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**Identification of a Novel Missense Mutation in *EDAR* Causing Autosomal Recessive Hypohidrotic Ectodermal Dysplasia with Bilateral Amastia and Palmoplantar Hyperkeratosis**

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**CONFLICT OF INTEREST DISCLOSURE**

The authors declare no conflict of interest.

Ectodermal dysplasias (EDs) are a large group of heritable complex conditions with more than 200 members and common clinical characteristics of anomalies of the hair, teeth, nails, and sweat glands with or without involvement of other organs <sup>1</sup>.

Anhidrotic or hypohidrotic ectodermal dysplasia (EDA/ HED) is the most common form of EDs which is characterized by the clinical triad of hypotrichosis (sparse hair), abnormal or missing teeth (anodontia or hypodontia), and deficient sweating (hypohidrosis or anhidrosis) <sup>2</sup>. Different modes of inheritance have been described for HED. X-linked HED (OMIM: 305100) is caused by mutations in ectodysplasin A gene (*EDA1*), whereas mutations in the EDA receptor (*EDAR*) and EDAR-associated death domain (*EDARADD*) genes result in autosomal dominant (OMIM:129490) and autosomal recessive (OMIM: 224900) forms <sup>3</sup>. These genes are involved in the NF- $\kappa$ B signaling pathway <sup>4</sup> and necessary for normal development of ectodermal organs both in human and mice <sup>5</sup>. Distinction of different forms of HED is clinically impossible and requires molecular analysis. Mutations in two other genes, *TRAF6* (TNF receptor-associated factor 6) and *WNT10A* (wingless-type MMTV integration site family, member 10A) have also been shown to cause HED <sup>3,6</sup>.

Using whole exome sequencing (Materials and Methods, see Supporting Information), we investigated the genetic basis of HED in an extended consanguineous Persian kindred in which the disease followed a pattern of autosomal recessive inheritance.

The female proband (II-1, Fig. 1.a), 16 years of age, was born to consanguineous healthy parents. The initial features included thin and sparse hair, lack of sweating and lacrimation, and frequent high fevers. On physical examination, she had pointed chin, sunken cheeks,

saddle-shaped nose, thick everted lips, conical teeth and hypodontia, dry skin, and absent limb and axillary hairs (Fig. 1). Lichenified eczematous plaques were noted on her back (Fig. 1.e). On thoracic ultrasonography, she, and her younger sister, had bilateral absence of breast development (amastia) whereas the pectoralis muscles were well-developed. The other unusual clinical finding was palmoplantar hyperkeratosis (Fig. 1.f-g). The sister (II-3) and male cousin (II-5) of the proband were also affected by HED with the same clinical features. Clinical findings are summarized in table 1. The growth and intellectual development of all patients were normal.

Whole exome sequencing of the main proband (II-I, Fig. 1.a) identified a novel homozygous missense mutation in *EDAR* (Fig. 2.a); c.338G>A (p.C113Y). This mutation replaces a highly conserved small cysteine with a large tyrosine at the position 113 (Fig. 2.b), which PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) predicts would be probably damaging (score=0.998). This mutation was absent in 150 control chromosomes. The other patients (II-3 and II-5) were homozygous for this mutation. In addition, all patients were homozygous for a non-synonymous polymorphism, c.1037C>T (p.T346M, rs150887314), resulting in a conserved polar threonine to nonpolar methionine substitution at 346<sup>th</sup> residue (Fig. 2.b), which was predicted to be probably damaging (score=1.000) by PolyPhen-2. Parents and healthy siblings in both families were heterozygous for the identified mutation and polymorphism.

The ectodysplasin A receptor gene (*EDAR*) is located on chromosome 2q11-q13 and contains 12 exons spanning over 94.9 kb. *EDAR* protein is a member of the tumour-necrosis factor receptor (TNFR) family that has a crucial role in the development of organs, especially those of ectodermal origin such as hair, glands, nails, teeth, and scales<sup>7</sup>. This protein is involved in the regulation of cell fate and morphogenesis during the development of ectoderm-derived structures. *EDAR* protein contains extracellular TNFR domains and an intracellular death domain (DD) region<sup>8</sup>. p.C113Y (c.338G>A) is located within the ligand-binding domain of *EDAR* and disrupts formation of disulfide bridge with a cysteine at position 93. This extracellular domain has been shown to play a critical role in specifically binding to the *EDAR* ligand, EDA-A1<sup>9</sup>.

The identified polymorphism, c.1037C>T (p.T346M, rs150887314), is located upstream of the death domain which is the potential site for interaction with *EDARADD* and other proteins. and involves in *EDAR*'s signalling<sup>5</sup>.

This is the second molecular study of a patient with HED and absence of breasts. The reported patient was an 18 year old female from Lebanon, with a homozygous *EDAR*

mutation IVS9+1G>A, who presented with the typical features of HED as well as absence of breasts <sup>10</sup>. She had never menstruated, whereas II-1 underwent normal menstruation. The other clinical differences were hyperconvex nails and hoarse voice in the Lebanese case.

Very recently, role of a unique TNF pathway in mammary gland morphogenesis has been identified. The authors suggest the EDA/EDAR/NF- $\kappa$ B pathway as a driver of hormone-independent ductal growth and branching <sup>11</sup>. Therefore, we can speculate that *EDAR* mutations can impact the breast development through this pathway and lead to mammary malformations.

As the cost of exome sequencing is tumbling, the application of this method is now very cost-effective, and indeed (3-4 times) cheaper, than the classical approach of linkage analysis followed by Sanger sequencing of candidate genes in the identified locus. Whole exome sequencing offers the advantage of directly analyzing the genome for homozygous variants thereby combining previous mapping and sequencing efforts. In addition, other genes of interest can be analyzed for variants that might cause the disease or influence its outcome. Accordingly, we checked the sequences for variants in other phenotype related genes including *EDA1*, *EDARADD*, *WNT10A*, *TRAF6*, but did not identify any pathogenic changes.

In summary, using whole exome sequencing, we describe a novel homozygous missense mutation in *EDAR* causing autosomal recessive HED associated with absence of breasts and palmoplantar hyperkeratosis.

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## TABLES

**Table 1: Review of Clinical Features of Our Patients**

Clinical Feature	II-1	II-3	II-5
Sex	female	female	male
Age (years)	16	10	13
Sparse eyelashes and/or eyebrows	+	+	+
Sparse axillary and/or pubic hair	+	+	+
Abnormal hair pattern	+	+	+
Hyperhidrosis (dry skin and overheating)	+	+	+
Dry skin	+	+	+
Palmoplantar hyperkeratosis	+	+	+
Erythematous atrophic patches on the face	-	-	-
Reduced papillae of the tongue and/or smooth tongue	-	-	-
Congenital absence of nails - Dystrophic finger/ toe nails	-	-	-
Absence of breasts	+	+	-
Anodontia, hypodontia or missing teeth	+	+	+
Saddle nose: flattened bridge of the nose with ozena	+	+	+
Recurrent respiratory infections	-	+	-



