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Missense mutation of the *EDA* gene in a Jordanian family with X-linked hypohidrotic ectodermal dysplasia: phenotypic appearance and speech problems

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ABSTRACT. Mutations in the *EDA* gene are responsible for X-linked hypohidrotic ectodermal dysplasia, the most common form of ectodermal dysplasia. Males show a severe form of this disease, while females often manifest mild to moderate symptoms. We identified a missense mutation (c.463C>T) in the *EDA* gene in a Jordanian family, using direct DNA sequencing. This mutation leads to an amino acid change of arginine to cysteine in the extracellular domain of ectodysplasin-A, a protein encoded by the *EDA* gene. The phenotype of a severely affected 11-year-old boy with this mutation included heat intolerance, sparse hair (hypotrichosis), absence of 17 teeth (oligodontia), speech problems, and damaged eccrine glands, resulting in reduced sweating (anhidrosis). Both the mother (40 years old) and the sister (10 years old)

were carriers with mild to moderate symptoms of this disease, while the father was healthy. This detailed description of the phenotype caused by this missense mutation could be useful for prenatal diagnosis.

Key words: EDA; Mutation; Ectodermal dysplasia; Jordan

INTRODUCTION

Ectodermal dysplasia (ED) is a hereditary disease characterized by a constellation of defects involving the teeth, skin, and appendicular structures, including the nails and the eccrine and sebaceous glands (Dhanrajani and Jiffry, 1998). The estimated disease incidence is 1 per 100,000 births (Zonana, 1993; Priolo and Lagana, 2001). X-linked anhidrotic ectodermal dysplasia (XLHED, OMIM 305100) is the most common form of ED (Lamartine, 2003). XLHED is characterized by reduced sweating (anhidrosis) and heat intolerance, sparse hair (hypotrichosis), and the absence of certain teeth (oligodontia or hypodontia) (Kupietzky and Houpt, 1995; Park et al., 1999). Hemizygous males show severe forms of the disease, while heterozygous females often manifest mild to moderate symptoms because of X-chromosome inactivation (Monreal et al., 1998; Vincent et al., 2001; Sekiguchi et al., 2005; Tao et al., 2006). XLHED is caused by mutations in the *EDA* gene (Kere et al., 1996; Bayes et al., 1998), which encodes ectodysplasin-A. Ectodysplasin-A belongs to type II transmembrane proteins of the tumor necrosis factor (TNF) family (Ezer et al., 1999; Wisniewski et al., 2002). By binding to its receptors, ectodysplasin-A activates NF-κB and c-Jun N-terminal kinase signaling cascades involved in the regulation of ectodermal morphogenesis (Yan et al., 2000; Cui and Schlessinger, 2006). Mutations in EDA receptors (*EDAR*, *EDARADD* and *NEMO*) were also shown to cause ectodermal dysplasia with autosomal mode of inheritance (Headon et al., 2001; Shimomura et al., 2004; Chassaing et al., 2006; Vinolo et al., 2006).

This study describes a novel missense mutation (T428C) in the *EDA* gene in a Jordanian family affected with XLHED. The mutation leads to an amino acid change of arginine to cysteine at codon 155. The phenotypes associated with this mutation are discussed in detail here, including skin, hair, nails, teeth, and speech problems.

MATERIAL AND METHODS

Subjects and clinical examinations

A family with XLHED (Figure 1) from Jordan was investigated in this study. All family members underwent examination at Jordan University of Science and Technology (JUST) Health Centre and King-Abdullah Hospital, Irbid, Jordan. Specialists performed the clinical examination of skin, hair, nail, sweat glands, and eyes, while pediatric dentists performed oral and dental examination clinically and radiographically for all subjects. In addition, a speech-language therapist carried out the speech evaluation. The consonants produced by the participants were evaluated in their free speech in the different word positions. Prior to starting the study, written informed consent was obtained from all participants in accordance with the requirements of the Institutional Review Boards of JUST.

DNA isolation

EDTA venous blood samples (3-5 mL) were obtained from participants. DNA was extracted from all samples using Wizard DNA Extraction Kit (Promega, Madison, USA) according to manufacturer instructions. The concentration of the isolated DNA was measured using SmartSpect™3000 (Bio-Rad, Hertfordshire, UK). DNA samples were stored at -20°C until used.

Mutation analysis

To screen for mutations, the eight exons of the *EDA* gene and splice junctions were amplified by polymerase chain reaction (PCR) from genomic DNA using primers designed from intronic sequences. The primer sequences and PCR conditions were performed as previously described (Huang et al., 2006). PCR-amplified fragments were purified using the spin column PCR products purification kit (Bio Basic Inc., Markham Ontario, Canada) and were visualized on 2% agarose gels to check for their purity and correct sizes. The fragments were then sequenced in an ABI Prism 3.1 automated sequencer, using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequence analysis was carried out using ChromasPro 1.49 (Technelysium Pty Ltd., Tewantin, Australia).

RESULTS

The pedigree of the XLHED family is shown in Figure 1. The family consists of two siblings, the father and the mother. The proband is an 11-year-old boy. He visited JUST Dental Health Centre for consultation regarding the absence of several teeth. He was diagnosed with ectodermal dysplasia at the age of 2 years; he had episodes of high temperature, smooth, soft, thin, and dry skin with widespread erythema, excoriation, and a history of eczema at a young age.

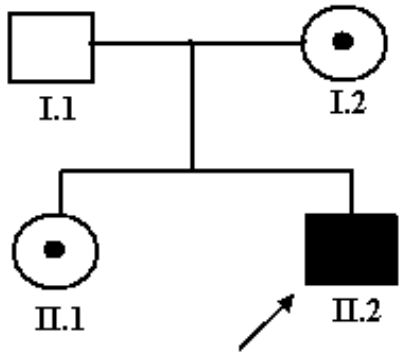

Figure 1. Pedigree of the family affected by the X-linked hypohidrotic ectodermal dysplasia. The arrow indicates the proband.

Figure 2 shows the proband's facial, oral, hair, and nail features. A saddle-shaped nose, periorbital wrinkling and increased pigmentation around the eyes and mouth marked the facial features (Figure 2A). In addition, the lips were protuberant and the ears situated obliquely on the head, causing them to stand out. The hair over the scalp was light brown, sparse and fine (Figure 2C). The eyebrows and eyelashes were almost lacking (Figure 2A). Fingernails and toenails were slightly defective. The shape of the skull resembled an inverted triangle with frontal bossing of the face and depression of the nasal bridge. The panoramic radiograph of the patient showed the absence of 17 permanent teeth including all lateral incisors, all first and second premolars, and all second permanent molars, in addition to the mandibular left first permanent molar (Figure 2D). The permanent mandibular central incisors appeared impacted and rotated on the OPT, and the maxillary primary second molars were retained. Most teeth present in the mouth had conical crowns (Figure 2B). The vertical dimension was reduced, and the palatal arch was high.

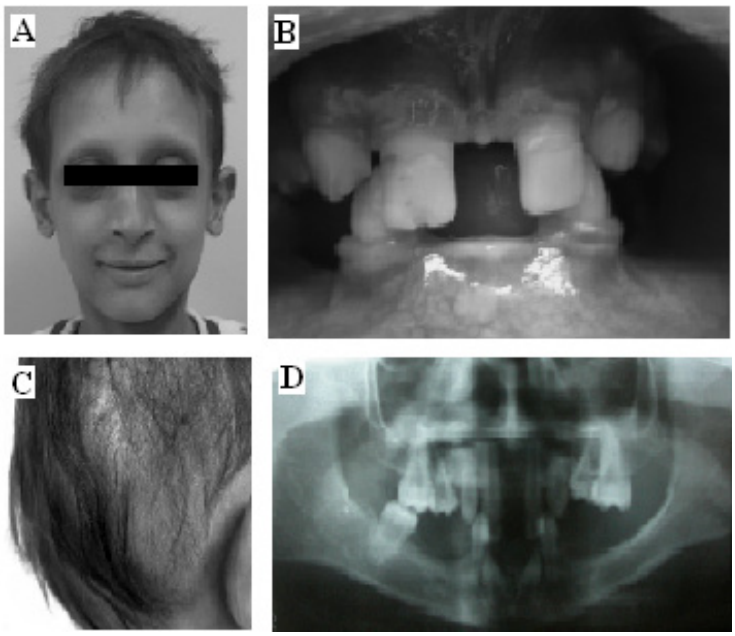

Figure 2. Phenotypic appearance of the affected boy in the family including the following features: **A.** absent eyebrows and eyelashes, a saddle nose, periorbital wrinkling and protuberant lips; **B.** abnormal shaped upper and lower permanent teeth; **C.** sparse scalp hair, and **D.** missing teeth.

Speech evaluation of the proband showed that he lisped the voiceless and voiced plain fricative /s/ and /z/, the voiceless postalveolar /Σ/ and the voiceless emphatic fricative /s≥/. In Arabic, emphatic sounds have primary and secondary places of articulation in contrast to their plain counterparts, which have the primary articulation only. /s/, for example, is produced with the primary articulation on the alveolar ridge. Its emphatic counterpart /s≥/ is produced with an additional secondary articulation with the tongue root

being retracted into the pharynx. The proband in the current study produced /s/, /z/, /Σ/, and /s≥/ in all word positions with lateral lisping where air escapes over the two sides of the tongue. These sibilant sounds are produced by directing air flow over the sharp edge of the teeth, causing turbulence. The central incisors of the proband are separated by a large diastema. This means that the barrier that these teeth create to cause turbulence is missing.

The proband has a healthy father and a mother and young sister who both had mild to moderate symptoms. The sister (10 years old) showed 5 teeth missing, including maxillary permanent lateral incisors, the mandibular permanent left lateral incisor, and the permanent mandibular central incisors. The retained maxillary primary lateral incisors were microdontic and peg-shaped, and the retained mandibular primary central incisors and left lateral incisor had a conical shape. Her scalp hair was of normal color but scant. The nails were slightly defective, but the skin appeared perfectly normal. The mother showed a similar degree of symptoms as the sister, except that the number of teeth was normal. Both the mother and the sister had no speech problems.

Amplified PCR fragments of all exons of the *EDA* gene from the proband and each member of his family were directly sequenced. Figure 3 shows part of the nucleotide sequence analysis of exon 3 of the *EDA* gene for the proband as well as his father, mother and sister.

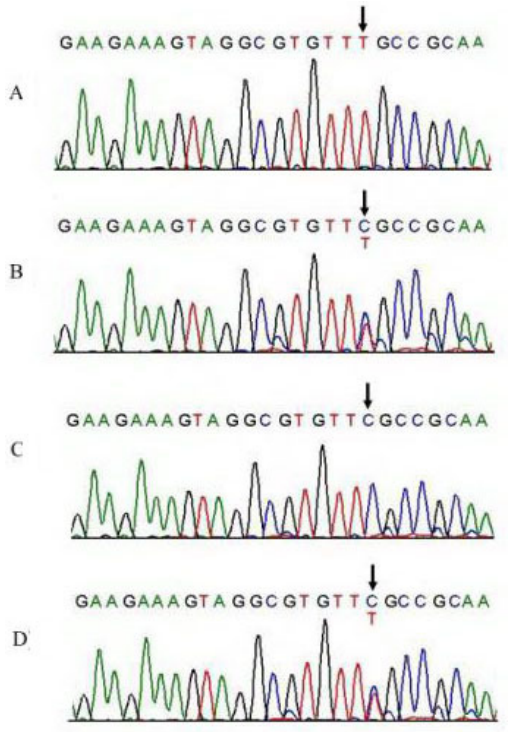

Figure 3. Nucleotide sequence analysis of exon 3 of the *EDA* gene for each member of the family under investigation. The part of the sequence harboring the mutation is shown: **A.** son (patient with the X-linked hypohidrotic ectodermal dysplasia); **B.** sister (carrier); **C.** father (normal), and **D.** mother (carrier). Son and father are hemizygous. Arrow indicates the mutation.

Analysis of the proband sequence revealed a missense mutation (T to C transition) at nucleotide position 463 in exon 3 of the *EDA* gene (Figure 3A), which changes codon 155 from arginine to cysteine (Arg155Cys). Hemizygosity at this position was demonstrated in the affected boy (Figure 3A), while heterozygosity at this position was demonstrated in the slightly affected mother (Figure 3D) and sister (Figure 3B). The mutation was not detected in the proband's father (Figure 3C). The nucleotide sequences at the other exons (1, 3-9) in all examined family members showed complete concordance (data not shown). The mother's family history (parents, five brothers and two sisters) was negative for features of ED indicating that it is *de novo* in the mother.

DISCUSSION

In this study, we identified a c.463C>T transition mutation in the *EDA* gene in a Jordanian family with XLHED. This mutation changes the basic amino acid arginine at codon 155 to uncharged cysteine. The phenotype resulting from this mutation was described including teeth, hair, skin, nails, and speech.

XLHED is characterized by varying degrees of abnormalities of teeth, sparse hair and absent or reduced sweating, which may lead to life-threatening hyperthermia (Yavuz et al., 2008). In addition, XLHED may have emotional consequences for affected individuals at an early age (Yavuz et al., 2006). The clinical findings of the affected male in the family examined fall within the scope of previous reports showing defective development of hair, teeth, nails, and skin (Ferguson et al., 1998; Chao et al., 2003; Lin et al., 2004; Na et al., 2004; Huang et al., 2006; Tao et al., 2006; Tariq et al., 2007; Fan et al., 2008). The defect in speech in the affected male could be due to the presence of the large diastema between the central incisors and the maladaptation in the production of the fricative sibilants (Sharma et al., 1978). In addition, diminished salivary flow and the hypotonicity of the perioral muscles that are common in XLHED patients may play a role in speech problems. In the obligate carriers (heterozygous), mother and sister, mild to moderate symptoms of the disease were observed confirming X-linked inheritance. This is consistent with most studies (Monreal et al., 1998; Sekiguchi et al., 2005; Tao et al., 2006; Vinolo et al., 2006). However, in some families, the carriers do not exhibit any clinical phenotype, suggesting phenotypic heterogeneity of this disease (Huang et al., 2006).

The *EDA* gene encodes the ectodysplasin-A protein, a type II transmembrane protein belonging to the TNF family (Ezer et al., 1999; Wisniewski et al., 2002). The signaling pathway mediated by ectodysplasin-A has important functions in the development of several organs and structures derived from the ectoderm, such as the skin, hair, nails, pituitary, mammary and sweat glands, nose, eyes, and the enamel of the teeth (Cui and Schlessinger, 2006; Cui et al., 2009). The extracellular domain of ectodysplasin-A contains furin, collagen and TNF-like domains. The TNF-like domain is necessary for receptor binding and the collagen domain for bundle formation, while the furin domain functions as a cleavage site for furin protease (Schneider et al., 2001). Mutations in any of these domains were reported to produce XLHED (Bayes et al., 1998; Ezer et al., 1999; Vincent et al., 2001; Chao et al., 2003). These mutations include small and large deletions (Vincent et al., 2001; Lin et al., 2004), frameshifts (Huang et al., 2006), insertions (Huang et al., 2006; Tariq et al., 2007) and substitution (Paakkonen et al., 2001; Na et al., 2004; Sekiguchi et al., 2005; Tao et al., 2006; Fan et al., 2008). The

mutation described in this study is located in the amino terminal, extracellular domain, an essential hydrophilic domain required for proper function of ectodysplasin-A. The location of this mutation and nature of the amino acid change (Asp155Cys) strongly imply that this is the disease-causing mutation in this family. The same mutation has been reported to cause ectodermal dysplasia (Fan et al., 2008). This mutation may affect the three-dimensional structure of ectodysplasin-A, and thereby the localization of the protein or its interaction with its receptors.

In summary, we identified a missense mutation of the *EDA* gene involved in XLHED, and we described the phenotypic changes associated with this mutation in affected and carrier individuals. The results should be useful in prenatal diagnosis for the family examined and in expanding the phenotypes of *EDA* mutations to include speech problems.

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