Amelogenesis imperfecta: enamel ultra structure and molecular studies

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Amelogenesis imperfecta (AI) is a hereditary disorder resulting in generalized defects in the enamel. The case reported here is of a seven-year-old male child with yellow color of all his teeth. Two of his primary molars were extracted due to dental abscess with advanced root resorption. Histologically hypoplastic enamel layer, positively birefringent, generalized pitting, roughness with irregular general cracked borders were observed. Scanning electron microscope, revealed extensive irregular, disorganized rough superficial enamel layer. The enamel was irregularly decussate with filamentous prisms accompanied by small rounded formations. The morphological and histological examination of the tooth revealed that this patient has the features of AI. For genetic study blood sample were collected from the patient and PCR analysis revealed that there is no mutation in exons 1-7 of AMELX gene on the X chromosome of the patient. Hence, it is probable that the AI of this patient is not X-linked. It is more likely to be an autosomal mutation.
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INTRODUCTION

Amelogenesis imperfecta is a hereditary disorder resulting in generalized defects in the enamel affecting primary or permanent dentition without evidence of systemic disorders.[1] This condition has been divided into four main types (hypoplastic, hypomaturation, hypocalcification and hypomaturation - hypoplasia with taurodontism) and fourteen subtypes based on clinical, histological, radiographic and genetic features. The hypoplastic types are characterized by deficiency in the quantity of enamel, which can be expressed clinically through fine enamel, or with grooves and pits on its surface. The hypocalcified types show enamel that has low mineralization, manifested clinically by pigmented, softened and easily detachable enamel. The hypomaturation types are associated with anomalies of the maturation stage during the formation of the enamel, resulting in an opaque and porous enamel.[2] The clinical appearance of these types of amelogenesis imperfecta ranges from thin enamel that is normal in color to enamel that is severely hypomineralized and readily abrades from the teeth as they erupt into the oral cavity.[3] Depending on the type of amelogenesis imperfecta the teeth can be extremely sensitive to thermal and chemical stimuli.

Genetic factors act with variable severity throughout the whole duration of amelogenesis that results in defects involving the whole enamel or are randomly distributed in it. The genetic inheritance pattern can be autosomal dominant, autosomal resessive or X – linked.[4] The genetic origin of the autosomal forms is still unknown, although the cause of X – linked amelogenesis imperfecta is definitely related to defects in the amelogenin gene, which is the principal protein related to the formation of human dental enamel. The human amelogenin gene is located at the distal region of the p22.1 - p22.3 short arm of the X chromosome and in the pericentromeric region of the Y chromosome. The determination of the human amelogenin gene in the p 22 region of the X chromosome, together with the discovery of the locus of amelogenesis imperfecta in the Xp22.2 region, support the association of this gene with the different amelogenesis imperfecta phenotype that have inheritance linked to this chromosome.[5]

In the present report, the dental, histopathological, scanning electron microscopic and genetic findings of a patient with amelogenesis imperfecta have been presented.

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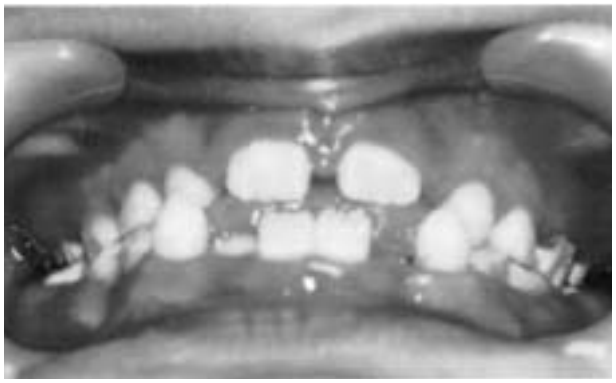

Figure 1. Upper and lower teeth showing enamel hypoplasia.

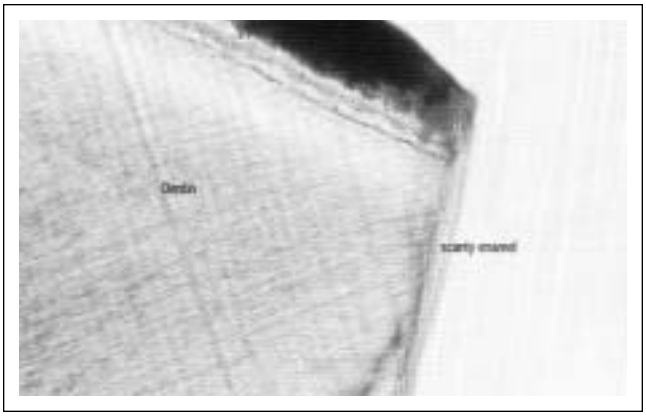

Figure 2. Ground section of tooth showing scanty enamel layer (X20).

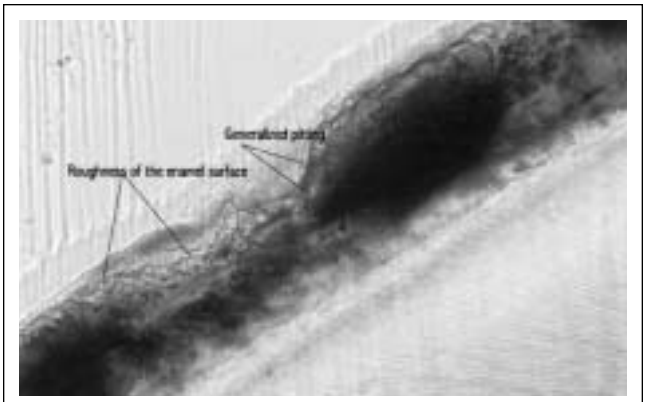

Figure 3. Ground section of tooth showing generalized roughness and pitting of the enamel layer (X 20).

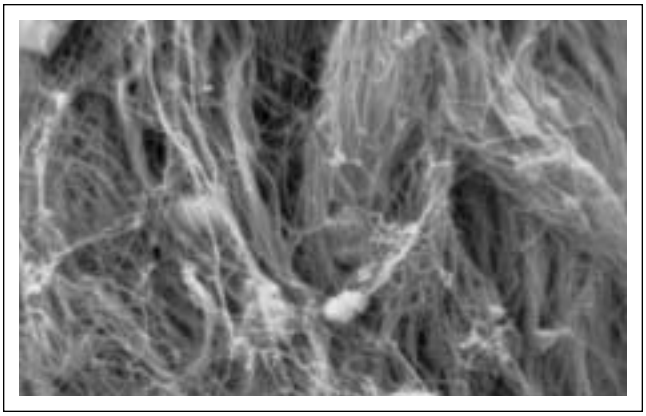

Figure 4. Scanning electron microscopy. Enamel with altered prisms, showing filamentous prisms accompanied by small rounded formations (X5500).

CASE REPORT

A seven years old male child reported to Hospital Universiti Sains Malaysia, School of Dental Sciences with a chief complaint of yellow color of all his teeth. The parents observed this problem with the eruption of the first few teeth in to the oral cavity. A detailed medical evaluation of the child revealed no relevant medical problems. Family history gave a clue to hereditary inheritance. It was observed that the parents did not have this problem but their children; two boys and one girl had yellow teeth. The eldest daughter had normal teeth. On further elucidating the family history the boy's mother revealed that her mother had the same problem.

Intra oral examination of the patient revealed mixed dentition with all the teeth showing enamel hypoplasia (Figure 1). Decayed teeth present were 54, 64, 74, 75, 85, 46. Restorations were done on 54, 64, 85 and 46. Decayed teeth 74 and 75 had symptoms of pain with apical abscess, intra oral radiograph revealed decay involving the pulp with advanced root resorption. Hence, 74 and 75 were extracted under local anesthesia and space maintainers were constructed. The extracted teeth were sent for histopathological and scanning electron microscopic examination. Blood samples were also collected from the patient for genetic study. All the subjects involved in this study signed a detailed informed consent form. The protocol was reviewed by the human ethical committee (School of Medical Sciences, Universiti Sains Malaysia).

MATERIAL AND METHODS

Extracted teeth were rinsed in water several times, and sectioned longitudinally into slices using diamond band cutting system (EXAKT), some slices were fixed in 10% neutral formalin solution, and processed as ground sections. Ground sections of the teeth were examined using image analyzer (KS 300, Zeiss). Other slices were immersed in 70% ethanol solution for several days at 4˚C. The teeth slices were processed for SEM according to the standard procedure, briefly, sections were dehydrated through a series of acetone and subjected to critical point drying for 30 minutes. The sections were then placed on to stubs using double-sided tap and coated with gold palladium in a sputter coater. Surface micrographs were taken using a Leica Cambridge S-360 scanning electron microscope (SEM).

Blood samples were collected from the amelogenesis imperfecta (AI) patient through venous blood collection method.

Table 1. Primer sequence and size of expected PCR product.

Exon	Primer	Sequence (5'-3')	Product size (bp)	References
1	AMEX1-F	TAA TCA CAA CAT ACA GCC TT		This study
	AMEX1-R	AGT AAC TAT TGA TGG CAT ATA G	335	This study
2	AMEX2-F	AAT TCA CAA ACA ATG GCT CC		This study
	AMEX2-R	AAA CAA GCT CCT GAA GTG TT	350	This study
3	AMEX3-F	AAC TAA AAA CGA ACC TCA AG		This study
	AMEX3-R	GGA TAA AGA ATC AAC ACA CT	308	This study
4&5	AMEX4-5-F	TGA GAG TAA TAA TAC TTG CC		This study
	AMEX4-5-R	TCC CAT TAA TGT CTG CAT GTG	430	11
6	AMEX6-F	ATA TTC CTA TAG CCA TAA TGG C		This study
	AMEX6-R	GCA TAT TGC TTG GAC TAA TAG	668	This study
7	AMEX7-F	TGG TTG TAC ATT GTT TTG AC		This study
	AMEX7-R	AGG AAA ATT TAA GTT TCA TT	298	This study

Genomic DNA isolation

Genomic DNA was extracted from the venous blood samples by phenol-chloroform and proteinase K digestion method. The DNA samples were quantitated using spectrophotometer and were further diluted using distilled water.

DNA sequences alignment

The genomic DNA sequences of amelogenin gene were obtained from Genbank and aligned with ClustalW software. The conserved and non-conserved regions of the alignments were visualized using Genedoc Software. Based on the conserved region on the alignment, specific primer sets were designed to amplify the exons 1-7 region of AMELX gene.

PCR amplification

The PCR was standardized using normal human genomic DNA and the primer sets to amplify exons of the AMELX gene (Table I). PCR was performed in 20ul reactions. Each reaction contained 20pmol for each forward and reverse primers, 0.16mM dNTPs, 2.5mM $MgCl_2$, 1X PCR buffer, 1 unit Taq DNA polymerase and DNA (~100ng). PCR was performed using Eppendorf mastercycler 5330, with an initial denaturation at 95˚C for 5 min, 30 cycles of denaturation at 95˚C for 30sec and annealing for 30sec at 52˚C for all of the exons, extension at 72˚C for 30sec, followed by an extra cycle of annealing at 52˚C for 30sec and a final extension at 72˚C for 5min. The PCR products were electrophoresed through 1.2% of low EEO agarose gel (Pronadisa) at 100v for 45min. The PCR products were purified using DNA gel extraction kit (Millipore, MA, USA).

DNA sequence analysis

The eluted PCR product was sent for DNA sequencing to a sequencing service center (Tech Dragon, Limited, Hong Kong). The DNA sequencing results from samples were assembled by using Contig Alignment software. The resultant contig was compared with the reference AMELX sequence (GenBank accession no.: AY040206) for mutations.

RESULTS AND DISCUSSION

The ground sections of the teeth revealed characteristic histological alteration include hypoplastic enamel layer, positively birefringent, generalized pitting, roughness with irregular general cracked border and porous enamel surface (Figures 2, 3). These histological features appeared in this case are compatible with previous reports.[6] Using scanning electron microscope, the sections presented extensive irregular, disorganized rough superficial enamel layer. Porosity and cracks were also observed among the surface, which was covered with ovoid or globular protrusions. The prism patterns in the enamel of teeth were irregularly decussate with filamentous prisms accompanied by small rounded formations (Figure 4). There have been a few reports in the literature on scanning electron microscope studies of the surface characteristic of amelogenesis imperfecta enamel,[7-10] the results of this study is compatible and support the previous scanning electron microscope observations about the amelogenesis imperfecta.

In several cases reported in the literature, the genetic background of AI is mostly due to X-linked amelogenesis imperfecta.[11-16] By PCR we were able to amplify exons 1-7 of the AMELX gene of the patient (Figure 5). However, by the sequencing, we found no mutations were observed in exons 1 to 7 of AMELX gene on the X chromosome (Table 2). The primers were designed specifically to detect only the exons on the X chromosome. Therefore, we are certain that the AMELX gene on the X chromosome contains no mutations. Hence, it is probable that the amelogenesis imperfecta of this patient is not X-linked. It is more likely to be an autosomal mutation.[14] The family pedigree of the patient can be found in Figure 6.

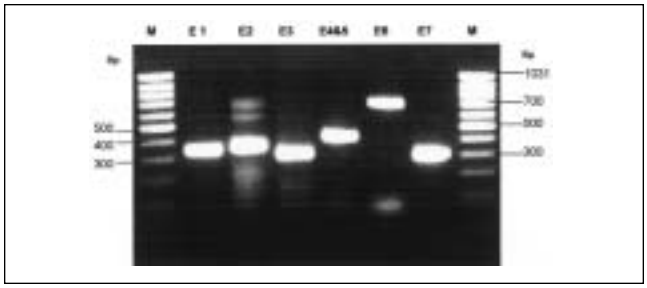

Figure 5. PCR products obtained from patient sample using AMELX exons **(E)** 1-7 specific primers.

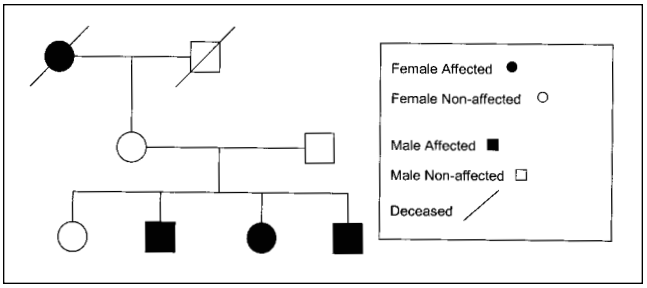

Figure 6. Pedigrees of the small kindred, showing that transmission of the AI phenotype is consistent with an autosomal recessive trait.

Table 2. The reference amelogenin sequence with the contig sequence showing no mutation in the protein

	Exon2	Exon3	Exon4	Exon5
AY040206	MGTWILFACLLGAAFAMP	LPPHPGYINFSYE	NSHSQAINVDRTAL	BLTPLKWYQSIRPP
Contig	MGTWILFACLLGAAFAMP	LPPHPGYINFSYE	NSHSQAINVDRTAL	BLTPLKWYQSIRPP
	Exon 6			
AY040206	YPSYGYEPMGGWLHHQIIPYLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPIQ			
Contig	YPSYGYEPMGGWLHHQIIPYLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPGQHSMTPIQ			
	Exon 6 continued			
AY040206	HHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLPPMLPDLTLEA			
Contig	HHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQPLPPMLPDLTLEA			
	Exon 6 continued	**Exon7**		
AY040206	WPSTDKTKREEV	D		
Contig	WPSTDKTKREEV	D		

CONCLUSIONS

The case reported here is a hypoplastic type of AI characterized by deficiency in the quantity of enamel, which is expressed clinically as fine enamel with pitting on the surface. SEM study on the surface of the enamel also confirmed hypoplastic changes. Genetic studies have ruled out the role of the X-linked mutation in this patient. Therefore, further studies on the family pedigree and autosomal mutation will be done.

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