Introduction

Ectodermal dysplasia (ED; MIM#305100) is one of more than 150 clinically distinct hereditary ectodermal disorders. Most of these disorders are rare and manifest variable defects in the development of the skin, hair, nails and teeth. Ectodermal dysplasia is characterized by a triad of signs comprising sparse hair (hypotrichosis), abnormal or missing teeth (oligodontia or anodontia), and an inability to sweat due to a lack of sweat glands (anhidrosis or hypohidrosis). The gene for X-linked anhidrotic ectodermal dysplasia (XLHED) has been mapped to Xq12–q13.1, and the part of the gene responsible for the disease, ectodysplasin-A1 (EDA1), has been isolated by positional cloning. The full-length sequence of the EDA1 transcript has recently been published. It consists of nine exons and produces a cDNA sequence of 5,307 bp in length, which is translated into a protein with 391 amino acids.

Here, we present a missense mutation in codon 215 of the EDA1 gene which leads to a glycine to arginine amino acid substitution, and a successful gene diagnosis in a Japanese family, leading to the detection of heterozygous status.

Case Report

The patient was a boy born in 2003, was first seen by a pediatrician at a general hospital, where HED was diagnosed based on (1) anhidrosis, (2) sparse hair, (3) dry skin, and (4) an edentulous jaw. As a result, he was referred to the Tokyo Dental College Chiba Hospital Department of Pediatric Dentistry for investigation of the relationship between his...
symptoms and mutation of the EDA1 gene. At the time of his first visit to our department, he was 1 year and 10 months old. This study was conducted with the approval of the Ethical Committee of Tokyo Dental College (Approval no. #21).

Earlier systemic findings at the general hospital had revealed evidence of fine hair growth from the parietal to frontal regions of the scalp, but none in the temporal and occipital regions. Although the eyebrows were also thin but the eyelashes were almost normal. The skin was dry. A sweat test revealed slight axillary sweating, but none in the trunk, nape or legs. Therefore, care was taken to prevent hyperthermia during the summer and there were no particularly large problems in everyday life for the patient. Some care had to be taken to prevent hyperthermia during the summer, but otherwise there were no particularly large problems in everyday life for the patient. Further examination at our department revealed no abnormalities in the morphology of the extremities, motor function, nutritional condition or developmental status. Oral examination revealed the eruption of the maxillary deciduous central incisors only (Fig. 1). Although a posteroanterior view revealed the presence of deciduous molar tooth germs, no succedaneous permanent teeth were observed (Fig. 2). The family medical history revealed congenital absence of the permanent teeth in the mother, but no abnormal findings in the father.

Fig. 1 Oral photographs of each member of the family under investigation
(a) the patient; (b) the father; and (c) the mother

Fig. 2 Posteroanterior view of the subject with ectodermal dysplasia taken at 1 year 10 months of age
Polymerase chain reaction amplification of samples

With informed consent, genomic DNA was isolated from oral buccal epithelial cells according to standard protocols. Polymerase chain reaction (PCR) fragments of 167–403 bp, corresponding to exons 1–9, were amplified using primers, as described by Monreal et al. In brief, 20 ng of DNA was amplified by PCR in a 40 μL polymerase chain reaction (PCR) containing 1X GeneAmp buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 4 pmol of each primer and 2.5 U AmpliTaq Gold® (Applied Biosystems, Foster City, CA, USA). Following an initial DNA denaturation step of 95°C for 9 min, 40 cycles of amplification were performed using the following cycling parameters: denaturation at 94°C for 30 s; and annealing/extension at 60°C for 1.5 min. A final extension at 72°C for 10 min followed the last cycle.

Nucleotide sequencing and mutation in the patient with ectodermal dysplasia

Amplified fragments from both the patient and the parents, were directly sequenced by using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin Elmer, Foster City, CA, USA). Reactions were analyzed in the ABI 373A automated DNA sequencer (Applied Biosystems). Nucleotide sequence analysis was carried out using the DNASIS-Mac computer program (Hitachi Software Engineering Co., Ltd., Yokohama, Japan).

Figure 3 shows the results of the nucleotide sequence analysis of exon 5 of the EDA1 for each member of the family. The sequence from the patient revealed a point mutation at nucleotide position 643 (G643A transversion) in exon 5 of the EDA1, which changes codon 215 from glycine to arginine (Gly215Arg). This point mutation causes a missense mutation. Heterozygosity was demonstrated in the mother, with both the wild and mutant types at the same nucleotide position. This mutation was not detected in the father. The nucleotide sequences of the amplified fragments from the patient, and the parents, showed complete accordance in exons 1, 2, 3, 4, 6, 7, 8 and 9 (data not shown).

Discussion

Anhidrotic ED was first described in 1848 by...
Thurnam and was first termed as “hereditary ectodermal dysplasia of the anhidrotic type” in 1929 by Weech. It is the most frequent type of ED and its incidence has been reported to be one in 100,000 births. A hereditary disease, it is classified into XLHED, autosomal dominant HED and autosomal recessive HED. In addition, a type with immunodeficiency has also been reported. Clinical findings are important for the diagnosis of ED. Anhidrosis (or hypohidrosis), sparse hair and congenital absence of teeth are major findings and the characteristic facial appearance, the so-called ‘dish face’, is characterized by a protruding forehead and mentum, a saddle nose and recession of the central portion of the face and cheeks are known features that are seen.

In the case of X-linked ectodermal dysplasia (XLED), males develop the symptoms and females become carriers. There is also a phenomenon known as called Lyonization, in which one of the two X chromosomes is inactivated at random. Therefore, in such cases, female carriers become mosaics of functionally normal cells and abnormal cells. Because of this, even carriers may develop varying degrees of symptoms depending on the percentage of abnormal cells among the progenitor cells of the teeth, hair and sweat glands. That is to say, females carries show a variety of symptoms ranging from those as severe as those observed in males to almost asymptomatic. However, in many cases although symptoms, such as hypohidrosis, hypotrichosis, and congenital absence or morphological abnormalities of the teeth (cone-shaped tooth) may be observed, they are less pronounced than in males. In our case, the mother was found to be a carrier and showed congenital absence of the teeth, although no hypohidrosis or hypotrichosis was not apparent.

The ectodysplasin-A (EDA) gene has been identified as the gene responsible for XLED. Six isoforms of the EDA gene occur by alternative splicing, including EDA-A1 (ectodysplasin-A1), with consists of 391 amino acids, and EDA-A2 (ectodysplasin-A2), which comprises 389 amino acids.

The locus for the EDA gene is on Xq12–q13.1 and more than 150 different mutation types have been reported. Many patients with XLED, there are many cases that show missense mutations or nonsense mutations in the functionally important parts of the EDA gene, namely, the TNF homology domain (exons 7–9), the collagen-like domain (exons 5, 6), and the furin recognition domain (exon 3). Our case also showed a missense mutation in exon 5, which is responsible for the collagen-like domain. It is believed that morphogenesis is impaired by such mutations, as the ability to bind with its receptor decreases due to mutations in the TNF homology domain, the formation of trimers of EDA is inhibited due to mutations in the collagen-like domain or the solubilization of EDA is inhibited due to mutations in the furin recognition domain. The inability to sweat may lead to life-threatening or brain-damaging hyperthermia. Therefore, early diagnosis and counselling for families are essential, including instructions on how to lower body temperature during hot weather or fever.

Clinically, no radical treatment is available at present, and palliative treatment is the only option. However, it has been reported that normal persistent ectodermal organogenesis, such as that of sweat glands, teeth and hair, was induced in new-born mice by only a short-term administration of recombinant EDA protein designed to pass through the placental barrier in pregnant EDA-mutant mice. Therefore, the development of treatments for this disorder is expected in the future.

References
7) Courtois, G., Smahi, A., Reichenbach, J., Döffinger,


