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*SK Naqvi*
*N Wasif*
*H Javaid*
*W Ahmad*

# Two novel mutations in the gene *EDAR* causing autosomal recessive hypohidrotic ectodermal dysplasia

**Authors' affiliations:**
*S.K. Naqvi, N. Wasif, H. Javaid, W. Ahmad,* Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

**Correspondence to:**
*Wasim Ahmad*
Department of Biochemistry
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad
Pakistan
E-mail: wahmad@qau.edu.pk

**Structured Abstract**

***Authors –*** Naqvi SK, Wasif N, Javaid H, Ahmad W

***Introduction –*** Hypohidrotic ectodermal dysplasia (HED) is a human heritable disorder characterized by sparse hair, reduced ability to sweat and hypodontia. The HED exhibits X-linked, autosomal recessive and autosomal dominant mode of inheritance. Mutations in four genes including *EDA*, *EDAR*, *EDARADD*, and *WNT10A* are known to cause hypohidrotic and anhidrotic ectodermal dysplasia.

***Materials and Methods –*** Genotyping of both affected and normal individuals of two consanguineous Pakistani families (A, B), showing autosomal recessive HED, was carried out using microsatellite markers linked to *EDAR* gene on chromosome 2q11-q13. To screen for mutations in the gene *EDAR*, all of its exons and splice junction were amplified and sequenced directly, using an automated DNA sequencer.

***Results –*** Genotyping using microsatellite markers analysis showed linkage of the two families to gene *EDAR* on chromosome 2q11-2q13. Subsequently, screening of all the 12 exons and splice junctions of gene *EDAR* revealed a novel missense mutation (c.1163T > C; p.Ile388Thr) in family A and a novel insertion mutation (c.1014insA; p.V339SfsX6) in family B.

***Conclusion –*** Our findings extend the body of evidence supporting the role of EDAR signaling pathway as a powerful regulator of development of ectodermal appendages.

**Key words:** gene *EDAR*; hypohidrotic ectodermal dysplasia; novel mutations

## Introduction

Hypohidrotic ectodermal dysplasia (HED, MIM 224900) is a rare congenital disorder characterized by abnormal development of hair (hypotrichosis), teeth (hypodontia), reduced ability of eccrine sweat glands (hypohidrosis), frontal bossing, flattened bridge of the nose with ozena, pointed chin, and thickness of lips. The HED exhibits X-linked, autosomal recessive, and autosomal dominant mode of inheritance. The X-linked HED results from mutations in ectodysplasin (*EDA*, MIM 300451) gene. Both autosomal forms of HED result from mutations in genes EDA-A1 receptor (*EDAR*, MIM 604095) and EDA-A1 receptor death domain (*EDARADD*, MIM 606603) (1, 2). The EDAR, a member of tumor necrosis

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factor (TNF) receptor family, is activated by its ligand EDA and uses EDARADD as an adaptor to build an intracellular NF-kB signal-transducing complex, which is necessary for normal development of ectodermal organs both in humans and in mice (3). Recently, it has been shown that mutations in gene *WNT10A* (MIM 257980), a member of Wnt-signaling pathway, result in various autosomal recessive forms of ectodermal dysplasias including onycho-odonto-dermal-dysplasia (OODD) and HED/EDA (4).

In this report, we investigated two Pakistani families with autosomal recessive form of HED. The families showed linkage to gene *EDAR* on chromosome 2q11-q13. Screening of the gene led to the identification of two novel disease causing mutations.

## Materials and methods

In the present study, we have presented two consanguineous Pakistani families (A and B) demonstrating autosomal recessive form of HED (Fig. 1). Approval of the study was obtained from Quaid-i-Azam University Institutional Review Board (IRB). Both the families contained three affected individuals each. The affected individuals of both the families showed typical clinical features of HED, including fine scalp hair, sparse to absent eyebrows and eyelashes, conical teeth, diminished sweating, dry skin, protruding prominent lips, and saddled-shaped nose (Fig. 2). Heterozygous carriers were clinically indistinguishable from unaffected normal individuals of the families.

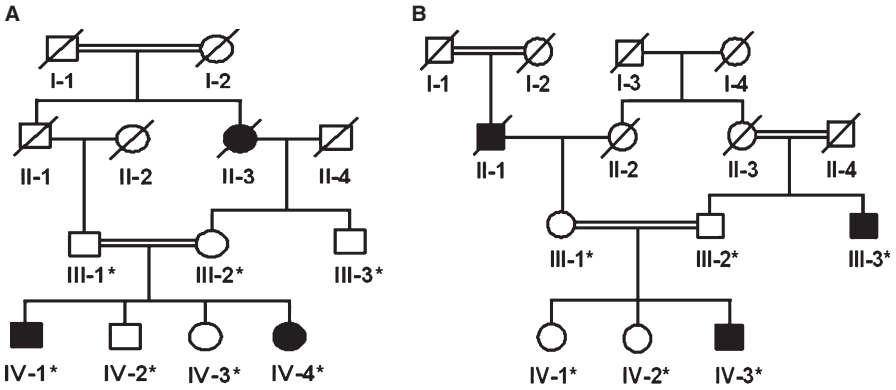


*Fig. 1.* Pedigrees of two Pakistani families (A and B) with autosomal recessive form of hypohidrotic ectodermal dysplasia. Affected men and women are indicated by filled squares and circles, respectively. Open symbols represent unaffected individuals. Symbols with star represent the samples available for the study. Double lines between figures are representative of consanguineous unions.

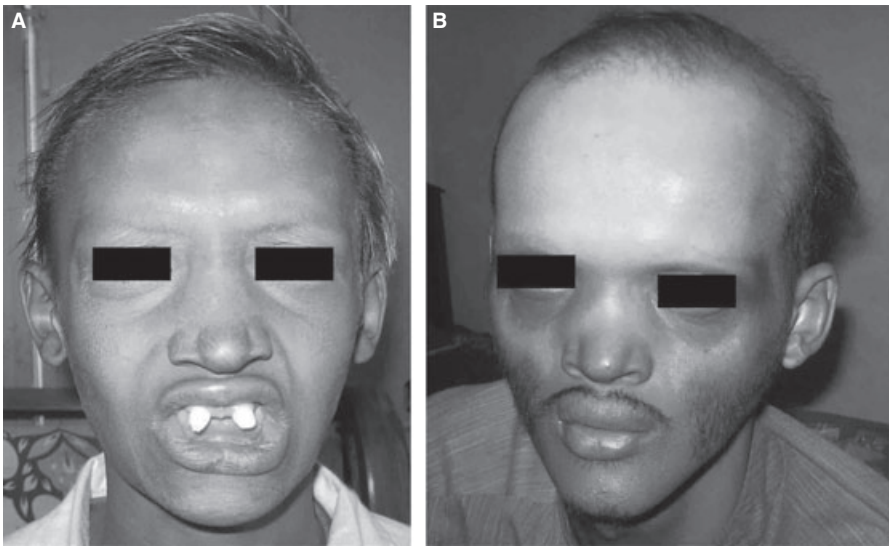


*Fig. 2.* Clinical findings in hypohidrotic ectodermal dysplasia. (A) An affected individual (IV-1) at 11 years of age in family A showing fine scalp hair, absent eyebrows and eyelashes, a flattened nose, prominent lips, permanent conical teeth, hyperpigmentation, and periorbital wrinkling. (B) An affected individual (IV-3) at 19 years of age in family B with sparse scalp hair, sparse beard and mustache, absent eyebrows and eyelashes, a saddle nose, periorbital wrinkling, prominent lips, and hyperpigmentation.

Genomic DNA was extracted from blood samples collected from two affected (IV-1, IV-4) and five unaffected individuals (III-1, III-2, III-3, IV-2, IV-3) of the family A and two affected (III-3, IV-3) and four unaffected (III-1, III-2, IV-1, IV-2) individuals of the family B using standard protocols. The PCR products were purified using the Rapid PCR Purification System (Marligen Biosciences, Ijamsville, MD, USA) and were sequenced in an ABI Prism 310 automated DNA sequencer, using the Big Dye Terminator Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA, USA) as described earlier (5).

## Results

Genetic linkage in both the families was established to *EDAR* gene (D2S1891, D2S340, D2S1889, D2S1893) on chromosome 2q11-q13. Twelve exons and splice-junction sites of the gene *EDAR* were PCR amplified from genomic DNA of affected and unaffected individuals of both the families. In family A, sequence analysis in the affected individuals revealed a novel missense mutation (c.1163T > C; p.Ile388Thr) in exon-12 of the gene. This missense mutation lies in a highly conserved death domain of EDAR substituting a conserved isoleucine with threonine residue. In family B, sequence analysis of exon-11 of the gene *EDAR* identified an insertion of A at nucleotide position 1014 (c.1014insA) leading to a frameshift and premature termination codon 16 bp downstream (p.V339SfsX6) in affected individuals (Fig 3). The two mutations were found in homozygous state in index patients and heterozygous state in obligate careers of the families. To ensure that the two novel mutations identified in the present families do not represent neutral polymorphism in the population, a panel of 200 unrelated unaffected ethnically matched control individuals was screened and the mutations were not identified outside the families.

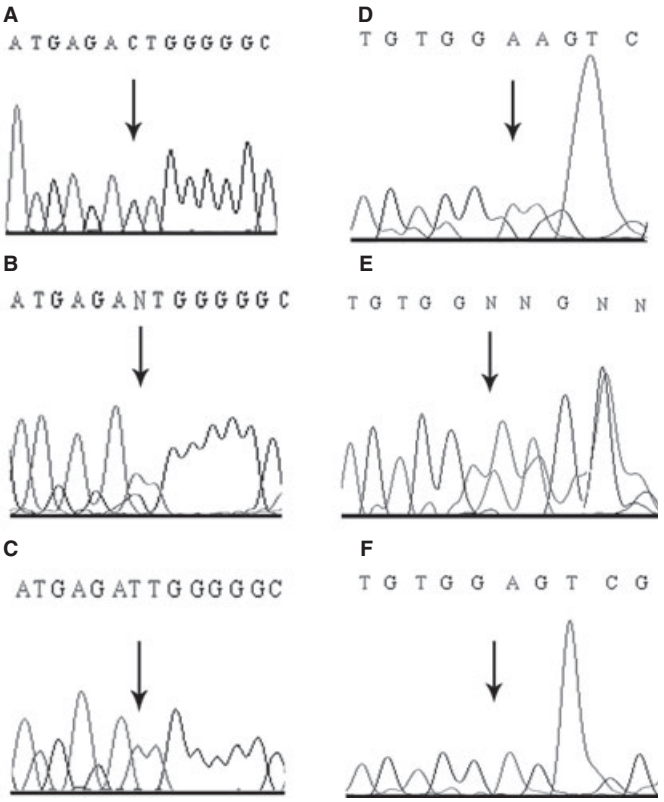


*Fig. 3.* Sequence analysis of two novel mutations c.1163T > C; p.Ile388Thr (left) and c.1014insA; p.V339SfsX6 (right) in the gene *EDAR*. The upper panels (A, D) represent the nucleotide sequences in the affected individuals, the middle panels (B, E) in the heterozygous carriers, and the lower panels (C, F) in the unaffected control individuals. Arrows indicate positions of the mutations.

## Discussion

Mutations in four genes including *EDA1*, *EDAR*, *EDARADD*, and *WNT10A* accounted for most of the cases with HED/EDA (4). The phenotypes associated with mutations in genes *EDA1*, *EDAR*, and *EDARADD* are consistent and indistinguishable; however, clinical expression of mutations in *WNT10A* gene is highly variable. The ectodysplasin A receptor (*EDAR*) gene encodes 448 amino acid protein, which is a member of TNF receptor family contains an N-terminal signal peptide, an extracellular domain, a single transmembrane region, and an intracellular death domain. According to Human Gene Mutation Database (HGMD, 2010), 33 mutations in the gene *EDAR* have been reported to date. In all such cases, it has been shown that mutant sequence variants in the gene *EDAR* result in HED with varying degree of abnormalities of teeth, hair, and eccrine sweat glands (5–14).

The missense mutation (p.Ile388Thr) identified in family A and insertion mutation (p.V339SfsX6) identified in family B, in the present study, are located in death domain (amino acids 209–448) of EDAR protein. The missense mutation is likely to obstruct the protein function by disturbing the EDA1-EDAR-EDARADD signaling pathway. However, the insertion mutation (p.V339SfsX6) causing frameshifting and introducing

premature termination codon in the spliced mRNA is very likely to result in aberrantly spliced *EDAR* mRNA, which may be degraded by the nonsense-mediated mRNA decay (NMRD) machinery (15). We were unable to observe difference in the severity of the phenotype in patient's carrying different mutations in the gene *EDAR* and no clear genotype–phenotype correlation emerged. However, severity of the phenotypes observed in our families was comparable to cases with autosomal recessive HED carrying mutations in the *EDAR* gene. Earlier, Chassaing et al. (6) also described that patients with two mutations suffered from anhidrosis and involved the abnormalities of hair, teeth, skin, and face, while patients with autosomal dominant HED suffered from hypohidrosis and display more variable expressivity. This study further supports the role of EDAR in regulating the development of ectodermal appendages.

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