype of ADHCAI similar to that in the five-generation family, and identified a *de novo FAM83H* nonsense mutation, p.Q452X in one of these patients.

Conclusion. We have identified a *FAM83H* mutation in two of six unrelated families with ADHCAI and found limited phenotypic variation of the enamel in these patients.

Introduction

Amelogenesis imperfecta (AI) is a hereditary dental anomaly that affects the enamel of the primary and the permanent dentition. The enamel phenotype in AI varies considerably, and the disease is characterized by extensive clinical diversity.

Lack of information on the underlying molecular background for the disease has impeded full understanding of the pathogenesis of the disease. In recent years, positive linkage of the autosomal dominant hypocalcified type of AI (ADHCAI) has been found to 8q24.3 by conventional linkage analysis and the underlying gene, *FAM83H*, has been identified[1–3].

The aims of this study were by means of a genome-wide scan to search for the gene underlying the phenotype in a Danish five-generation family and to study the intrafamilial phenotypic variation in 14 affected family members.

Material and methods

Study population

A study on a five-generation family was initiated in 2004 at the School of Dentistry, Aarhus University, Denmark. The family consisted of 41 members and was identified because two family members affected by AI were referred for dental treatment. A pedigree

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of the family is presented in Fig. 1. Clinical and radiological findings after examination of 28 (77.7%) of 36 invited family members have been reported previously[4], and the family was diagnosed as being affected by ADHCAI according to the criteria by Witkop[5]. Following the identification of the mutated gene in the five-generation family, patients from five other and unrelated families were included in the genetic analysis to determine the occurrence of *FAM83H* mutations among Danish ADHCAI families.

The research protocol was approved by The Central Denmark Region Committees on Biomedical Research Ethics (protocol # 20050017). Written information about the project was sent to all family members. The participants or guardians gave written informed consent before entering the study.

Clinical and radiographic examination

The clinical and radiographic examination has previously been described in detail[4]. The results on the phenotypic variation among family members presented in this report are necessarily limited to a description of clinical and radiographic phenotype of affected individuals with teeth that were not covered by composite fillings, cast crowns or other types of dental treatment making assessment impossible. Of a total of 14 affected subjects, six had no teeth and wore dentures, and two had full coverage of all teeth. The remaining six individuals had one or more teeth which could be assessed. The clinical examination included recording of colour, transparency of the enamel, characteristics of the enamel surface and posteruptive enamel breakdown.

In individuals with teeth present in the oral cavity, a full-mouth periapical survey consisting of 14 conventional radiographs was conducted. The radiographic examination included an evaluation of the thickness of the enamel as well as an evaluation of the contrast between enamel and dentine.

Genotyping and linkage analysis

Samples were available from 23 family members, of which 14 are affected with ADHCAI. Genomic DNA was isolated from peripheral blood leucocytes using standard techniques. The 14 affected individuals were genotyped using the Affymetrix Human Mapping 10K Xba142 array (AROS; Applied Biotechnology, Skejby, Denmark). The genotypes were uploaded to the BCSNP database (Biocomputing Platforms Ltd, Espoo, Finland), and a multipoint linkage analysis was performed with Merlin (version 1.0.1) under a model of autosomal dominant inheritance with full penetrance. The disease allele frequency was set to 0.0001, and the Marshfield genetic map distances and

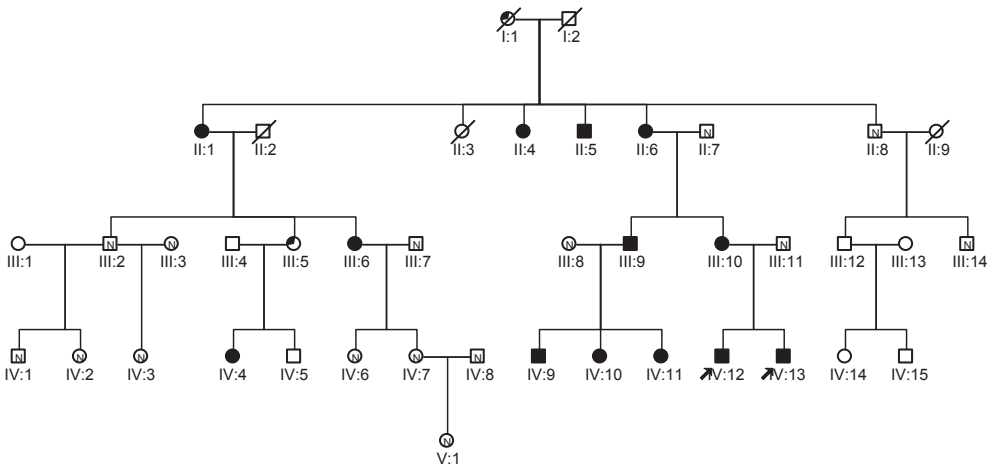

Fig. 1. A pedigree chart of the five-generation family consisting of 41 family members. Males are marked by squares and females by circles. Filled symbols: affected individuals. Clear symbols with 'N' included: not affected. Partially filled symbol: reported to be affected. Clear symbols: individuals reported to be unaffected, not included. Arrow: the proband. Symbols with crossing: deceased.

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Affymetrix SNP allele frequencies for Caucasian were used. Confirmation of linkage was performed by genotyping all family members where DNA was available (14 affected and nine unaffected) with eight microsatellite markers (D8S1836, D8S1050, D8S1024, D8S1712, D8S1128, D8S2334, D8S1842, and D8S529).

Mutational analysis

The entire coding sequence of *FAM83H* was PCR amplified using primers and PCR conditions as previously described[2]. Sequencing was performed in both directions using ABI PRISM Big Dye Terminator Labelling Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, USA) as recommended by the manufacturer with the same primers as for PCR, and run on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Nucleotides have been numbered with nucleotide no. 1 being the first nucleotide in the *FAM83H* initiation codon ATG (NM_198488; http://www.ncbi.nlm.nih.gov/entrez/).

Results

Clinical and radiographic findings

Only six individuals from generation IV had permanent teeth in which the enamel could be assessed (Table 1). Only one of the additional five families included later proved to have a mutation in the gene, *FAM83H*. The clinical and radiographic findings of this patient (a 12-year-old boy) are included in the description of the phenotypic characteristics of the affected members of the five-generation family.

In those areas where the enamel surface was intact, the clinical features of the enamel showed limited variation between the patients: the colour of the enamel was light-yellow with whitish, cloud-like patterns, and the normal translucency of enamel was absent (Figs 2 and 3). Posteruptive breakdown was seen in all patients soon after the eruption of incisors, premolars and molars (Table 1, Fig. 2). In all types of teeth, a zone with enamel of apparently normal thickness was found along the gingival margin, most pronounced in premolars and molars (Fig. 2d). In premolars and molars, the tip of the cusps appeared intact. In molars this resulted in a tent-like appearance with imaginary 'tent poles' forming the cusp tips and loosely supporting the enamel layer as if it was tent canvas (Fig. 3). The enamel surface of the molar crown was very irregular. After a longer period of exposure in the oral cavity, e.g., in a 28-year-old woman (Fig. 1, IV:4), the surfaces of the molar crowns were regular and smooth, as if the tip of cusps had been lost, reducing the height of the crown and resulting in a flat occlusal surface exposing the dentine (Fig. 2e).

In all six individuals, the vast majority (80% or more) of assessable teeth had reduced radiographic contrast between enamel and dentine, and 50% or less of the teeth in these six individuals showed reduced enamel thickness (Table 2).

Table 1. Phenotype and dental treatment status in permanent teeth of six dentate amelogenesis imperfecta-affected members of the five-generation Danish family.

Person ID*	Gender	Age in years	No. and type of teeth present in the oral cavity	No. of permanent teeth with partial or full coverage of crowns	No. of permanent teeth available for clinical assessment	Severity of posteruptive enamel loss	Surface roughness	Discoloration
IV:4	Female	28	22 PT, 0 pt	20 FC	2	+++	Smooth	Brown/black
IV:9	Male	16	27 PT, 0 pt	4 SSC, 18 CC	5	++	Rough	Yellow
IV:10	Female	14	24 PT, 3 pt	2 SSC, 16 CC	6	++	Rough	Yellow
IV:11	Female	12	24 PT, 0 pt	11 CC	13	++	Rough	Yellow
IV:12	Male	12	24 PT, 2 pt	4 SSC, 7 CC	13	++	Rough	Yellow
IV:13	Male	9	12 PT, 11 pt	4 SSC, 4 CC	4	++	Rough	Yellow

*Number refers to person ID in Fig. 1.

+, mild loss; ++, moderate loss; +++, severe loss; PT, permanent teeth; pt, primary teeth; FC, full crown (metal or ceramic); CC, composite crown or composite veneer; SSC, stainless steel crown.

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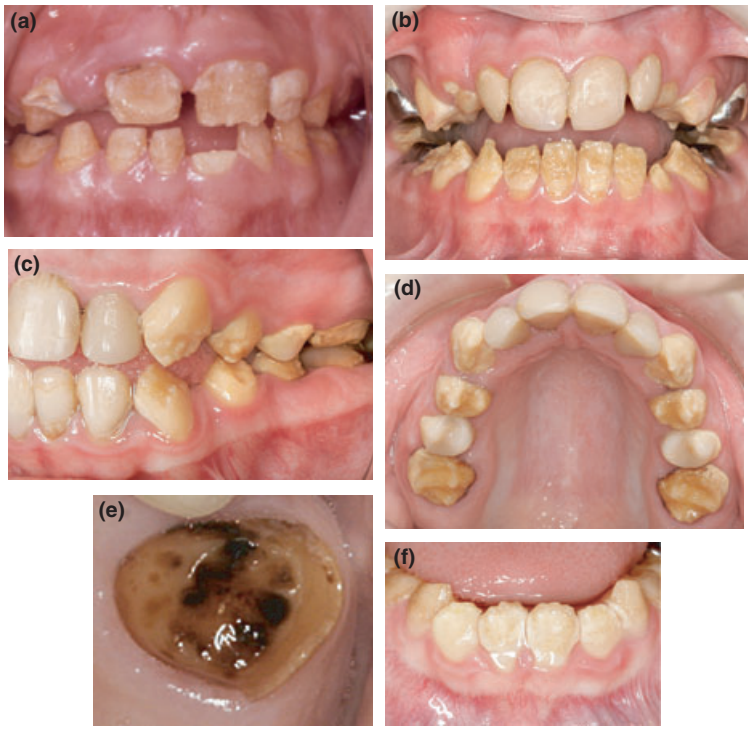

Fig. 2. Clinical photographs of teeth in five amelogenesis imperfecta-affected family members of the five-generation Danish family. The photographs show extensive discoloration and degradation of enamel. (a) Permanent incisors and canines in 6-year-old boy IV:9. (b) Frontal view of upper and lower dental arches of 11-year-old boy IV:12. (c) Permanent teeth of 13-year-old girl IV:10. Some of the teeth have been treated with temporary composite crowns or fillings. (d) Occlusal view of maxillary teeth in 13-year-old girl IV:10. (e) Maxillary molar of 28-year-old female IV:4. Extensive posteruptive enamel loss and discoloration of the remaining enamel are seen. (f) Mandibular incisors of 8-year-boy IV:13.

Linkage analysis

From the genome-wide linkage scan, including 14 affected family members, one single locus for AI in the five-generation family was identified with a maximum lodscore of 3.6 in a 25-cM region on chromosome 8q24.21–q24.3 (Fig. 4). All other chromosomal regions displayed lodscores below 0.5.

A follow-up linkage and haplotype analyses using eight microsatellite markers in 14 affected as well as nine unaffected family members narrowed down the region of interest to an approximately 15 cM region on distal part of chromosome 8q (maximum lodscore 4.5) because of a recombination event in two unaffected individuals (data not shown).

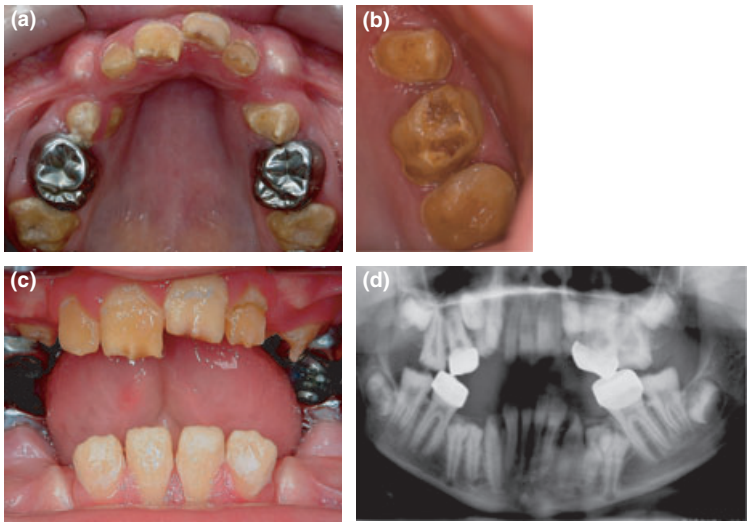

Fig. 3. Clinical photographs and a radiograph of permanent teeth in an unrelated 12-year-old amelogenesis imperfecta-affected boy with a *de novo* p.Q452X mutation in *FAM83H*. (a) Occlusal view of the maxillary teeth. (b) Occlusal view of left-side maxillary premolar and two molars. A stainless steel crown has been removed from the permanent first molar. (c) Frontal dental view with molars in occlusion. (d) Panoramic radiograph illustrating the crowding of teeth in addition to impacted teeth.

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Table 2. Radiographic variation of assessable permanent teeth present in the oral cavity of six amelogenesis imperfecta-affected family members.

Person ID	Number of teeth with lack of contrast between enamel and dentine of total number of assessable teeth	Number of teeth with reduced enamel thickness of the total number of assessable teeth
IV:4	–*	–*
IV:9	12/15	0/12
IV:10	16/18	2/17
IV:11	13/15	6/16
IV:12	17/18	9/18
IV:13	22/22	0/8

*No teeth possible to assess.

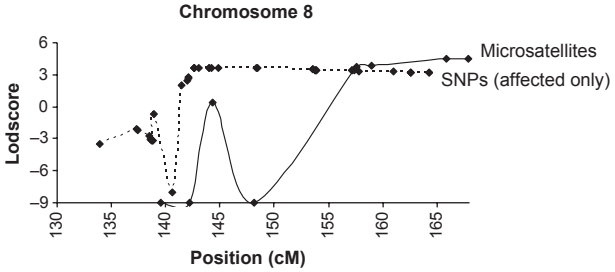

Fig. 4. A genome-wide linkage analysis was performed to locate the gene for autosomal dominant hypocalcified amelogenesis imperfecta in the five-generation family. Significant linkage was found on chromosome 8q24.3 using 14 affected individuals and SNP array data. Linkage was confirmed using microsatellite markers, and by including more family members, the lodscore increased to 4.5.

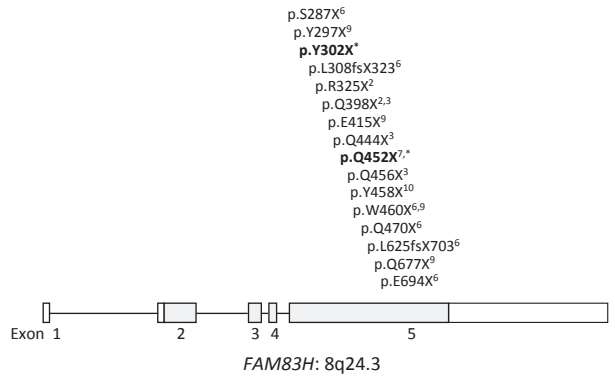

Fig. 5. Distribution of the 16 different *FAM83H* mutations identified so far. The two nonsense mutations identified in this study are marked with an asterisk.

The disease haplotype was identified in all affected individuals and in none of the unaffected family members indicating 100% penetrance of the mutation in this family.

Mutational analysis

Sequencing of the *FAM83H* gene located in the area identified by the linkage analysis revealed a single-base substitution in exon 5, c.906T>G in heterozygous form, changing codon 302 for tyrosine to a stop codon (p.Y302X; Fig. 5).

Sequencing of *FAM83H* in five other unrelated patients revealed in one patient a single-base substitution, c.1354C>T in heterozygous form, also situated in exon 5, changing codon 452 for glutamine to a stop codon (p.Q452X). This mutation was not detected in the unaffected parents of the patient, indicating a de novo mutation in the proband. No other putative diseases causing *FAM83H* mutations were detected.

Discussion

This study reports on a novel *FAM83H* nonsense mutation, p.Y302X, in a Danish five-generation family with ADHCAI. The phenotypic variation in the affected family members harbouring this particular mutation was limited.

The clinical examinations showed that teeth in all affected individuals with assessable teeth, except one, had rough surfaces. The woman with smooth tooth surfaces also presented brown and black discoloration of the enamel. These findings could be explained by this woman being the oldest individual with assessable teeth; thus, it could be expected that exposed enamel will smoothen off and that discoloration gradually may increase over time. Concerning all other evaluated characteristics, very limited phenotypic variation was observed. In all types of teeth, a zone with enamel of apparently normal thickness was found along the gingival margin. This finding is in contrast to the reporting of a unique phenotype with the affected enamel being localized to the cervical 1/3 of the teeth in most affected individuals in two of seven ADHCAI-affected families studied[6].

Since the first description of *FAM83H* mutations as a possible cause of ADHCAI, 16 different mutations have been reported on (Fig. 5). All mutations are truncating (14 nonsense and 2 frameshifts) and all positioned within exon 5.

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The de novo p.Q452X mutation identified in one of the patients presented in the present report has previously been described[7].

As suggested by Wright and co-workers[6], unique phenotypes appear to be associated with specific *FAM83H* mutations. The phenotypic appearance of the unrelated boy with a de novo mutation (p.Q452X) in exon 5 of *FAM83H* varied in some aspects from the ADHCAI phenotype seen in the five-generation family. In comparison with the five-generation family, the morphological disturbances of the dentition were more extended, e.g., with a clear tent-like appearance of both premolars and molars. In addition, this boy has eruptional disturbances of teeth not seen in the five-generation family at all. It should, however, be mentioned that loss of space may have occurred because of early extractions of primary teeth in this patient. Nevertheless, it is reasonable to expect the process of dental eruption to be disturbed in this patient is because of AI, and presence of such findings in patients with AI has previously been reported on in the literature (for systematic review, see Ref. 8).

In conclusion, we have identified a *FAM83H* mutation in two of six unrelated families with ADHCAI and found limited phenotypic variation of the enamel in these patients.

What this paper adds

- Knowledge about a novel mutation in *FAM83H* in patients with ADHCAI
- Knowledge about limited phenotypic variation of enamel in family members with a novel nonsense mutation, p.Y302X.

Why this paper is important to paediatric dentists

- To inform and counsel parents to children with ADHCAI
- Knowledge of the clinical features of ADHCAI is important for proper treatment planning

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Contributions

All authors have been actively involved in the overall designing and execution of the study and have contributed to the preparation of the manuscript. Further, H. Gjørup, S. Poulsen and D. Haubek carried out the procedures related to the visit of the participants in the clinic. Blood samples were collected at Aarhus University Hospital. L.G Jensen, I. Juncker, M. Nyegaard, A. Børglum, and J.M. Hertz did the genetic analyses.

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