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Communication

A novel mutation A1270G of the *EDA1* gene causing Tyr343Cys substitution in ectodysplasin-A in a family with anhidrotic ectodermal dysplasia✪

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The structure of the *EDA1* gene was investigated in a patient with anhidrotic ectodermal dysplasia. Sequence analysis revealed a novel A1270G transition in exon 9 of the *EDA1* gene in the patient and his uncle, whereas the patient's mother and grandmother were heterozygotes. This mutation resulted in Tyr343Cys substitution in the extracellular domain of the *EDA1* gene product — ectodysplasin-A. The additional Cys343 was located between Cys332 and Cys346 and formed with Cys352 a cluster of four closely situated residues that could potentially form disulfide bonds. This mutation might affect the tertiary structure of the receptor-binding domain of ectodysplasin-A and precipitate the clinical symptoms of anhidrotic ectodermal dysplasia.

The characteristic phenotype of ectodermal dysplasia (McKusick, 1998) comprises *oligodontia*, *hypotrichosis* and *hyperthermia* due to a lack of sweat glands. These symptoms are mostly due to mutations of the *EDA1* gene, located on the X chromosome. The longest *EDA1* gene transcript encodes ectodysplasin-A, a 391 amino-acid type II transmembrane protein with the C-terminus projecting outwards (Kere *et al.*, 1996; Bayes *et al.*, 1998;

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Abbreviations: SSCP, single-stranded conformation polymorphism; TNF, tumor necrosis factor.

Monreal *et al.,* 1998). This protein promotes cell adhesion to the extracellular matrix, a function consistent with its role in epithelial-mesenchymal interactions responsible for differentiation of skin appendages (Mikkola *et al.*, 1999). The interaction of this protein with its receptor (EDAR) regulates enamel knot formation in tooth morphogenesis (Tucker *et al.*, 2000). The extracellular domain of ectodysplasin-A contains a C-terminal region, conserved in the tumor necrosis factor (TNF) ligand family, an interrupted collagen-like motif comprising 19 (Gly-X-Y) repeats (Ezer *et al.,* 1999), and a consensus cleavage site, recognized by the protease, furin (Elomaa *et al.*, 2001), releasing the ligand from the cell surface (Chen *et al.*, 2001). The C-terminal region includes three conserved cysteine residues (332, 346 and 352) facilitating trimerization and transport of ectodysplasin-A to the cell membrane (Bayes *et al.*, 1998; Schneider *et al.,* 2001).

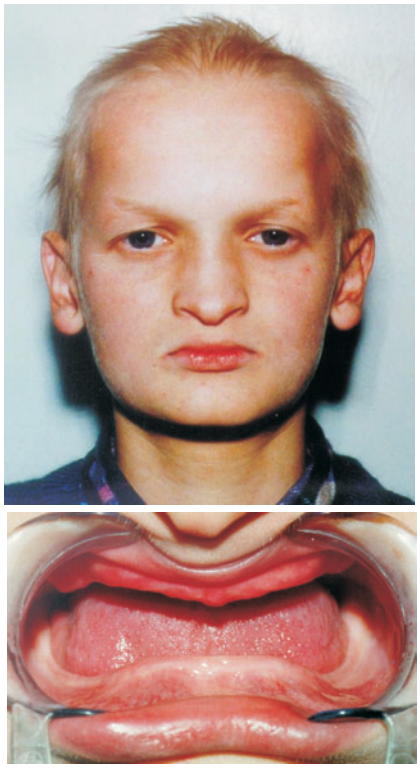


Figure 1. Appearance of the patient.

A. Facial; B. Oral cavity. Note sparse hair, signs of facial *dysmorphism* and lack of teeth in maxilla and mandible.

MATERIALS AND METHODS

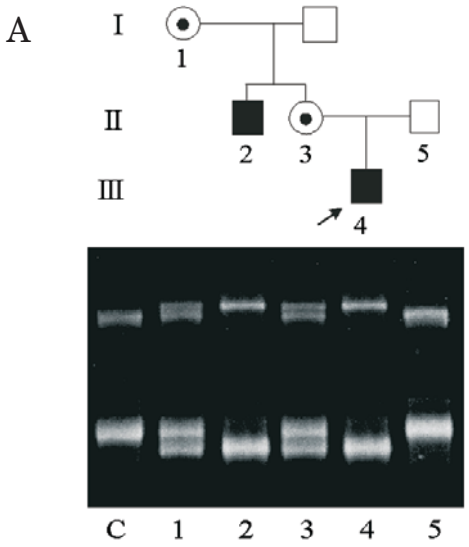
Genomic DNA was isolated from peripheral blood leukocytes and exons 1–9 of the *EDA1* gene were amplified by PCR (Bayes *et al.*, 1998). The amplification products were subjected to single-stranded conformation polymorphism analysis (SSCP) and direct sequencing was conducted using fmol DNA Sequencing System and Cy5 labeled primer: 5′ttc tgt caa ttc acc aca ggg 3′.

DESCRIPTION OF THE CASE

The patient was the first child of healthy, unrelated parents. He was born at full term following normal delivery. At the age of 18 months, anhidrotic ectodermal dysplasia was diagnosed based on thin, dry skin, sparse hair and characteristic features of facial *dysmorphism* (Fig. 1A and B). Visual dental examination revealed four conical, decidual teeth in mandible and maxilla and a panoramic X-ray (not shown), a lack of tooth buds. Although no episodes of elevated body temperature were evidenced, Gibson-Cook test (Mikkola *et al.,* 1999) showed reduced secretion of sweat. Several members of the patient's family were also investigated but in father, mother and grandmother no features of ectodermal dysplasia were evidenced. However, the patient's uncle exhibited typical symptoms of ectodermal dysplasia including a lack of over ten decidual teeth and a conical shape of some of teeth, but he refused to be inspected in detail and photographed.

RESULTS AND DISCUSSION

The SSCP analysis in the patient (Fig. 2A) demonstrated an abnormal mobility of an amplified fragment of exon 9 of the *EDA1* gene. Sequence analysis (Fig. 2B) revealed a novel



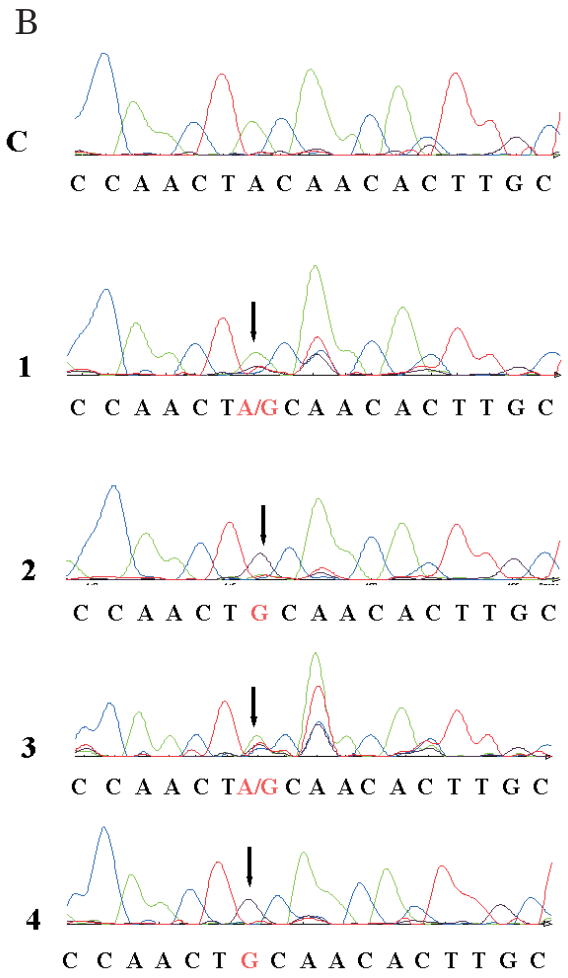


Figure 2. Single-stranded conformation polymorphism (A) and sequence (B) analysis of the amplified fragments of exon 9 of the *EDA1* gene.

Note abnormal mobility of single-stranded DNA fragments (A). Arrows indicate position of the mutation in the sense strand (B). 1, grandmother; 2, uncle; 3, mother; 4, patient; 5, father; C, control (healthy individual). Symbols on the pedigree correlate with appropriate lanes.

A1270G transition resulting in a Tyr343Cys substitution in the extracellular domain of ectodysplasin-A in the patient and his uncle (hemizygotes), but not in his father. The patient's mother and grandmother were heterozygous carriers of this mutation.

To date, 54 different mutations of the *EDA1* gene have been described (Kere *et al.*, 1996; Bayes *et al.*, 1998; Monreal *et al.*, 1998; Kobielak *et al.*, 2001; Schneider *et al.*, 2001). Most were clustered in functional regions encoded by exons 1, 3, 8 and 9. A smaller number of mutations, resulting in 18bp to 36bp deletions were located in exon 5 encoding (Gly-X-Y) repeats (Bayes *et al.*, 1998; Monreal *et al.*, 1998; Kobielak *et al.*, 2000). However, to date no mutations causing the Tyr343Cys substitution have been reported. It has been suggested that the collagen-like motif of ectodysplasin-A is responsible for the interaction with other molecules of the same receptor to form a triple helix stabilized by three conserved cysteine residues (332, 346 and 352) (Bayes *et al.*, 1998). Two of these residues are involved in the formation of a disulfide bond.

We postulate that Cys343 substituted for tyrosine might be involved in the formation of an additional disulfide bond with one of the adjacent cysteines (332, 346 or 352). This might affect the tertiary structure of the protein, impair binding of the ligand to its receptor and precipitate the symptoms of anhidrotic ectodermal dysplasia.

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