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A point mutation of the ED1 gene in a Japanese family with X-linked hypohidrotic ectodermal dysplasia

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Summary. X-linked hypohidrotic ectodermal dysplasia (EDA) is characterized by the hypoplasia or absence of hair, teeth and sweat glands. In this study, the authors investigated the ED1 gene in a Japanese family with X-linked hypohidrotic ectodermal dysplasia. The only affected male fulfils the diagnostic criteria for this disorder. His parents were not consanguineous and both of them were healthy. After informed consent, genomic DNA was isolated from the peripheral blood lymphocytes or oral buccal epithelial cells of all members of the family. A polymerase chain reaction fragment containing exon 9 of the ED1 gene was amplified using primers. The patient's amplified fragment, as well as those from his father, mother and sister, were directly sequenced. The sequence from the patient revealed a point mutation (G1149A) in exon 8 of the ED1 gene, which changes codon 291 from glycine to arginine. Heterozygosity was demonstrated in his mother and sister. This mutation has not been reported previously. The amino acid substitution is predicted to disrupt the transmembrane domain, which strongly implies that this is the disease-causing mutation in the family.

Introduction

The disorder, now called anhidrotic (or hypohidrotic) ectodermal dysplasia (EDA; MIM#305100) is one of more than 150 clinically distinct hereditary ectodermal dysplasias [1]. Most of these syndromes are rare and manifest variable defects in the development of the skin, hair, nails and teeth. Ectodermal dysplasia is one of the more common types and is characterized by a triad of signs comprising sparse hair (hypotrichosis), abnormal or missing teeth (hypodontia or anodontia), and an inability to sweat because of the lack of sweat glands (anhidrosis or hypohidrosis). The inability to sweat may lead to life-threatening or brain-damaging hyperthermia, and thus, early diagnosis and counselling of families are essential, including instructions for lowering the body temperature during hot weather or fever.

The gene for EDA has been mapped to Xq12–q13, and a part of the gene responsible for the disease, ED1, has been isolated by positional cloning [2]. The full-length sequence of the ED1 transcript has recently been published [3,4]. It consists of nine exons, resulting in a cDNA of 5307 bp, which is translated into a protein with 391 amino acids.

This paper describes a novel mutation in codon 291 of the ED1 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. This study was conducted with the approval of the Ethical Committee of Tokyo Dental College, Chiba, Japan.

Case report

In 1992, a 1·7-year-old boy presented at the paediatric dental clinic of the Tokyo Dental College

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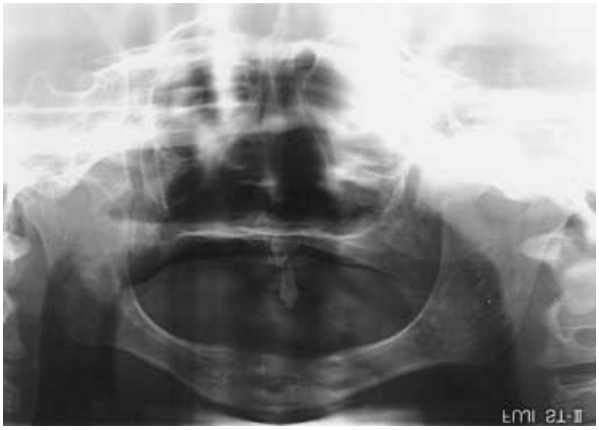

Fig. 1. Panoramic radiograph of the subject with ectodermal dysplasia taken at 5 years of age.

Chiba Hospital for consultation regarding the delayed eruption of his primary teeth. He had fine, curly and sparse hair, sparse eyebrows, a saddle nose, and mild frontal bossing. The subject had a recurrent fever of 38 °C, and the absence of sweating since birth was reported. Figure 1 shows a panoramic radiograph of the patient that was taken when he was 5 years of age. He had only a primary central incisor and a permanent central incisor bud in the maxilla. The other primary and permanent tooth buds were not present in either his maxilla and mandible. He had a father, mother and young sister, all of whom were healthy. Figure 2 shows an oral photograph of each member of the family. At 8 years of age, the subject only had a single maxillary permanent central incisor, and this was an abnormally shaped tooth. His mother had peg-shaped maxillary permanent lateral incisors. The number and shape of his father and sister's teeth were normal. He was diagnosed with hypohidrotic ectodermal dysplasia (EDA) on the basis of his characteristic facial and oral appearance and hypohidrosis.

Polymerase chain reaction amplification of samples

After informed consent, genomic DNA was isolated from peripheral blood lymphocytes or oral buccal epithelial cells according to standard protocols. Polymerase chain reaction fragments of 167–403 bp, corresponding to exons 1–9, were amplified using primers, as described by Monreal *et al*. [3]. In brief, 20 ng of DNA was amplified in a 40-μL polymerase chain reaction (PCR) reaction containing 1X GeneAmp buffer, 0·2 mM dNTPs, 1·5 mM $MgCl_2$, 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,

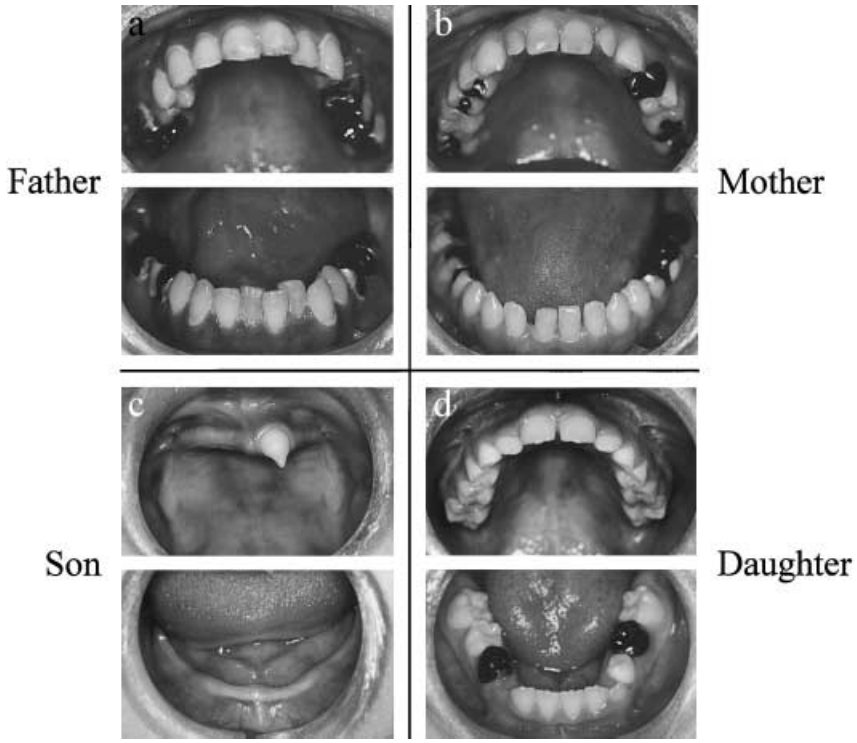

Fig. 2. Oral photographs of each member of the family under investigation: (a) the father; (b) the mother; (c) the son; and (d) the daughter.

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

The patient's amplified fragments, as well as those from his father, mother and sister, 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 analysed in an 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 8 of the ED1 gene for each member of the family. The sequence from the patient revealed a point mutation at nucleotide position 1149 (G1149A transversion) in exon 8 of the ED1 gene, which changes codon 291 from glycine to arginine (Gly291Arg). This point mutation causes the missense mutation. Heterozygosity was demonstrated in the patient's mother and sister. Thus, his mother and his sister were heterozygotes with wild and mutant types at the same nucleotide position. The mutation was not detected in his father. The nucleotide sequences of the amplified fragments of the subject, and his father, mother and sister, showed complete accordance in exons 1, 2, 3, 4, 5, 6, 7 and 9 (data not shown).

DNA diagnosis by means of PCR-MspA1I digestion and detection of the G78A mutation by restriction analysis

The DNA fragment of 239 bp containing exon 8 of the ED1 gene was obtained for each individual of the family by PCR amplification and was subsequently digested with Mspa1I (Promega Co., Madison, WI, USA). The digested samples were examined by electrophoresis in 5% polyacrylamide gel and visualized by silver staining methods.

MspA1I digestion of the 239 bp PCR products containing exon 8 of the ED1 gene enabled the authors to distinguish between the mutant (239 bp fragment) and the wild-type allele (131 and 108 bp fragments). There is a different number of fragments because the mutation removed a cutting site for the enzyme, which cuts at the sequence C(A/C)GC(G/T)G, with the mutation changing the sequence from CAGCGG to CAGCAG. The patient, his mother and his sister had this mutation, whereas his father did not (Fig. 4). His mother and sister had a mutated allele on one X-chromosome and a normal allele on

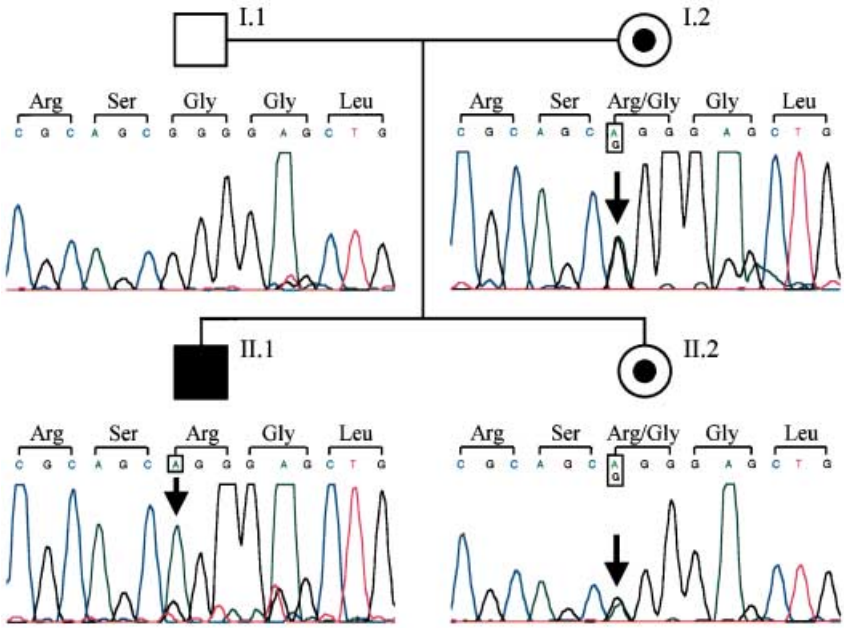

Fig. 3. Nucleotide sequence analysis of exon 8 of the ED1 gene for each member of the family under investigation.

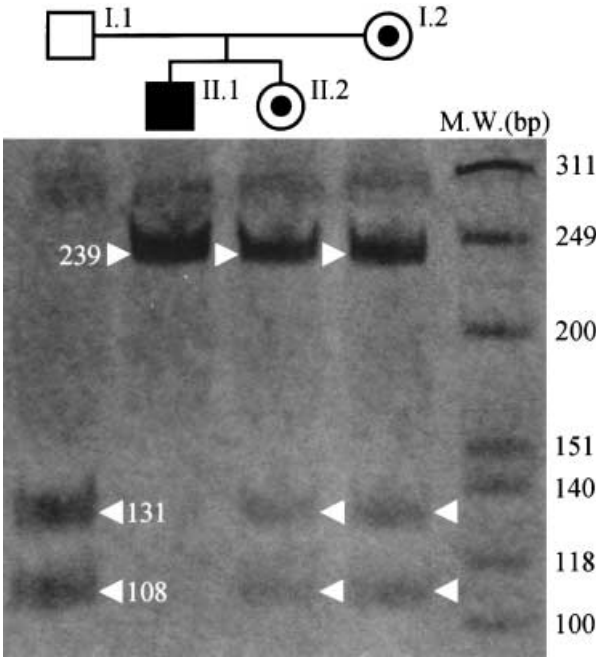

Fig. 4. MspA1I digestion of the 239-bp polymerase chain reaction products containing exon 8 of the ED1 gene. The mutant (239-bp fragment) and the wild-type allele (131- and 108-bp fragments) can be distinguished from each other.

the other. This mutation was not detected in genomic DNA from the control subjects tested.

Discussion

The ED1 gene is expressed in various tissues such as the heart, kidney and pancreas, as judged by Northern blotting [2]. *In situ* hybridization with skin showed positive signals in epidermal cells, the outer root sheath of the hair follicles and sweat glands [2]. The protein encoded by the ED1 gene is a 150-kDa product, and is predicted to possess at least three functional domains: a transmembrane domain, a TNF-like domain and a positively charged domain [5]. So far, 37 different mutations have been identified [1,3,4,6–10], four with small deletions, five with gross deletions, four with insertions, one with deletion/insertion, one with altered splicing, and 22 with point mutations. In addition, a number of reported missense mutations have been detected predominantly in three domains. The precise function of the ED1 protein is still obscure, apart from the well-known clinical phenotype its mutations cause. However, based on these mutation data, one can assume that the domains are likely to play the important role in the involvement of ED1 in the epithelial-mesenchymal signalling pathway.

In this report, the missense mutation (G1149A) was found in exon 8, and it results in an amino acid substitution of the ED1 protein. This amino acid substitution is predicted to disrupt the transmembrane domain [2], which strongly implies that this is the disease-causing mutation in the subject's family.

Acknowledgements

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Résumé. La dysplasie ectodermique anhidrotique liée à l'X (EDA) est caractérisée par l'hypoplasie ou l'absence de cheveux, dents et glandes sudoripares. Dans cette étude, nous avons évalué le gène ED1 dans une famille japonaise avec dysplasie ectodermique anhidrotique liée à l'X. Le seul garçon atteint correspondait aux critères diagnostics de cette affection. Ses parents n'étaient pas consanguins et étaient tous les deux sains. Après consentement éclairé, de l'ADN génomique a été isolé des lymphocytes du sang périphérique ou de cellules épithéliales buccales de tous les membres de la famille. Un fragment contenant l'exon 8 du gène ED1 a été amplifié par PCR à l'aide de primers. Le fragment amplifié du patient ainsi que ceux du père, de la mère et de la soeur ont été directement séquencés. La séquence du patient a révélé une mutation ponctuelle (G1149A) dans l'exon 8 du gène ED1, qui change le codon 291 de la glycine à l'arginine. L'aspect hétérozygote a été démontré chez la mère et la sœur. Cette mutation n'a pas été décrite précédemment; cette substitution amino acide est censée rompre le domaine transmembranaire, ce qui suppose fortement qu'il s'agit de la mutation responsable de l'atteinte de la famille.

Zusammenfassung. Die X-chromosomale anhidrotische ektodermale Dysplasie ist charakterisiert durch die Hypoplasie oder Abwesenheit von von Haaren, Zähnen und Schweißdrüsen. Die vorliegende Studie untersuchte das ED1-Gen bei einer Familie mit X-chromosomaler anhidrotischer ektodermaler Dysplasie. Der einzige Betroffene war

männlich, er erfüllte die genannten diagnostischen Kriterien. Seine Eltern waren nicht consanguin und beide gesund. Nach Aufklärung und Einwilligung wurden DNA-Proben aller Familienmitglieder isoliert aus peripheren Blut-Lymphozyten oder bukkalen Mukosazellen. Ein Fragment, welches Exon 8 des ED1-Gens enthielt, wurde amplifiziert und jeweils von Vater, Mutter, dem Betroffenen und seiner Schwester direkt sequenziert. Die Sequenz des Patienten zeigte eine Punktmutation (G1149A) in Exon 8 des ED1-Gens, wodurch im Codon 291 Glycin durch Arginin ausgetauscht wird. Bei Mutter und Schwester wurde Heterozygosität festgestellt. Diese Mutation wurde zuvor nicht beschrieben, der Aminosäurenaustausch führt vermutlich zu einer Veränderung in der Transmembrandomäne, was es sehr wahrscheinlich macht, dass diese Mutation in dieser Familie als die Krankheitsursache des Patienten angesehen werden kann.

Resumen. La displasia ectodérmica hipohidrótica ligada al cromosoma X (DEA) se caracteriza por hipoplasia o ausencia de pelo, dientes y glándulas sudoríparas. En este estudio investigamos el gen ED1 en una familia japonesa con displasia ectodérmica hipohidrótica ligada al cromosoma X. El único varón afectado cumple los criterios diagnósticos de esta alteración. Sus padres no eran cosanguíneos y ambos estaban sanos. Después del consentimiento informado, se aisló el DNA genómico a partir de linfocitos en sangre periférica o de células epiteliales de la mucosa yugal de todos los miembros de la familia. Se amplificó usando primers, un fragmento de la reacción en cadena de la polimerasa, que contenía exon 8 del gen ED1. Se secuenciaron directamente el fragmento amplificado del paciente así como del padre, de la madre y su hermana. La secuencia del paciente reveló una mutación puntual (G1149A) en exon 8 del gen ED1, que cambia el codon 291 de glicina a arginina. La heterocigocidad se demostró en su madre y su hermana. Esta mutación no ha sido informada previamente; se piensa que la sustitución del aminoácido alteraría el dominio de la transmembrana, lo que implica que esta es la mutación causante de la enfermedad en la familia.

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