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Mutations in *EDA* and *EDAR* Genes in a Large Mexican Hispanic Cohort with Hypohidrotic Ectodermal Dysplasia

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Dear Editor:

Ectodermal dysplasias (ED) encompass nearly 200 different genetic conditions identified by the lack, or dysgenesis, of at least two ectodermal derivatives, such as hair, nails, teeth, and sweat glands. Hypohidrotic/anhidrotic ED (HED) is the most frequent form of ED and it can be inherited as an X-linked (XL)-HED (MIM 305100), autosomal recessive (AR)-HED (MIM 224900), or autosomal dominant (AD)-HED (MIM 229490) condition. HED is caused by mutations in any of the three ectodisplasin pathway genes: ectodisplasin (*EDA*), which encodes a ligand for the second gene, the EDA receptor (ectodysplasin A-receptor, *EDAR*), and *EDARADD*, an intracellular signaling for this pathway. HED is characterized by a triad of clinical features including absent or diminished eccrine sweat glands, missing and/or malformed teeth, and thin, sparse hair. It also includes dryness of the skin, eyes, airways, and mucous membranes, as well as other ectodermal defects and, in some cases, fever, seizures, and rarely, death.

XL-HED is caused by mutations in the *EDA* gene, located on chromosome Xq12-q13.1, which encodes a signaling molecule of the tumor necrosis factor (TNF) superfamily. AR- and AD-HED are caused by mutations in the *EDAR* gene, located on chromosome 2q11.q13 or the EDAR-Associated Death Domain encoding gene, *EDARADD*, located on chromosome 1q42-q43[1].

Several mutations in the *EDA*, *EDAR*, and *EDARADD* genes have been described as causing HED in different populations. The XL-HED form is the most common and is responsible for 90% of all HED cases[2-6].

The three forms of HED are clinically indistinguishable. To date, a comprehensive evaluation of HED in the Mexican Hispanic population has not been undertaken. In the present study, we aimed to characterize the mutations in *EDA*, *EDAR*, and *EDARADD* genes present in Mexican Hispanic patients with HED.

Male and female patients (35 families) from different geographical regions of Mexico with features suggestive of HED were enrolled in the study (Fig. 1). Index cases and their parents were screened for missing or malformed teeth, thin or sparse hair, and nail changes; all subjects answered questions about sweating, heat intolerance, fever, seizures, and family history of siblings deceased due to unknown fever

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Fig. 1. Mutations in ectodisplasin (*EDA*) and ectodysplasin A-receptor (*EDAR*) genes cause hypohidrotic/anhidrotic ED (HED). (A) Index case (arrow) from X-linked pedigree shows multiple male affected offspring. Two of them died of fever during the first month of birth. Mutation analysis revels a homozygous c.1049G>A, p.G350D mutation. Male and female index cases (arrows) from a family with Autosomal Recessive HED. This patient has an heterozygous mutation in Exon 3 c.212 G>A, p.71 C>Y. The clinical findings of these two patients with HED show a sparse hair, frontal bossing, saddle nose, periocular hyperpigmentation and enlarged lips are evident. In our cohort there was no difference between the two forms of inheritance. White symbols indicate unaffected indiviudals, black squares and circles denote affected, dots indicate obligate carried females, the propositus are in arrows. (B) X-linked-HED, clinical variability in male patients analayzed. They show the clasical triad of HED, dental abnormalities, anhidrosis/hypohirosis and thin sparse hair. Some of them has normal hair appaerance and recessive HED female patient with minor clincal aspect of HED.

during the first year of life.

The protocol was approved by the ethics and research committees of the University Hospital of the Autonomous University of Nuevo Leon (DE 12-007) and Yale University School of Medicine. After obtaining informed consent, peripheral blood of patients and family members was drawn and DNA was extracted using a standard phenol-chloroform protocol.

All exons of *EDA*, *EDAR*, and *EDARADD* genes were screened using specific primers as described by Cluzeau et al.[2], 2011; polymerase chain reaction amplification was performed using Amplitaq Gold (Applied Biosystems, Foster City, CA, USA) or Kapa 2G Fast polymerase (Kapa Biosystems, Wilmington, MA, USA).

Sequencing reactions were carried out using Big Dye Terminator v3.1 (Applied Biosystems) and resulting DNA fragments were sequenced using the 3130xl Genetic Analyzer (Applied Biosystems). Sequencing quality control and assembly were performed using Sequence Scanner V.1.0 (Applied Biosystems) and Phred, Phrap, and Consed or Sequencher software (Gene Codes, Ann Arbor, MI, USA)[7].

A total of 40 patients from 35 families were tested for *EDA*, *EDAR*, and *EDARADD* mutations. Sequence analysis of all coding exons of *EDA*, *EDAR*, and *EDARADD* genes identified 16 different mutations in 21 (60%) families (Table 1)[3,4,8-10].

Sixteen different mutations were present; fifteen mutations were found in the *EDA* gene, 5 had previously been reported, and 10 were novel. One known mutation was found in the *EDAR* gene.

No mutations were found in the *EDARADD* gene in the remaining kindreds (14) analyzed. *WNT10A* analysis was not performed.

Upon review of the clinical features in the affected patients and female carriers, there was no phenotypic correlation with genotype (data not shown). Ten obligate female carriers displayed dental abnormalities (one missing tooth) that were independent of specific mutations; we speculate that this may be due to random X-inactivation.

In studying a Mexican Hispanic Cohort (see results Table 1), we have found that 60% bear mutations in *EDA* or *EDAR*, but not in *EDARDD*. Of the 16 identified inherited mutations, ten were novel. Of these novel mutations in *EDA*, p.E36ter is a nonsense mutation that produces a truncated protein; the other nine missense mutations lie in conserved regions of the protein including the furin cleavage site and the TNF-like domain where previous disease-causing mutations have been identified. The nonsense mutations p.E36ter and p.Q128ter produce a truncated form of the ectodisplasin protein, subject to nonsense-mediated decay[3]. Missense mutations p.R150K, p.R155C, and p.R156H disrupt the furin cleavage site, whereas p.G299R, p.C346Y, p.C346W, and p.T378M are predicted to disrupt protein function[4-6].

Two novel deletion mutations were present in this population: c.del 546-581 (p.183-194 del) and c.887-900 del, p.297-301delFSx4 as one splice site mutation, c.793+1 G>C.

Thirty-three mutations, mainly missense mutations, *EDAR* gene have previously been reported[11]. We found a homozygous c.G212A, p.C70Y mutation in exon 3 of *EDAR* in

Table 1. Mutations identified in 35 Mexican Hispanic families with hypohidrotic ectodermal dysplasia (ED)

Family	Exon	Coding sequence variant	Protein change	Mutation type	Reference
1	EDA-1	**c.106G>T**	**p.E36Ter**	Nonsense	
2	EDA-1	c.382C>T	p.Q128ter	Nonsense	Schneider et al.[3]
3	EDA-3	**c.448G>A**	**p.E150K**	Missense	
4	EDA-3	c.463C>T	p.R155C	Missense	Vincent et al.[4]
5	EDA-3	c.467G>A	p.R156H	Missense	Monreal et al.[9]
6	EDA-4	**c.Del 546-581**	**p.183-194del**	Deletion	
7	EDA-5	**c.574G>C**	**p.G192R**	Missense	
8	EDA-6	**c.793+1 G>C**		Splice site	
9	ED-A7	**Del 887-900**	**p.297-301delFSx4**	Deletion	
10	EDA-8	**c.894G>C**	**p.G299R**	Missense	
11	EDA-8	c.911A>G	p.Y304C	Missense	RamaDevi et al.[10]
12	EDA-9	**c.1037G>A**	**p.C346Y**	Missense	
13	EDA-9	**c.1038C>G**	**p.C346W**	Missense	
14	EDA-9	**c.1049G>A**	**p.G350D**	Missense	
15	EDA-9	c.1133C>T	p.T378M	Missense	Schneider et al.[3]
16	EDAR 3/11 AR-HED*	c.212 G>A	p.C71Y	Missense	Moya-Quiles et al.[8]

*Autosomic recessive hypohidrotic/anhidrotic ED (HED). *EDA* ten novel mutations are show in bold. Fifteen mutations in different domains of the *EDA* gene were found. Four non-related families have the same recurrent mutation p.G3650D in the tumor necrosis factor domain.

an affected father-daughter pair with AR HED. This mutation lies within the ligandbinding domain of EDAR and is crucial for binding to the ligand[8]. A novel recurrent mutation p.G350D was found in four kindred, and the previously reported p.R155C was found in three kindred[3]. This suggests possible independent founder mutations in the Mexican Hispanic population.

These first findings in the Mexican Hispanic population expand the mutation spectrum in *EDA* and *EDAR* in HED, and confirm the clinical diagnosis in a large cohort. Recurrent mutations suggest possible founder mutations that may aid in genetic counseling and prenatal diagnosis in at-risk pregnancies. We also found that newborn febrile seizures are a significant cause of perinatal lethality, with 8 deaths in our cohort. An additional benefit of our study is the identification of potential candidates for replacement therapy with recombinant EDA protein (Edimer Pharmaceuticals, Cambridge, MA, USA)[12].

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