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SHORT COMMUNICATION

A novel de novo frame-shift mutation of the *EDA* gene in a Chinese Han family with hypohidrotic ectodermal dysplasia

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Abstract Hypohidrotic ectodermal dysplasia (HED) is characterized by severe hypohidrosis, hypotrichosis, and hypodontia. It can be inherited in autosomal dominant, autosomal recessive, or X-linked patterns. Mutations in the *EDA* gene, which encodes ectodysplasin-A, are responsible for X-linked HED (XLHED). In the present study, we identified a Chinese Han family with XLHED. Direct DNA sequence analysis of the entire coding region and exon–intron boundaries of *EDA* identified a novel de novo mutation, c.573_574insT, in two affected males and one carrier female. Restriction fragment length polymorphism (RFLP) analysis showed that the mutation was not present in 200 controls. The 1-bp insertion mutation resulted in a frameshift, which causes premature termination of EDA polypeptide and truncation of the EDA protein. These results suggest that the c.573_574insT mutation of the *EDA* gene is a cause for XLHED in the family. To the best of our knowledge, this is the first de novo insertion mutation of *EDA* described for XLHED.

Keywords EDA · Frameshift mutation · RFLP · X-linked hypohidrotic ectodermal dysplasia

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Introduction

Hypohidrotic ectodermal dysplasia (HED) is found to occur worldwide, with an estimated incidence of 1 per 100,000 births (Zonana 1993). Ectodermal dysplasias typically affect the hair, teeth, nails, and/or skin. HED is primarily characterized by the partial or complete absence of certain sweat glands (eccrine glands), causing the lack of or diminished sweating (anhidrosis or hypohidrosis), heat intolerance, and fever, abnormally sparse hair (hypotrichosis), and the absence (anodontia or hypodontia) and/or malformation of certain teeth. It can be inherited in autosomal dominant, autosomal recessive, or X-linked patterns. However, X-linked HED (XLHED; OMIM 305100)

is the most common form of HED (Pinheiro and Freire-Maia 1994). If unrecognized, XLHED is one of the causes of fever of unknown origin, repeated bronchitis, and sudden death during infancy and early childhood (Zhang et al. 2003).

XLHED is caused by mutations in the *EDA* gene (Kere et al. 1996), which encodes ectodysplasin-A, a type II transmembrane protein of 391 amino acids. Ectodysplasin-A has a furin site, a collagen-like sequence in the linker region between the transmembrane helix and the tumor necrosis factor (TNF) ligand motif (Schneider et al. 2001; Wisniewski et al. 2003). Ectodysplasin-A is involved in the regulation of ectodermal morphogenesis. By specific binding to the ectodysplasin receptor (EDAR), a member of the TNF receptor family, EDA may activate the NF-κB and c-Jun N-terminal kinase pathways (Yan et al. 2000). Mutations in *EDAR* (Chassaing et al. 2006), *EDARADD* (Cui et al. 2002), or *NEMO* (Vinolo et al. 2006) are also reported to be associated with anhidrotic ectodermal dysplasia.

In this study, we studied a Chinese Han family with XLHED. Using linkage analysis, we mapped the disease locus to chromosome Xq12–q13, where the *EDA* gene is located. By direct DNA sequence analysis, we identified a novel de novo 1-bp insertion (c.573–574insT) in exon 4 of *EDA* that causes XLHED.

Subjects and methods

Study participants and the isolation of genomic DNA

We analyzed a Chinese Han family with XLHED (Fig. 1a). The proband (III-1) was a 21-year-old male (Fig. 1b). He had typical triad of the disorder, including hypohidrosis, hypotrichosis, and anodontia, and suffered from recurrent idiopathic fever, which was more frequent and more obvious in the summer and during eating. His scalp hair and eyelashes were sparse, thin, and dry, and his eyebrows were scanty to absent. Sparse hair distributions were also noted in the beard and pubic areas, axillae, and trunk. He also had characteristic facial appearance, including a prominent forehead, high and wide cheekbones and narrow lower half of the face, a depressed nasal bridge, large and conspicuous nostrils, thickened lips, and a large chin. Yellow-brown hyperpigmentation was observed around the periorbital area, nose, and mouth. He was found totally anodontia since his birth. However, his physical development was normal, and no abnormalities in his finger and toe nails were identified. His younger brother

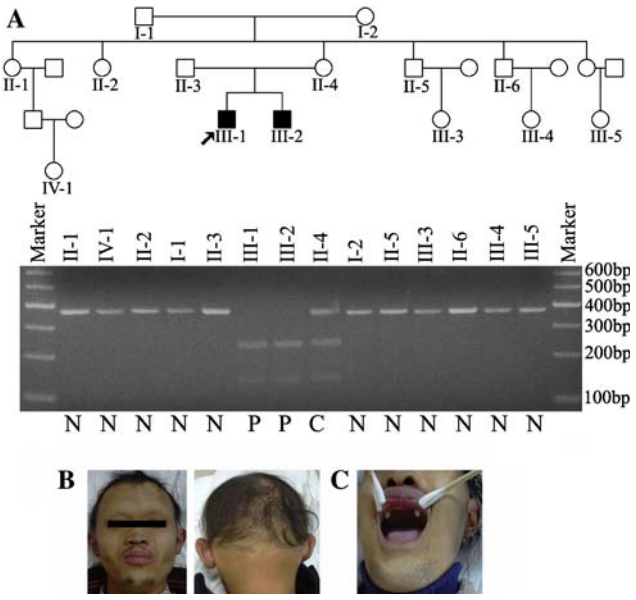

Fig. 1 **a** The pedigree structure and results of the restriction fragment length polymorphism (RFLP) analysis of a Chinese family with X-linked hypohidrotic ectodermal dysplasia (XLHED). RFLP analysis showed that the 1-bp insertion cosegregated with two affected individuals, III-1 and III-2, who were hemizygous for the c.573–574insT mutation. The female carrier (II-4) was heterozygous for the mutation, and other normal family members carried the wild type allele. Note that the wild type allele is represented by the 368-bp DNA band, and the mutant allele is shown as two bands of 237 and 132-bp in sizes, respectively. **b** Clinical features of the affected individual III-1 in the Chinese family (21-year-old male with hypohidrosis, hypotrichosis, and anodontia). The patient appeared to be prematurely aged. **c** Clinical features of the affected individual III-2 (19-year-old male with similar symptoms and clinical onsets as III-1, except that he has two conical-shaped teeth)

(III-2) had similar symptoms and clinical onsets, except that he had two conical-shaped teeth (Fig. 1c).

Informed consent was obtained from each participating family member or their legal guardian. Genomic DNA was extracted from the peripheral blood with Wizard Genomic DNA Purification Kit (Promega, Madison, WI).

Linkage analysis

The possible involvement of the *EDA* gene in the HED family was initially examined by linkage analysis with markers *DXS991* and *DXS986*. Genotyping was carried out using an ABI 3100 Genetic Analyzer and allele-typing was performed using the GeneMapper 2.5 software (Applied Biosystems, Foster City, CA).

DNA sequence analysis and mutation detection

The entire coding region and exon–intron boundaries of *EDA* were PCR-amplified and sequenced as de-

scribed previously (Wang et al. 2005). The primers used are shown in Table 1.

RFLP analysis

Since mutation c.573–574insT generated a novel *Nco* I restriction enzyme site (CCATGG) in exon 4 of the *EDA* gene, we PCR-amplified exon 4 and the flanking sequences from all family members and 200 normal controls. The PCR products were digested with Nco I (TaKaRa, Dalian, China) at 37°C for 4 h, and separated on a 2% agarose gel.

Paternal testing

To confirm the familial relationship of the mutation carrier with her parents, we performed genotyping analysis using a set of informative microsatellite markers from various chromosomes, including *D8S1179, D21S11, D7S820, D3S1358, D13S317, D16S539, D2S1338, D19S43, D18S51, D5S818, CSF1PO, AMEL, FGA, TPOX, TH01,* and *vWA*. Transmission analysis for each marker was then carried out.

Results

Linkage analysis of the Chinese XLHED family with two markers on chromosome X suggested that the *EDA* gene was responsible for the disease phenotype (data not shown). Direct DNA sequence analysis identified a novel 1-bp insertion, c.573–574insT, in exon 4 of *EDA* (GenBank accession number NM_001399) (Fig. 2). This insertion resulted in a frame-shift that causes replacement of the C-terminal 200 amino acids of EDA (starting with amino acid residue G_{192}) with a short polypeptide with 47 unrelated amino acid residues. The predicted mutant protein lacks the part of the collagen domain and the entire TNF-homology domain.

Direct DNA sequence and RFLP analyses showed that the 1-bp insertion co-segregated with HED in the family (Fig. 1a). Further RFLP analysis did not detect the c.573–574insT mutation in 200 normal controls. These results suggested that the c.573–574insT mutation of *EDA* is not a rare polymorphism, but a causative mutation for XLHED in the Chinese Han family.

We did not find the c.573–574insT mutation from individuals I-1 and I-2, but their daughter, individual II-4 in the family, carried the 1-bp insertion, and transmitted this mutation to her two sons (Fig. 1a). Paternal testing with multiple informative microsatellite markers from various chromosomes confirmed the familial relationship of individual II-4 with both parents. These results demonstrate that the c.573–574insT insertion is a de novo mutation.

Discussion

In this study, we identified a novel 1-bp insertion, c.573–574insT, in the *EDA* gene in a Chinese Han family with XLHED. The mutation occurred in two affected males as a hemizygous state, and in the carrier female as a heterozygous state. It was not present in other normal family members or 200 normal controls. Furthermore, the mutation arose de novo. These results strongly suggest that the c.573–574insT mutation

Table 1 Primers used for polymerase chain reaction (PCR) amplification of the human ectodysplasin-A (*EDA*) gene

Exon	Sequence (5′–3′)	Tm (°C); fragment (bp)
Exon1–1	Forward: AGAGGTCGTGAACGGCTGAGG Reverse: CGCAACTCTAGGTAGCAGCACAAC	54; 264
Exon1–2	Forward: GCCTGCTCTTCCTGGGTTTCTT Reverse: TCCCTGGTCCTGCCCTCTAAAT	54; 400
Exon2	Forward: GCTGGTTTTTTATGTTGGCTATGAC Reverse: CCACCATGCCCTACCAAGAAG	52; 266
Exon3	Forward: TTTGCAGTGTCTTGGGGATCC Reverse: GCAGGGAGAAGAACAAGGAAGAAT	53; 346
Exon4	Forward: CCGAGATCGTGCCACTGAACT Reverse: CCCCATCTCCACCGTTTGAA	53; 368
Exon5	Forward: TCAGGTGAGGGGAAAAGGAAGT Reverse: GGGCTGTGAGTGAAAACCGTC	52; 240
Exon6	Forward: AGGGGAGAGGGATCAGAATTG Reverse: AGGCTGGGTGATTATTTGGAG	50; 256
Exon7	Forward: TGCCTCGATTATTCTGACATGTACTG Reverse: CCCAAAGCAGGAAGTTAGCCATT	53; 300
Exon8	Forward: CCCCACCCTCTCTTTCCTCTCTTC Reverse: GGCTGCAACACCAATACACCTCAC	56; 412

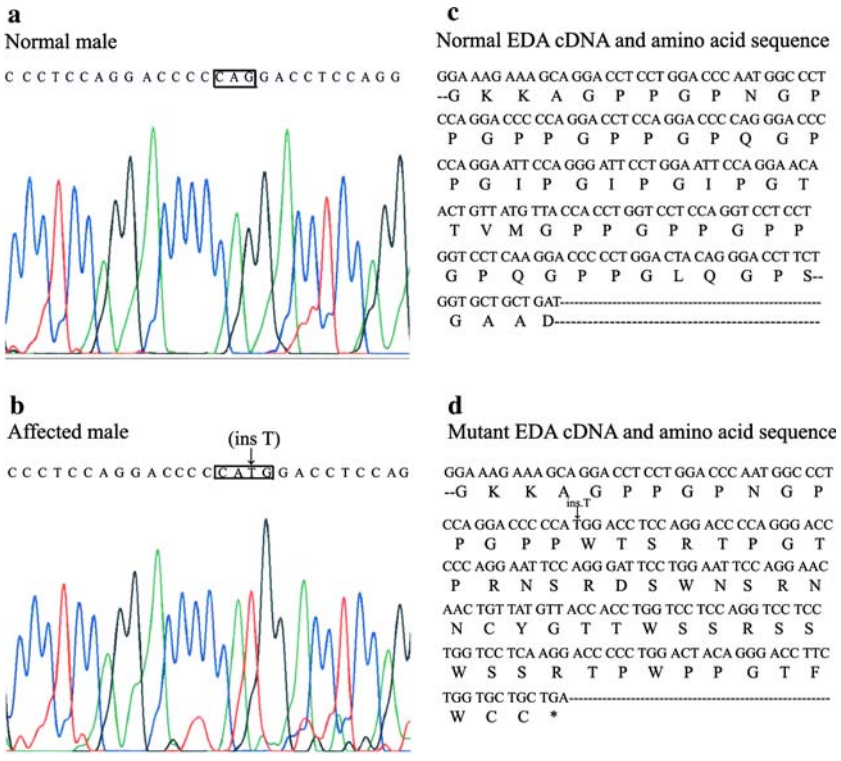

Fig. 2a–d Identification of a novel 1-bp insertion in the *EDA* gene. **a** DNA sequence of exon 4 of the *EDA* gene from a normal male (II-3 in Fig. 1a). **b** DNA sequence from an affected male (III-1) showing the c.573–574insT mutation. **c** Normal cDNA and protein sequences of the EDA collagen domain. **d** The c.573–574insT mutation resulted in a frame-shift, leading to replacement of the C-terminal 200 amino acids of EDA with an aberrant polypeptide with 47 unrelated amino acids. The insertion occurs at codon 192

causes the disease in the family, and it represents the first de novo insertion identified in the *EDA* gene.

The ectodysplasin-A protein contains several domains: a small N-terminal intracellular domain followed by the transmembrane domain, and a larger C-terminal extracellular domain, which contains a furin site, a collagen-like domain (containing 19 Gly-X-Y repeats), and a TNF homology domain. The furin domain functions as a cleavage site for a furin protease. The collagen domain is crucial for multimerization of EDA trimers. The TNF homology domain consists of 10 predicted anti-parallel β-sheets linked by variable loops, as in other members of the TNF family, and is necessary for the homotrimerization of ligands and its binding to the receptor (Ezer et al. 1999). The c.573–574insT insertion resulted in a frame-shift that truncates the EDA protein by 200 amino acid residues. The resultant mutant protein lacks the part of the collagen-like domain and the TNF-homology domain. Thus, the 1-bp insertion may significantly affect the function(s) of the EDA protein by disrupting two important functional domains.

Recently, one novel R65G mutation in the *EDA* gene was identified in a Mongolian family with only hypodontia, and without other HED manifestations (Tao et al. 2006), whereas another mutation G291R was reported in a Japanese family with hypohidrosis, hypotrichosis, and hypodontia (with only a primary central incisor and a permanent central incisor bud in the maxilla) (Sekiguchi et al. 2005), suggesting phenotypic heterogeneity of this syndrome. It is interesting to note that heterozygous mutation carriers of XLHED may have variable clinical features, displaying minor or moderate degrees of hypodontia, hypotrichosis, and hypohidrosis. In some families, the obligated carriers did not exhibit any clinical phenotype. The affected members of the family studied here displayed severe EDA symptoms of hypodontia (anodontia), hypotrichosis, and hypohidrosis, but these clinical features were not demonstrated in their heterozygous mother.

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