Turkish Ectodermal Dysplasia Cohort: From Phenotype to Genotype in 17 Families

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Keywords

Ectodermal dysplasia · *EDA* · *EDAR* · *EDARADD* · *WNT10A*

Abstract

Hypohidrotic or anhidrotic ectodermal dysplasia (HED/EDA) is characterized by impaired development of the hair, teeth, or sweat glands. HED/EDA is inherited in an X-linked, autosomal dominant, or autosomal recessive pattern and caused by the pathogenic variants in 4 genes: *EDA*, *EDAR*, *EDARADD*, and *WNT10A*. The aim of the present study was to perform molecular screening of these 4 genes in a cohort of Turkish individuals diagnosed with HED/EDA. We screened for pathogenic variants of *WNT10A*, *EDA*, *EDAR*, and *EDARADD* through Sanger sequencing. We further assessed the clinical profiles of the affected individuals in order to establish phenotype-genotype correlation. In 17 (63%) out of 27 families, 17 pathogenic variants, 8 being novel, were detected in the 4 well-known ectodermal dysplasia genes. *EDAR* and *EDA*

variants were identified in 6 families each, *WNT10A* variants in 4, and an *EDARADD* variant in 1, accounting for 35.3, 35.3, 23.5, and 5.9% of mutation-positive families, respectively. The low mutation detection rate of the cohort and the number of the *EDAR* pathogenic variants being as high as the *EDA* ones were the most noteworthy findings which could be attributed to the high consanguinity rate.

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Ectodermal dysplasias (EDs) belong to a large clinically and genetically heterogeneous and growing group of disorders characterized by the impaired development of 2 or more ectoderm-derived structures, involving hair, teeth, nails, or exocrine glands [Freire-Maia, 1977; Lamartine, 2003; Bohring et al., 2009; Visinoni et al., 2009]. Hypohidrotic or anhidrotic ectodermal dysplasia (HED/EDA), the most frequent form of ED, is characterized by a clinical triad comprising abnormalities of

the hair (hypotrichosis), teeth (anodontia, hypodontia/oligodontia, misshapen teeth), and sweat glands (anhidrosis or hypohidrosis) [Cluzeau et al., 2011]. Dysmorphic features (frontal bossing, periorbital wrinkling and hyperpigmentation, depressed nasal bridge, and prominent lips) and mammary agenesis may also be associated with HED/EDA [Cluzeau et al., 2011; Svendsen et al., 2014].

HED is predominantly inherited in an X-linked pattern (XLHED; OMIM 305100) [Chassaing et al., 2010; Cluzeau et al., 2011]. XLHED is due to mutations in the *EDA* gene (ectodysplasin-A), which maps to Xq12q13.1 and encodes ectodysplasin (*EDA*; OMIM 300451), a member of the tumor necrosis factor (TNF) family [Bayés et al., 1998]. Autosomal dominant (AD; OMIM 129490) and autosomal recessive (AR; OMIM 224900) forms of HED/EDA are encountered less frequently and result from mutations in the *EDAR* or in the *EDARADD* genes [van der Hout et al., 2008; Plaisancié et al., 2013]. Moreover, recently, it has been shown that mutations in the *WNT10A* gene are responsible for a broad spectrum of autosomal recessive ED [Cluzeau et al., 2011]. *EDAR*, encoding the EDA receptor, maps to 2q11q13 (OMIM 604095), and *EDARADD*, encoding the EDAR-associated death domain gene, maps to 1q42q43 (OMIM 606603). As EDA, both of the proteins encoded by these genes belong to the TNF-like EDA signaling pathway [Headon et al., 2001; Cui and Schlessinger, 2006]. Pathogenic variants in these 3 genes (*EDA*, *EDAR*, and *EDARADD*) result in indistinguishable phenotypes since the involved gene products all participate in the EDA-NF- κ B signaling pathway, which is necessary for the initiation, formation, and differentiation of the skin appendages [Cluzeau et al., 2011; Svendsen et al., 2014]. In contrast, mutations in the *WNT10A* gene (OMIM 606268), encoding a protein that participates in the WNT/ β -catenin/Lef-1 signaling pathway, have been shown to cause a highly variable phenotype, ranging from isolated tooth agenesis to ED syndromes such as odonto-onycho-dermal dysplasia (OODD; OMIM 257980) and Schöpf-Schulz-Passarge syndrome (SSPS; OMIM 224750) [Adaimy et al., 2007; Bohring et al., 2009; Cluzeau et al., 2011; Kantaputra et al., 2014]. The classic Wnt/ β -catenin signaling pathway has been shown to play an essential role in odontogenesis, ectodermal development, and epidermal homeostasis [Tardieu et al., 2017].

The aim of the present study was to perform molecular screening to identify pathogenic variants in the *EDA*, *EDAR*, *EDARADD*, and *WNT10A* genes in a cohort of clinically diagnosed HED/EDA Turkish patients.

Patients and Methods

Patients

A total of 33 patients from 27 families with a clinical diagnosis of HED/EDA were included in the study. All patients were evaluated by expert clinical geneticists in the Medical Genetics Department, Istanbul Medical Faculty, Istanbul University. At least 3 generation pedigrees were constructed to define possible mode of inheritance, and further individuals were invited for clinical and dental evaluation.

Mutation Analysis

Genomic DNA was isolated from peripheral blood of the index patients and family members using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche, Basel, Switzerland). Primers were designed to cover all coding exons and exon-intron boundaries of *EDA* (NM_001399.4), *EDAR* (NM_022336.3), *EDARADD* (NM_145861.2), and *WNT10A* (NM_025216.2). Sequencing primers are available from the authors upon request. Bidirectional DNA sequencing was performed in the DNA from index patients using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems, Bedford, MA, USA). Identified variants were then screened in additional family members for segregation validation. One hundred control chromosomes from a healthy Turkish population were checked for the presence of all novel variants identified in this study. Population genetic data from the 1000 Genomes (<http://grch37.ensembl.org/index.html>), Database of Single Nucleotide Polymorphisms (dbSNP; <https://www.ncbi.nlm.nih.gov/snp>), and ExAC (<http://exac.broadinstitute.org/>) databases were also used as controls to rule out population polymorphisms. Domain information for *EDA*, *EDAR*, *EDARADD*, and *WNT10A* was retrieved from Uniprot (<http://www.uniprot.org/>). In silico analyses to predict the pathogenicity of the novel variants were performed using the PolyPhen-2 [Adzhubei et al., 2010] and MutationTaster2 [Schwarz et al., 2014] programs.

Results

Thirteen out of the 27 families were consanguineous. Pedigree analysis suggested probable autosomal recessive inheritance in 17 families, probable X-linked recessive inheritance in 8 families, and probable autosomal recessive or X-linked recessive inheritance in 2 families. No variants were found in 10 families, although phenotypic features of the affected patients are typical for ED (online suppl. Table 1; for all suppl. material, see www.karger.com/doi/10.1159/000499325). One patient who had a cleft lip and palate was clinically diagnosed with cleft lip/palate-ectodermal dysplasia syndrome (CLPED; OMIM 225060), but no mutation was detected in the related *PVRL1* gene. Whole exome sequencing is planned for these 10 families to elucidate the molecular basis of the disease. Novel genes may be associated with their etiology.

Fig. 1. Domains of ED-related genes (*WNT10A*, *EDA*, *EDAR*, and *EDARADD*) and the mutational distribution. Novel mutations identified in this study are in bold print.

In 17 (63%) of the 27 families, variants were detected in at least 1 of the 4 known ED genes. Mutations and phenotypic features of the mutation-positive families are summarized in Table 1. The *EDAR* and *EDA* pathogenic variants were identified in 6 families each, the *WNT10A* in 4, and the *EDARADD* in 1, accounting for 35.3, 35.3, 23.5, and 5.9% mutation-positive families, respectively. The pedigrees of the mutation-positive families are shown in online supplementary Figure1.

EDAR

Five novel *EDAR* mutations were identified in 6 index patients from 6 families and the variants segregated consistent with an autosomal recessive inheritance. In 5 families, mutations were at homozygous state and in 1 at compound heterozygote state. Three families were consanguineous. The remaining 3 families were apparently not consanguineous, but the parents of each index patient originated from the same villages, suggesting possible consanguinity. The c.276–277insGCC change was predicted to be disease-causing by MutationTaster. The remaining alterations in the *EDAR* gene were missense variants predicted to be possibly or probably damaging as well as disease-causing according to in silico analyses. The c.71C>A (p.Ala24Asp) variant, detected in 3 index

patients, resides in exon 3 and is predicted to affect the signal peptide region of the corresponding protein. The c.276–277insGCC and c.245G>A variants are located in an extracellular cysteine-rich domain and are assumed to alter ligand binding [Zonana and Huttner, 2016]. The c.1011T>G and c.1012G>A variants in exon 11 detected in the *EDAR_F4* proband correspond to the intracellular domain of the protein and were found to be in the transposition according to segregation analysis (Fig. 1) confirming compound heterozygosity.

All patients carrying *EDAR* variants presented with hypo/anhidrosis, sparse hair, and facial dysmorphism to varying degrees, comprising periorbital wrinkling and hyperpigmentation. They also exhibited missing primary and permanent teeth and conical permanent incisors. The mean number of congenitally missing permanent teeth was 21 (range 17–26). Parents carrying these variants in the heterozygous state were clinically evaluated and found to exhibit no ectodermal phenotype.

EDA

A total of 6 variants were identified in *EDA*: 5 missense variants and 1 variant leading to the loss of a stop codon. One missense variant, c.1156G>C (p.Gly387Arg), was novel and predicted to be probably damaging and dis-

Fig. 2. Clinical photographs and radiographs. Frontal facial view (1), intraoral view (2), and panoramic radiograph (3). **A, B** WNT10A_F2 siblings showing normal primary dentition and severely affected permanent dentition. **C** EDA_F3 proband showing facial dysmorphism and severe oligodontia. **D, E** EDARADD_F1 siblings showing severe facial dysmorphism and severe oligodontia. **F** EDAR_F5 proband showing moderate oligodontia.

ease-causing by in silico analyses. c.1156G>C resides in the TNF-like domain of the gene and is assumed to impair receptor binding (Fig. 1). The c.463C>T [Monreal et al., 1998], c.756T>G [Clauss et al., 2010], c.467G>A [Clauss et al., 2010], c.466C>T [Clauss et al., 2010], and c.1174T>C [Yang et al., 2015] variants identified in the remaining 5 patients had been reported previously.

All EDA mutation-positive affected males had the classic XLHED phenotype, including severe hair, sweat gland, and teeth anomalies (Fig. 2). Three of the 6 obligate carrier females displayed mild teeth and hair anomalies, and 3 had no symptoms. The mean number of permanent congenitally missing teeth in affected males was 21.6 (range 12–27).

WNT10A

A total of 5 different pathogenic variants in WNT10A, 2 being novel, were identified in 5 patients from 4 families. Three known variants (c.433G>A, c.682T>A, and c.310C>T) were missense [Bohring et al., 2009; Cluzeau et al., 2011; Yang et al., 2015]. The WNT10A_F4 patient showed compound heterozygosity for 2 variants, 1 missense (c.86T>G), and 1 frameshift insertion (c.103–104-insGCCTGCT). The c.86T>G variant is located in exon 1, which encodes for the signal peptide, and the c.103–104-insGCCTGCT change in exon 1 is assumed to encode a truncated protein (Fig. 1). Both variants were predicted to

be disease-causing and/or possibly damaging by in silico analyses.

Patients with WNT10A pathogenic or possibly pathogenic variants did not have facial features such as periorbital wrinkling or hyperpigmentation (Fig. 2). Hair sparsity was milder when compared with the patients who have mutations in other ED/HED genes, while the severity of hypo/anhydrosis did not differ. The mean number of permanent congenitally missing teeth was 19.2, with permanent tooth agenesis being more severe than primary tooth agenesis.

EDARADD

Sequencing of the EDARADD gene led to the identification of only 1 previously reported variant [Headon et al., 2001] in 2 siblings born to first-degree cousins. The p.Glu152Lys (NM_145861) variant was reported as p.Glu142Lys (NM_080738) by Headon et al. [2001] due to different protein isoforms being used. Both siblings exhibited severe oligodontia of the primary and permanent teeth. They also exhibited an abnormal crown morphology in the primary and permanent dentition as well as skeletal Class III malocclusion due to maxillary hypoplasia. The siblings showed severe facial dysmorphism, sparse hair, dry skin, and spoon-shaped nails. The male sibling had anhidrosis, and the affected female sibling had hypohidrosis (Fig. 2).

Table 1. Phenotype and genotype of HED/EDA cohort

Family No.	Gender	Cons	Hair	Sweating/skin	Nail	Teeth	Other findings	Genomic change	Zygosity	Protein change	Exon	Predicted effect	Mutation type	Variant classification	References
EDA_F1	M	-	++	A	-	O+, CI	FD, hyperkeratosis	c.463C>T	Hom	p.Arg155Cys	2	Impaired furin cleavage	Missense	Pathogenic	Monreal et al., 1998
EDA_F2	M	-	+	H, D+	-	O, CI	FD	c.756T>G	Hom	p.His252Gln	6	Impaired receptor binding	Missense	Pathogenic	Clauss et al., 2010
EDA_F3	M	-	++	H, D	-	O, CI	FD	c.467G>A	Hom	p.Arg156His	2	Abolished furin cleavage	Missense	Pathogenic	Clauss et al., 2010
EDA_F4	M	+	+	-	-	O, TI	FD	c.1156G>C	Hom	p.Gly386Arg	8	Impaired receptor binding	Missense	Likely pathogenic	Novel
EDA_F5	M	-	+	A, D+	+	O+, CI	FD	c.466C>T	Hom	p.Arg156Cys	2	Abolished furin cleavage	Missense	Pathogenic	Clauss et al., 2010
EDA_F6	M	+	++	A, D+	-	O+, CI	FD	c.1174T>C	Hom	p.Ter392Gln	8	Impaired receptor binding	Stop lost	Pathogenic	Hashiguchi et al., 2003
EDAR_F1	M	+	+	D	-	O+, CI	FD, palmar hyperhidrosis	c.71C>A	Hom	p.Ala244Asp	3	Altered subcellular location	Missense	Likely pathogenic	Novel
EDAR_F2	M	-	++	A, D	-	O+, CI, Class III mal	FD, psychomotor retardation	c.276-277insGCC	Hom	p.92insAla	4	Possibly affecting ligand binding	Insertion	Likely pathogenic	Novel
EDAR_F3	F	+	+	H, D+	-	O+, CI, Class III mal	FD, palmar hyperhidrosis	c.71C>A	Hom	p.Ala244Asp	3	Altered subcellular location	Missense	Likely pathogenic	Novel
EDAR_F4	F	+	+	H	+	O+, CI	FD, clinodactyly, postaxial polydactyly	c.1011T>G c.1012G>A	Comp het	p.Cys337Trp p.Gly338Arg	11	Unknown	Missense/missense	Likely pathogenic/likely pathogenic	Novel
EDAR_F5	M	-	++	A, D	-	O+, CI	FD	c.71C>A	Hom	p.Ala244Asp	3	Altered subcellular location	Missense	Likely pathogenic	Novel
EDAR_F6	M	-	+	H	+	O+, CI	FD	c.245G>A	Hom	p.Gly82Glu	4	Possibly affecting ligand binding	Missense	Likely pathogenic	Novel
EDARADD_F1-1	M	+	++	A, D	+	O+, Class III mal	FD, blue sclera, palmar hyperhidrosis	c.454G>A	Hom	p.Glu152Lys	6	Impaired NF-kappa-B activation	Missense	Pathogenic	Headon et al., 2001, reported as p. Glu142Lys
EDARADD_F1-2	F	+	++	H, D	+	O+, Class III mal	FD, palmar keratosis	c.454G>A	Hom	p.Glu152Lys	6	Impaired NF-kappa-B activation	Missense	Likely pathogenic	
WNT10A_F1	F	+	+	H, D	-	O	-	c.433G>A c.682T>A	Comp het	p.Val145Met p.Phe228Ile	1 3	Unknown	Missense/missense	Pathogenic/pathogenic	Cluzeau et al., 2011, Bohring et al., 2009
WNT10A_F2-1	M	+	+	H, D+	-	O*, TI	-	c.433G>A	Hom	p.Val145Met	1	Unknown	Missense	Pathogenic	Cluzeau et al., 2011
WNT10A_F2-2	M	+	+	H, D	-	O*, TI	-	c.433G>A	Hom	p.Val145Met	1	Unknown	Missense	Pathogenic	
WNT10A_F3	F	-	+	D	+	O+, CI, Class III mal	Palmar hyperhidrosis	c.310C>T	Hom	p.Arg104Cys	2	Impaired disulfide bond	Missense	Pathogenic	Yang et al., 2015
WNT10A_F4	F	+	+	H, D	+	O*	Atopic dermatitis	c.86T>G c.103-104insGCCTGCT	Comp het	p.Leu29Arg p.Ala35Glyfs*11	1 1	Impaired signaling/truncated protein	Missense/frameshift insertion	Likely pathogenic/pathogenic	Novel/novel

A, anhidrosis; AR, autosomal recessive; CI, conical incisors; Comp het, compound heterozygous; Cons, consanguinity; D, dryness; Dr+, severe dryness; FD, facial dysmorphism; H, hypohidrosis; Hom, homozygous; mal, malocclusion; O, oligodontia; O+, severe oligodontia; TI, tapered incisors; -, negative; +, positive. Number of + reflects the degree of severity regarding the feature. * Only in permanent dentition.

Discussion

Twenty-seven families clinically diagnosed with HED/EDA were included in the cohort. Four known ED genes, *EDA*, *EDAR*, *EDARADD*, and *WNT10A*, were sequenced, and 17 pathogenic variants, 8 being novel, were identified in 63% (17/27) of the cohort. Cluzeau et al. [2011] examined all 4 ED-related genes in a large cohort of 65 unrelated patients, 61 of which presented with HED/EDA, and concluded that the 4 ED genes accounted for 92% of their cases. In a recent cohort of 133 Italian ED individuals, pathogenic changes were identified in 106 subjects (80%). *EDA*, *WNT10A*, *EDAR*, and *EDARADD* variants were detected as 45, 25, 7.5, and 1.5%, respectively [Guazzarotti et al., 2015]. In another large cohort study, 124 European HED patients were investigated by Sanger sequencing and MLPA analyses of *EDA*, *EDAR*, *EDARADD*, *TRAF6*, and *EDA2R*, revealing pathogenic changes in 101 out of 124 HED individuals (81.5%). Patients with pathogenic variants in the *EDA*, the *EDAR*, and the *EDARADD* genes constituted 88, 9, and 3% of the cases, respectively [Wohlfart et al., 2016a]. Compared to these previous large cohorts, the cohort reported herein exhibited a lower variant detection rate that can partially be explained by the limitations of Sanger sequencing, which cannot detect exonic deletions, duplications, or regulatory sequence variants. Furthermore, given the high rate of consanguinity in our cohort, the lower variant detection rate may also stem from variants in as yet unidentified novel genes with autosomal recessive inheritance patterns. In another ED cohort of 40 patients from 35 Mexican families, *EDA*, *EDAR*, and *EDARADD* genes were sequenced, and 16 different variants were identified only in 21 families (60%) [Salas-Alanis et al., 2015]. The *WNT10A* gene was not included in the study, so this may have led to the lower mutation detection rate. The vast majority of HED/EDA cases carry mutations in *EDA*, with a reported prevalence of 58% [Cluzeau et al., 2011] and 88% [Wohlfart et al., 2016a]. To date, more than 300 pathogenic variants in *EDA* have been reported (Human Gene Mutation Database) [Stenson et al., 2003]. The present study revealed a lower variant rate in *EDA* (35.3%) compared to those previously reported cohorts. *EDAR* variants in our study were detected in 35.3% of the patients, similar to the rate of *EDA* variants. This is in contrast to previous studies, which reported the incidence of *EDAR* variants at 9% [Wohlfart et al., 2016b] and 16% [Cluzeau et al., 2011]. The high rate of *EDAR* variants in the present study is probably associated with the high incidence

of consanguineous marriages in our cohort, which increases the likelihood of an autosomal recessive inheritance pattern.

The phenotypes associated with *EDA*, *EDAR*, and *EDARADD* variants are indistinguishable, and the clinical features among these patients were typical, so that a diagnosis of ED was often possible even after a cursory examination. Ectodermal symptoms including hypo/anhydrosis, sparse hair, and oligodontia were consistently present in all cases; facial dysmorphism and dry, translucent, and velvety skin were also frequent features. In contrast, the phenotypic features of our patients carrying *WNT10A* variants were consistent with those reported by Cluzeau et al. [2011]. Although patients harboring *WNT10A* variants have a marked dental phenotype, they show no dysmorphic facial characteristics suggestive of a clinical diagnosis of HED. Bergendal et al. [2016] suggested that most *WNT10A* mutation-positive patients exhibited a thin upper lip as a dysmorphic feature, although in our small cohort, only 2 patients with the *WNT10A* variants had thin upper lips. The most significant feature of individuals carrying *WNT10A* changes is reported to be severe oligodontia in permanent dentition with apparently normal or comparatively less disturbed primary dentition [Bohring et al., 2009]. Our findings further support this observation. In the present study, 3 out of 5 individuals (F1, F2-1, and F2-2) carrying the *WNT10A* variants had a full set of deciduous dentition, whereas permanent dentition showed severe oligodontia. The other 2 *WNT10A* variant carriers exhibited permanent dentition that was more severely affected than primary dentition. Another typical dental feature of patients with the *WNT10A* mutations is the presence of tapered permanent incisors [Bohring et al., 2009; Bergendal et al., 2016]. In the present study, 3 patients carrying the *WNT10A* variants showed tapered incisors.

Conclusion

In this study, we assessed the presence of pathogenic variants in 17 HED/EDA families. Eight novel and 9 known variants were identified in the *EDA*, *EDAR*, *EDARADD*, and *WNT10A* genes, expanding the mutational spectrum of HED/EDA. The rate of *EDAR* variants was found to be higher than that observed in previous studies. Thus, in populations with a high rate of consanguinity, if pedigree analyses rule out an X-linked mode of inheritance, we highly suggest that *EDAR* be the first

gene to be tested in HED/EDA. We also suggest that the *WNT10A* gene should be the first to be considered if the patient has severe oligodontia in permanent dentition and mildly affected or unaffected primary teeth with no facial dysmorphism.

Acknowledgment

We are grateful to the patients and their families for participating in this study.

Statement of Ethics

The study has been conducted in accordance with the Declaration of Helsinki, and ethical approval has been obtained from the local ethic committees of the Istanbul Medical Faculty at Istanbul University (2008/1194 for CRANIRARE and 2012/743-1061 for CRANIRARE-II) and the Koç University (2015. 120.IRB2.047 for CRANIRARE-II). All participants and/or their legal representatives gave written informed consent both for molecular testing and publication of accompanying photographs.

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Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by the Programme Hospitalier de Recherches Cliniques (PHRC) and the Scientific and Technological Research Council of Turkey (TUBITAK), grants 108S418 and 112S398 to H.K., and the overall consortiums, CRANIRARE and CRANIRARE-2, of the European Research Area Network projects (E-RARE).

Author Contributions

A. Smahi and H. Kayserili conceived and designed the experiments. Z. Oya Uyguner, E. Bal, and S. Hadj-Rabia performed the experiments. Y. Guven, U. Altunoglu, E. Yücel, and E. Bal analyzed the data. M. Koruyucu, E. Bahar Tuna, S. Çıldır, and O. Aktoren contributed to data acquisition. Y. Guven and H. Kayserili wrote the paper. All authors gave final approval and agree to be accountable for all aspects of the work.

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