EDA mutation as a cause of hypohidrotic ectodermal dysplasia: a case report and review of the literature

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ABSTRACT. Ectodermal dysplasia (ED) represents a collection of rare disorders that result from a failure of development of the tissues derived from the embryonic ectoderm. ED is often associated with hair, teeth, and skin abnormalities, which are serious conditions affecting the quality of life of the patient. To date, a large number of genes have been found to be associated with this syndrome. Here, we report a patient with hypohidrotic ED (HED) without family history. We identified that this patient's disorder arises from an X-linked HED with a mutation in the *EDA* gene (G299D) found by whole-exome sequencing. In addition, in this paper we summarize the disease-causing mutations based on current literature. Overall, recent clinical and genetic research involving patients with HED have uncovered a large number of pathogenic mutations in *EDA*, which might contribute to

a full understanding of the function of *EDA* and the underlying mechanisms of HED caused by *EDA* mutations.

**Key words:** Hypohidrotic ectodermal dysplasias; Whole exome sequencing; *EDA*

INTRODUCTION

The ectoderm is one of the three germinal layers established during embryogenesis, and is important for the development of sweat glands, hair, nails, nerves, and enamel. Although ectodermal dysplasias (EDs) are not common human disorders, over one hundred and fifty different types of congenital defects of certain ectodermal structures and their accessory appendages, which can be divided into 11 clinical subgroups, have been reported to date (Sepulveda et al., 2003). The subtype of hypohidrotic ED (HED) is the most common form, and is estimated to affect 1 in 100,000 newborns (Keller et al., 2011). The severity of HED has been found to vary between different patients. HED might result in considerable social and psychological problems and is also a source of great financial burden for affected families (Norderyd, 2012). However, there have not been any effective and economic methods for the treatment of HED worldwide until recently (Ioannidou-Marathiotou et al., 2010). Evidence suggests that HED is a typical genetic disease, and currently at least 5 genes have been found to be associated with HED (Cluzeau et al., 2011). Therefore, HED-related gene screening and prenatal gene diagnosis are necessary for affected individuals. Recently, some studies have been indicated that whole-exome sequencing represented an economic and effective tool for the diagnosis of genetic diseases for patients with or without family history as the cost of high throughput sequencing continues to decrease. Here, we report a patient with HED without family history carrying an *EDA* mutation identified by whole-exome sequencing, and we present a review of the literature of HED caused by *EDA* mutations.

MATERIAL AND METHODS

Case presentation

The affected boy, 7 years of age, was seen at the Key Laboratory of Guilin 181st Hospital for genetic counseling with the chief complaint of abnormal appearance and an absence of teeth. The boy presented with features of HED and had been diagnosed when he was two years old by other physicians. On our physical examination, it was observed that he had thin and scanty hair, nearly absent eyebrows, and his skin was smooth and dry with small wrinkles. The boy was found to have three teeth erupted in the oral cavity on intraoral examination; radiographic examination confirmed that two teeth were still emerging, and the roots of the erupted teeth were very short and conical (Figure 1). The boy found it difficult to tolerate hot summery days, and his parent revealed that the boy used to live under the air conditioner in summer to combat the heat. A biopsy of abdominal skin stained by hematoxylin-eosin in Shenzhen People's Hospital indicated the presence of a few lymph cells infiltrated into the dermis, but no sweat glands, hair follicles, sebaceous glands, or other skin appendages were found (Figure 2). The couple also indicated that no family history of HED was present and that no features of HED had been ever identified in other family members during routine physical examinations.
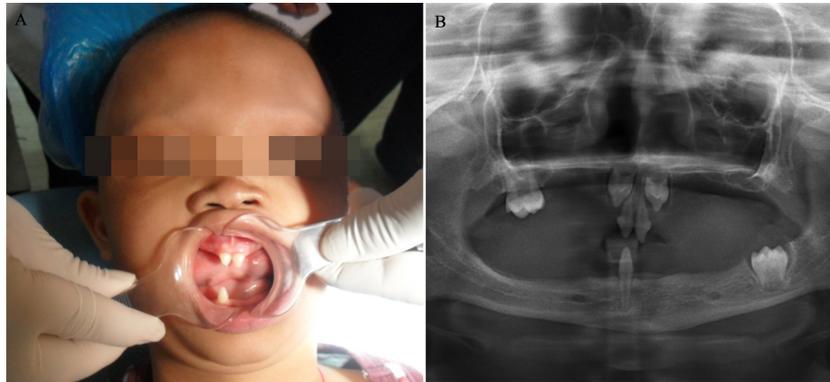
Figure 1. Oral examination of the affected boy. **A.** Erupted teeth in the oral cavity. **B.** Radiographic examination of the teeth.
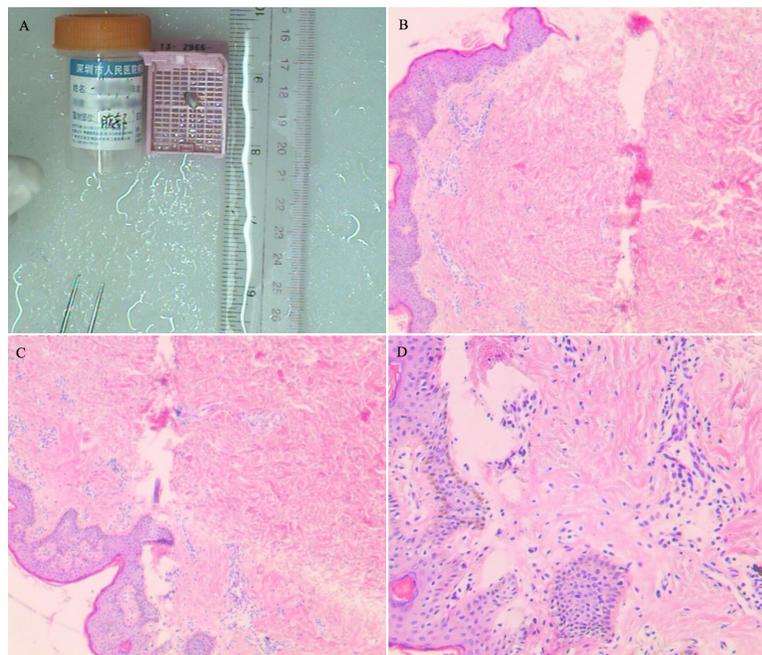
Figure 2. Biopsy of abdominal skin with hematoxylin-eosin (H&E) staining. **A.** Abdominal skin taken from the affected boy. **B.** and **C.** 4X H&E. **D.** 10X H&E.

Mutation analysis

In order to identify the genetic type of HED, blood sample was obtained from the affected boy, and standard cytogenetic and molecular procedures were performed. We sequenced the whole exome of the affected boy according to our previous study (Sui et al., 2013). The variants were called and filtered by the National Center for Biotechnology Information (NCBI) dbSNP Build 132, the 1000 Genomes Project, HapMap, and the YH database. And the candidated mutation was confirmed by conventional Sanger sequencing.

RESULTS

Karyotype analysis with G banding showed that the affected boy was a normal male. The mean depth of the target region was 148.24X, and the whole-exome sequencing identified a mutation in *EDA* (NM_001399.4: c.896G>A, p.G299D), which was a previously reported mutation associated with HED, conventional Sanger sequencing conformed that the mutation was not present in the affected boy's parent or in our 3 normal control. Based on the clinical features, the result of biopsy, whole-exome sequencing, and the literature of HED, we suggest that *EDA* (G299D) might be a disease-related mutation in this affected Chinese boy.

DISCUSSION

HED (MIM 305100) is the most prevalent form of ED. Given the high complexity of developmental control during embryogenesis,it is expected that considerable genetic heterogeneity underlies HED. To date, molecular research has shown that 64 genes and 3 chromosomal regions are involved in EDs (Visinoni et al., 2009). Among these, *EDA*, *EDAR*, *EDARADD*, *TRAF6*, *WNT10A*, and *NEMO* (or *IKKG*) have been found to be associated with HED (Cluzeau et al., 2011), with *EDA*, *EDAR*, *EDARADD I*, and *WNT10A* accounting for 90% HED, and *EDA* mutations identified in more than 50% HED (Cluzeau et al., 2011).

EDA is also known as *ED1*, *HED*, *EDA1*, *HED1*, *ODT1*, *XHED*, *ECTD1*, *XLHED*, and *STHAGX1*. Its product, ectodysplasin-A (EDA), was found to be a member of the tumor necrosis factor (TNF) superfamily of ligands. EDA contains a transmembrane domain, putative furine cleavage site, collagen subdomain, and a TNF-homologous domain. In the literature, over 100 different variations in *EDA* have been reported in patients with or without EDs. Although a great number of the *EDA* mutations might represent null mutations and thus be without clear genotype-phenotype correlation (Mikkola, 2009), at least 82 variations have been identified as pathogenic mutations associated with HED in the NCBI ClinVar database (http://www.ncbi.nlm.nih.gov) and in published papers (Table 1) (Pääkkönen et al., 2001; Schneider et al., 2001; Cluzeau et al., 2011). In addition, *EDA* mutations have been found in patients with EDs worldwide, including in India, Brazil, and China (Schneider et al., 2001; Visinoni et al., 2009; Cluzeau et al., 2011). In the spectrum of deleterious *EDA* mutations, the most frequent event was found to be missense mutations; followed by deletions and nonsense mutations causing premature truncation of the EDA protein; the rest of the mutations were shown to affect splice sites or represent in-frame deletions (Pääkkönen et al., 2001). From the structure of the EDA protein and of the reported mutations, we find that the majority of residues affected by missense mutations occur in the TNF homology domain, collagen domain, and the furin recognition sequences (Schneider et al., 2001). In our patient, the mutation (G299D) is in the TNF homology domain, and the residue is located in an amino acid sequence which is absent in the EDA-A2 protein. Some studies have indicated that the mutations associated amino acid in the known TNF-like ligands were highly conserved (Pääkkönen et al., 2001). Mutation of G299D might therefore affect receptor binding, and result in an interruption of the interactions of EDA with both EDAR and XEDAR, which might play important roles in the EDA-NF-kB pathway (Aradhya and Nelson, 2001; Kurban et al., 2010). In the literature, the ectodysplasin/NF-κB and Wnt/β-catenin pathways are well known to be involved in the early steps of the development of ectodermal appendages (Figure 3) (Clauss et al., 2008), and it has been hypothesized that the rest of the hitherto unknown mutations related to HED might lie in proteins contained in either of these two pathways (Gat et al., 1998; Cluzeau et al., 2011).

**Table 1.** *EDA* mutations found in patients with HED in the literature.

No.	Mutation (Nucleotide)	Variant type	Predicted protein changes	Predicted effect on protein (domain)
1	2T>C	Missense	M1T	
2	46insC	Frameshift	FS18	
3	60–61ins8	Frameshift	FS 20	
4	67C>T	Nonsense	Q23X	
5	121–122insC	Frameshift	FS 41	Transmembrane domain
6	160C>T	Missense	H54Y	Transmembrane domain
7	164C>G	Missense	L55R	Transmembrane domain
8	181T>C	Missense	Y61H	Transmembrane domain
9	−181?_396+?del	Deletion	p.?	
10	183C>G	Nonsense	Y61X	Transmembrane domain
11	187G>A	Missense	E63K	Transmembrane domain
12	206G>T	Missense	R69L	
13	252delT	Frameshift	FS85	
14	272dupG	Frameshift	S91Rfs	
15	329C>A	Nonsense	S110X	
16	347T>A	Nonsense	L116X	
17	358G>T	Missense	E120X	
18	361delG	Deletion	M214NfsX26	
19	382C>T	Nonsense	Q128X	
20	394C>T	Nonsense	Q132X	
21	396+1G>A	Splice donor	p.?	
22	396+2T>G	Splice donor	p.?	
23	457C>T	Missense	R153C	Furin subdomain
24	463C>T	Missense	R155C	Furin subdomain
25	466C>A	Missense	R156S	Furin subdomain
26	466C>T	Missense	R156C	Furin subdomain
27	467G>A	Missense	R156H	Furin subdomain
28	474A>T	Missense	K158N	Furin subdomain
29	502+1G>A	Splice donor	p.?	
30	503–?_1176+?del	Splice donor	p.?	
31	526+5G>T	Intron	p.?	
32	546_581del36	Deletion	In-frame 183_194del (GlyXY)34	Collagen subdomain
33	553_588del36	Deletion	In-frame 185_196del (GlyXY)34	Collagen subdomain
34	562_589del28	Deletion	188_197del, FS198	Collagen subdomain
35	566G>A	Missense	G189E	Collagen subdomain
36	572_589del18	Deletion	In-frame 191_196del (GlyXY)32	Collagen subdomain
37	573_590del	Deletion	G192_Q197del	Collagen subdomain
38	595_613del19	Deletion	199_204del, FS205	Collagen subdomain
39	599-600insC	Frameshift	FS201	Collagen subdomain
40	607C>T	Missense	P203S	Collagen subdomain
41	619G>A	Missense	G207R	Collagen subdomain
42	620G>T	Missense	G207V	Collagen subdomain
43	626C>T	Missense	P209L	Collagen subdomain
44	653G>A	Missense	G218D	Collagen subdomain
45	656_673del18	Deletion	In-frame 218_223del (GlyXY) 32	Collagen subdomain
46	659_676del18	Deletion	In-frame 220_225del (GlyXY) 32	Collagen subdomain
47	663_697del35	Deletion	221_233del, FS234	Collagen subdomain
48	671G>C	Missense	G224A	Collagen subdomain
49	676C>T	Nonsense	Q226X	Collagen subdomain
50	A>G at 706–2	Splice	Altered splicing	
51	730C>T	Nonsense	R244X	
52	755A >T	Missense	H252L	TNF homology subdomain
53	764G>A	Missense	G255D	TNF homology subdomain
54	766C>T	Nonsense	Q256X	TNF homology subdomain
55	789_825del	Deletion	K263DfsX5	TNF homology subdomain
56	809delT	Frameshift	V270Gfs	TNF homology subdomain
57	G822>A	Nonsense	W274X	TNF homology subdomain
58	822delG	Frameshift	W274Cfs	TNF homology subdomain
59	822G>T	Missense	W274C	TNF homology subdomain
60	826C>T	Missense	R276C	TNF homology subdomain
61	G871>T	Missense	G291W	TNF homology subdomain
62	G871> A	Missense	G291R	TNF homology subdomain

Continued on next page

Table 1. Continued.

No.	Mutation (Nucleotide)	Variant type	Predicted protein changes	Predicted effect on protein (domain)
63	G892>C	Missense	D298H	TNF homology subdomain
64	G895>A	Missense	G299S	TNF homology subdomain
65	G15>A	Splice	Altered splicing	TNF homology subdomain
66	902A>G	Missense	Y301C	TNF homology subdomain
67	948delC	Frameshift	p.?	TNF homology subdomain
68	959A>G	Missense	Y320C	TNF homology subdomain
69	961G>T	Nonsense	E321X	TNF homology subdomain
70	968T>G	Missense	V323G	TNF homology subdomain
71	991C>T	Nonsense	Q331X	TNF homology subdomain
72	1028A>G	Missense	Y343C	TNF homology subdomain
73	1037G>A	Missense	C346Y	TNF homology subdomain
74	1045G>A	Missense	A349T	TNF homology subdomain
75	1067G>A	Missense	A356D	TNF homology subdomain
76	1067C>T	Missense	A356V	TNF homology subdomain
77	1070G>C	Missense	R357P	TNF homology subdomain
78	1122C>A	Missense	S374R	TNF homology subdomain
79	1132A>C	Missense	T378P	TNF homology subdomain
80	1133C>T	Missense	T378M	TNF homology subdomain
81	1141G>A	Missense	G381R	TNF homology subdomain
82	789_825del	Deletion	K263DfsX5	TNF homology subdomain

HED = hypohidrotic ectodermal dysplasia.
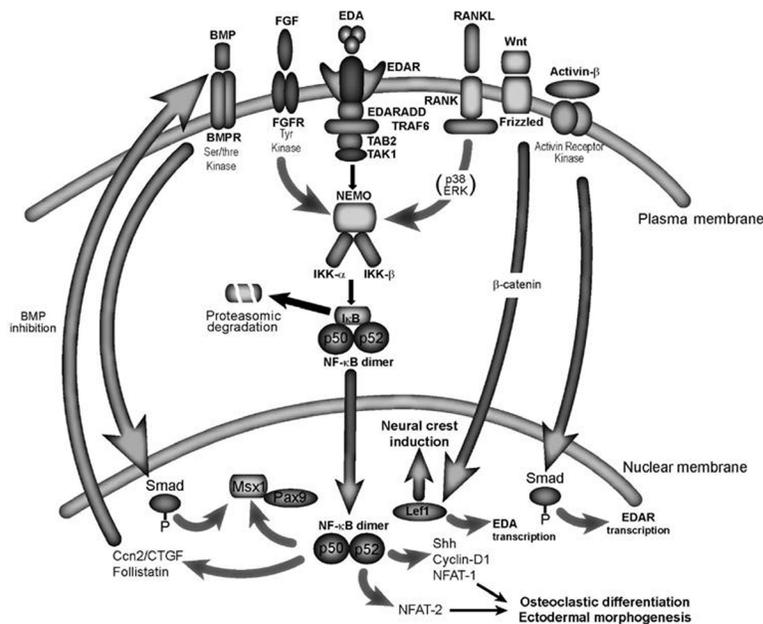
Figure 3. Overview of EDA involvement in the development of ectodermal appendages (Clauss et al., 2008).

HED can be diagnosed by three cardinal features: hypotrichosis (sparseness of scalp and body hair), hypohidrosis (reduced ability to sweat), and hypodontia (congenital absence of teeth) (Wright et al., 2014). For the majority of patients with HED caused by homozygous mutation of *EDA*, the classic symptoms can be found after infancy, such as peeling skin, periorbital hyperpigmentation, heat intolerance, and uncommonly elevated body temperatures (Blüschke et

al., 2010). However, diagnosis might be delayed until their teeth fail to erupt or erupt abnormally at the expected age, as seen in our patient who was not diagnosed until two years of age. For female carriers with heterozygous mutations of *EDA*, their features on clinical examination might be mild; for example, including some sparseness of the hair, sweat dysfunction in some part of the skin, and a small number of missing or abnormal teeth (Cambiaghi et al., 2000). Phenotypes associated with *EDA* mutations vary, and might present in the clinic ranging from nonsyndromic hypodontia to classic HED. Some studies have indicated that most pathogenic *EDA* mutations causing nonsyndromic hypodontia were missense mutations associated with the TNF domain; and a great number of pathogenic mutations causing HED were thought to be loss of function mutations (Cambiaghi et al., 2000). In our patient, although the mutation in EDA (G299D) is a missense mutation in the TNF domain, the affected boy has a classic form of HED; therefore, the residue associated with the mutation might interrupt the interaction between EDA and EDAR, thereby impairing its role in the EDA-NF-kB pathway.

For genetic counseling, HED related gene screening is needed. In the literature, Sanger sequencing and multiplex ligation-dependent probe amplification have been commonly used for *EDA* mutation discovery in both research and clinical studies (Lexner et al., 2008). Recently, whole-exome sequencing has been reported to be an economical and rapid tool for genetic diagnosis, and it has been used to diagnose types of disorders similar to ED (Haghighi et al., 2013). In this study, we performed whole-exome sequencing to screen ED-associated genes in a boy affected with HED, and identified that an EDA (G299D) mutation might be the primary cause of the disorder in this patient. This finding demonstrated that whole-exome sequencing is well developed and might be a useful tool for ED-associated mutation identification in patients with HED with or without family history.

In summary, genetic studies involving patients with HED have uncovered a large number of pathogenic mutations in *EDA*, which might be helpful to establish a full understanding of the function of *EDA*. In addition, whole exome sequencing might be useful to identify the type of ED in clinical practice. However, HED is a highly complex and heterogeneous disease. Therefore, genotype-phenotype correlation in different patients needs to be a focus of further research.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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