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Research Article

Only four genes (*EDAI*, *EDAR*, *EDARADD* and *WNT10A*) account for 90 % of hypohidrotic/anhidrotic ectodermal dysplasia cases

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Abstract

Hypohidrotic and anhidrotic ectodermal dysplasia (HED/EDA) is a rare genodermatosis characterized by abnormal development of sweat glands, teeth and hair. Three disease causing genes have been hitherto identified, namely i) *EDA1* accounting for X-linked forms, ii) *EDAR*, and iii) *EDARADD*, causing both autosomal dominant and recessive forms. Recently, *WNT10A* gene was identified as responsible for various autosomal recessive forms of ectodermal dysplasias, including onycho-odonto-dermal dysplasia (OODD) and Schöpf-Schulz-Passarge syndrome. We systematically studied *EDA1*, *EDAR*, *EDARADD* and *WNT10A* genes in a large cohort of 65 unrelated patients, of which 61 presented with HED/EDA. A total of 50 mutations (including 32 novel mutations) accounted for 60/65 cases in our series. These four genes accounted for 92 % (56/61 patients) of HED/EDA cases: i) *EDA1* gene was the most common disease causing gene (58 % of cases), ii) *WNT10A* and *EDAR* were each responsible for 16 % of cases. Moreover, a novel disease locus for dominant HED/EDA mapped to chromosome 14q12-q13.1. While no clinical differences between patients carrying *EDA1*, *EDAR* or *EDARADD* mutations could be identified, patients harboring *WNT10A* mutations displayed distinctive clinical features (marked dental phenotype, no facial dysmorphism), helping to decide which gene should be first investigated in HED/EDA.

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Keywords: HED/EDA; *EDA1*; *EDAR*; *EDARADD*; *WNT10A*.

Introduction

Ectodermal dysplasia (ED) is a clinically and genetically heterogeneous condition characterized by abnormal development of two or more of the following ectodermal-derived structures: hair, teeth, nails, and sweat glands [Freire-Maia, 1977; Lamartine, 2003; Visinoni, et al., 2009]. Anomalies in other organs and systems may also be observed [Pinheiro and Freire-Maia, 1994; Dhanrajani and Jiffry, 1998; Priolo, et al., 2000]. Anhidrotic or hypohidrotic ectodermal dysplasia (HED/EDA), the most common phenotype of ED, is characterized by a triad of signs comprising sparse hair (hypotrichosis), abnormal or missing teeth (anodontia or hypodontia) and inability to sweat (anhidrosis or hypohidrosis). Typical clinical manifestations also include dryness of the skin, eyes, airways and mucous membranes presumably due to the defective development of several exocrine glands. HED/EDA can be associated with dysmorphic features (forehead bumps, rings under the eyes, everted nose and prominent lips) and occasionally with absent nipples.

The most frequent form of HED/EDA (MIM305100) results from mutations in the *EDA1* gene, located on chromosome Xq12-q13.1 and encoding ectodysplasin (MIM300451), a member of the Tumor Necrosis Factor (TNF) family [Kere, et al., 1996; Bayes, et al., 1998]. Mutations in the EDA receptor encoding gene *EDAR*, located on chromosome 2q11-q13 (MIM604095), or in the EDAR-Associated Death Domain encoding gene *EDARADD*, located on chromosome 1q42-q43 (MIM606603), have been shown to cause autosomal recessive and dominant HED forms respectively [Monreal, et al., 1999; Headon, et al., 2001; Bal, et al., 2007]. These three forms are clinically indistinguishable, probably because they alter a single signal transduction pathway.

Indeed, the binding of ectodysplasin to its receptor *EDAR*, allows the recruitment of *EDARADD* as an adapter to activate the NF- κ B signalling pathway [Yan, et al., 2000; Koppinen, et al., 2001; Kumar, et al., 2001]. This pathway is necessary for initiation, formation and differentiation of

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2 skin appendages [Mikkola, et al., 1999; Laurikkala, et al., 2002; Mustonen, et al., 2004; Mou, et
3
4 al., 2006; Schmidt-Ullrich, et al., 2006; Pummila, et al., 2007].

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6 On the other hand, loss-of-function and missense mutations in the *WNT10A* gene (chromosome
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8 2q35, MIM257980) have been shown to cause odonto-onycho-dermal dysplasia, a rare form of
9
10 ectodermal dysplasia [Adaimy, et al., 2007; Nawaz, et al., 2009]. Subsequently, *WNT10A*
11
12 mutations have been reported in various forms of ectodermal dysplasia, including in three
13
14 patients with sweating anomalies [Bohring, et al., 2009; Nagy, et al., 2010; van Geel, et al.,
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16 2010].

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18 In order to evaluate the impact of *EDA1*, *EDAR*, *EDARADD* and *WNT10A* mutations in
19
20 HED/EDA, we have sequenced all four genes in a large cohort of 65 unrelated patients. Among
21
22 them, 61 patients had HED/EDA. Our study shows that only four genes account for more than 90
23
24 % of cases in HED/EDA.

25 26 27 **Patients and Methods**

28 29 **Patients**

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31 Since 2002, a total of 65 patients were recruited by the departments of Dermatology and Genetics
32
33 of Necker-Enfants Malades Hospital. Patients included in this study presented abnormalities of at
34
35 least two of the three following ectodermal structures: teeth, hair and sweat glands. Most of those
36
37 patients (53/65) presented the classical triad of HED/EDA phenotype. The relatives of the
38
39 probands occasionally presented abnormalities of only one epidermal structure. The great
40
41 majority of cases were familial cases (48/65) of Caucasian origin (45/65). A total of 9/65 patients
42
43 were born to related parents. Informed consent for DNA analysis and reproduction of the
44
45 photographs was obtained from all individuals concerned.

46 47 **Mutation detection**

1 DNA was extracted from patients' blood leukocytes using the Illustra DNA extraction kit
2
3 BACC3 (GE Healthcare), following manufacturer's instructions. All exons and at least 60 base
4
5 pair of flanking intronic sequences of the *EDAI* (NM_001399.4), *EDAR* (NM_022336.3),
6
7 *EDARADD* (NM_080738.3) and *WNT10A* genes (NM_025216.2) were amplified by PCR using
8
9 specific primers (see Supp. Table S1). Both DNA strands were sequenced using the Big Dye™
10
11 Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems). Sequence
12
13 variations were numbered with +1 corresponding to the A of the ATG translation initiation codon
14
15 in the cDNA reference sequence, or with the initiation codon as codon 1 in the proteic reference
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17 sequence, according to journal guidelines (www.hgvs.org/mutnomen).

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19 For Quantitative Multiplex PCR of Short fluorescent Fragments analysis (QMPSF), exon 18 of
20
21 *MLH1* gene (E. coli MutL Homolog 1) or exon 3 of *GFAP* gene (Glial Fibrillary Acidic Protein)
22
23 were amplified as positive controls, along with each exon of the *WNT10A* gene (see Supp. Table
24
25 S1). PCR products were migrated using an ABI3130 sequencer (Applied Biosystems), and
26
27 analyzed with GeneScan Analysis software version 3.7 (Applied Biosystems).

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29 A genome-wide scan for one large family carrying no known mutation was undertaken with 382
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31 pairs of fluorescent oligonucleotides of the Genscan Linkage Mapping set, version II (average
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33 spacing of 10 cM, Perkin-Elmer Cetus) under conditions recommended by the manufacturer. To
34
35 refine the novel HED locus identified, additional microsatellites DNA markers from chromosome
36
37 14q region were studied. After amplification, PCR products were pooled with GeneScan 400D
38
39 ROX size standard ladder (0.3 µl; Applied Biosystems), and analyzed on an ABI3130 sequencer.

42 Results

43
44 A total of 31 *EDAI* mutations, including 20 novel mutations were identified in 35/65 cases of our
45
46 cohort (Table 1, Figure 1A, Supp. Figure S1A). Most of those mutations were missense
47
48 mutations (24/31), located in exons 3 and 5 to 9. The male patients carrying *EDAI* mutations
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1
2 were severely affected, as they all displayed anomalies in the three ectodermal structures. Carrier
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4 females were occasionally moderately affected, but two displayed unusually severe symptoms
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6 (patients EDA1-F19 and EDA1-F24, Figure 1B1-2).

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8 As to the *EDAR* gene, a total of 10 mutations, including 5 novel mutations, were identified in
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10 10/65 patients (Table 1, Figure 1A, Supp. Figure S1B). Five mutations were dominantly, and five
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12 recessively inherited. They were mostly located in exons 9 to 12 (7/10).

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14 Interestingly, the dominant mutations were all located close to the *EDAR* Death Domain (DD,
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16 two missense mutations, one single residue duplication and two frameshift mutations).

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18 Clinical features of these patients were indistinguishable from those observed in *EDA1* patients.

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20 However, individuals carrying dominant *EDAR* mutations were less severely affected than their
21
22 recessive counterparts (Figure 1C and D). This was particularly true with respect to sweating
23
24 (Table 1).

25
26 As to the *EDARADD* gene, only one novel dominantly inherited missense mutation was found in
27
28 a patient with a moderate HED phenotype (NM_080738.3: c.328G>T, p.D110Y; Table 1, Figure
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30 1E1-2). This mutation located near the *EDARADD* Death Domain (Supp. Figure S1C), probably
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32 altered its interaction with *EDAR* and/or multimerization of *EDARADD*.

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34 As to the *WNT10A* gene, a total of 8 mutations were identified in 14/65 cases (9 familial and 5
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36 sporadic cases; Table 2, Figure 2, and Supp. Figure S1D). They included 5 novel missense
37
38 mutations and the first *WNT10A* duplication identified to date. The p.F228I mutation was
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40 prevalent in our cohort (10/14 patients) and found in homozygote or compound heterozygote
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42 patients. None of those mutations were found in 150 control chromosomes from healthy
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44 Caucasian individuals. QMPFS analysis of the four exons of the *WNT10A* gene in six
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46 heterozygote patients failed to detect any intragenic deletion (not shown).

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48 Interestingly, most patients carrying *WNT10A* mutations (10/14) presented with sweating
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50 anomalies, including total anhidrosis for two of them (*WNT10A*-F03 and S01). However,

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2 comparison of those patients with HED/EDA cases harboring mutations in the ectodysplasin
3 pathway allowed us to identify slight differences in their respective phenotypes. Indeed,
4 dermatological features (anomalies of hair and sweat glands) were less severe and none of the
5 patients carrying *WNT10A* mutations presented facial dysmorphism. Their dental phenotype
6 consisted in microdontia, while teeth agenesis was more frequent in patients carrying mutations
7 in the ectodysplasin pathway (Table 2, Figure 1F1-3 and 1G). The other four patients with
8 *WNT10A* mutations presented with incomplete form of OODD or unclassified form of ED (Table
9 2).

10
11 Finally, among the 5/65 unexplained cases of our cohort, one patient was the proband of a four-
12 generation French family, presenting an autosomal dominant HED (patient HED-F01, Supp.
13 Table S2). Genome-wide scan analysis led to the mapping of this novel disease gene to a 5 cM
14 interval on chromosome 14q12-q13.1 (Z_{max} = 4.8; Supp. Figure S3). The strongest candidate
15 genes have been excluded by sequencing analysis (*PAX9, NFKBIA, PSMA6, SNX6, MBIP*). None
16 of the remaining known genes mapping to this region were involved in either ectodysplasin or
17 Wnt signalling pathways.

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32 Discussion

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34 Here we show that four genes (*EDA1*, *EDAR*, *EDARADD* and *WNT10A*) accounted for 92 % of
35 cases in a series of 65 unrelated patients, of which 61 presented with HED/EDA. *EDA1* mutations
36 were prevalent (58 % of cases). *WNT10A* mutations were more frequent than *EDAR* mutations in
37 our cohort, but both genes are each responsible for 16 % of HED/EDA cases (Figure 1A). We
38 also report here on a novel disease gene mapping to chromosome 14q12-q13.1 and accounting for
39 autosomal dominant HED/EDA.

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Deleted: but *WNT10A* was the second most frequent disease gene (21 %). Indeed, *WNT10A* mutations were more frequent than *EDAR* mutations in our cohort (Figure 1A)

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41 The phenotypes associated with *EDA1*, *EDAR*, and *EDARADD* mutations were indistinguishable
42 and consistently included hypohidrosis or anhidrosis, sparse hair, and oligodontia with abnormal
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conical teeth, frequently associated with dryness of skin, eczema and facial dysmorphism. Yet, the clinical expression of *WNT10A* mutations was highly variable. *WNT10A* gene was first involved in OODD syndrome (MIM257980) and subsequently in Schöpf-Schulz-Passarge syndrome (MIM224750) [Adaimy, et al., 2007; Bohring, et al., 2009]. We confirmed the involvement of *WNT10A* gene in OODD or OODD-like forms of ED (3/14 patients), and unclassified ED (1/14; Table 2). Moreover, 10/14 patients of our cohort harboring *WNT10A* mutations presented with anomalies corresponding to HED/EDA, ie hypohidrosis or anhidrosis associated with teeth and hair anomalies. Thus our results expanded the spectrum of *WNT10A* mutations to HED/EDA phenotype. The variability of phenotypes associated to *WNT10A* mutations is not understood yet. The type of mutations (nonsense or missense) and their functional effects could explain this broad spectrum of phenotypes.

Our study gives support to the high incidence of *EDA1* mutations (35/61, 58 %), and the scarcity of *EDAR* mutations in HED/EDA (10/61, 16 %) [Vincent, et al., 2001; Chassaing, et al., 2006; van der Hout, et al., 2008]. Interestingly, while *WNT10A* was reportedly known to account for various forms of ED, we provide here the first evidence for its very high incidence in HED/EDA cases (10/61, 16 %) [Adaimy, et al., 2007; Bohring, et al., 2009; Nawaz, et al., 2009; Nagy, et al., 2010].

Yet, the mode of inheritance of *WNT10A* mutations remains unclear. While eight patients were homozygotes or compound heterozygotes, only one heterozygous mutation was identified in six patients. Although a single mutation could probably explain the moderate cases (*WNT10A*-F08, -F09 and -S04), the three severely affected patients may carry a second unidentified mutation (in intronic or regulatory sequences of *WNT10A*). Alternatively, a dominant mode of inheritance with variable penetrance could also explain the severe phenotype of these patients.

A higher proportion of tooth anomalies has been previously described in males harboring heterozygous *WNT10A* mutations [Bohring, et al., 2009]. These sex-biased manifestations were

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not found in our cohort. Dental abnormalities were the most frequent manifestation in heterozygous patients in both sexes. This difference may result from the high frequency of the p.C107X mutation in the German/Turkish cohort [Bohring, et al., 2009].

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Most of the HED mutations reported here were missense mutations, located in functionally important domains of the protein (furin cleavage site, collagenous and TNF domains for Ectodysplasin; Death Domain for EDAR and EDARADD). Interestingly, all dominant mutations reported to date in EDAR were located in its C-terminal region near its Death Domain. These mutations probably exert a dominant negative effect on the wild-type allele *via* the formation of non-functional multimers, unable to recruit EDARADD. All missense EDARADD mutations are located in (or very close to) the Death Domain of EDARADD, probably impairing its interaction with EDAR.

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The involvement of the ectodysplasin/NF- κ B and Wnt/ β -catenin pathways in ectodermal appendage development has long been known [Kere, et al., 1996; Gat, et al., 1998]. Because the two pathways are involved in early steps of ectodermal placode development, one can hypothesize that the 10 % hitherto unknown mutations may lie in either of these two pathways.

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In conclusion, WNT10A gene should be considered as a candidate gene for HED/EDA, especially in the clinical condition of microdontia, sweating anomalies, and absence of facial dysmorphism.

Alternatively, patients with the classical triad of HED/EDA signs and facial dysmorphism, should be first studied for *EDA1* gene, except in non-X-linked familial cases. If no mutation is identified in *EDA1*, *EDAR* is the next gene to consider, before WNT10A and EDARADD.

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For Peer Review

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Legends to figures

Figure 1: Mutation distribution and clinical appearance of HED/EDA patients.

A: Frequencies of identified mutations for each known gene in our series of 61 HED/EDA patients. B1-B4: severely affected female patient (EDA1-F19) at age 6 years (B1, B2) and her brother at age 5 years (B3, B4). C: severely affected patient EDAR-F01 at age 3 years. D: moderately affected patient EDAR-F05 at age 30 years. E1-E2: EDARADD-F01 at age 9 years. F1-F3: WNT10A-F03 at age 16 years. G: WNT10A-S04 at age 5 years. [Pictures collection from the department of Dermatology, Necker-Enfants Malades Hospital, Paris.](#)

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Figure 2: Pedigrees of the 9 families carrying mutations in *WNT10A* gene.

Mutations identified in each individual are indicated, WT designated a second wild-type allele. Filled symbols represented severely affected individuals, half-filled symbols moderately affected individuals. The question mark (?) indicated an undetermined status, an asterisk (*): no DNA available.

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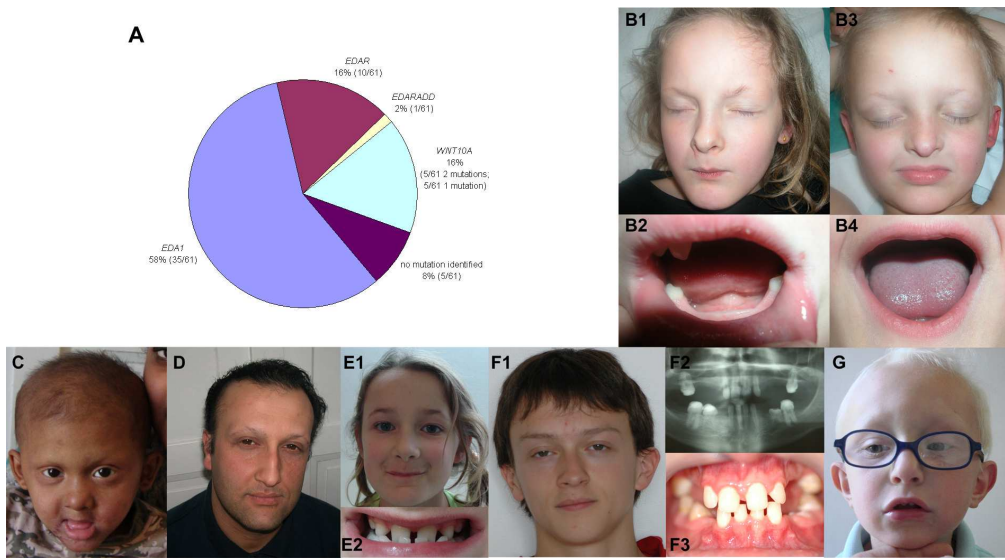
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Figure 2

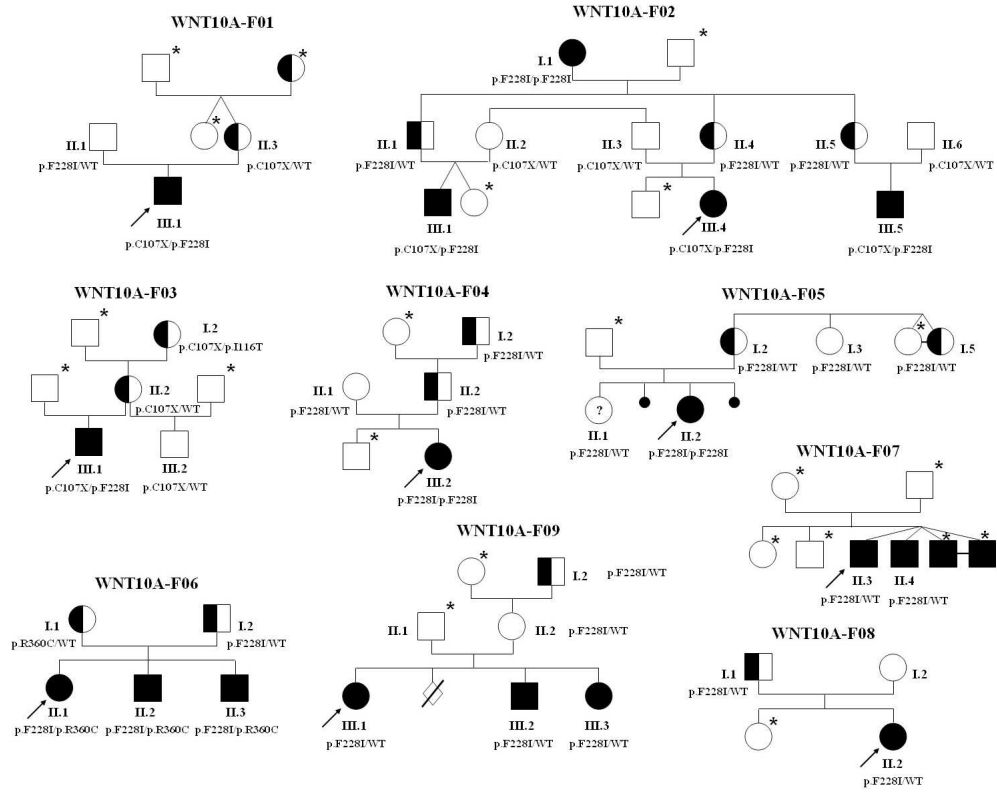


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Table 1: Clinical description and mutations in HED/EDA patients with mutations in EDA1, EDAR and EDARADD genes.

Proband's number	Sex, age (year)	Transmission, origin	Hair	Teeth	Sweating	Other signs	Nucleotide changes	Predicted proteic changes
<i>EDA1 (previously described mutations)</i>								
EDA1-F01	M, 10	Martinique	++	++	++	Eczema	c.466C>T (Bayes et al., 1998; Monreal et al., 1998)	p.R156C
EDA1-F02	M, 5	Algeria, CS	++	+++	++	Eczema, dry skin, facial dysmorphism	c.467G>A (Monreal et al., 1998)	p.R156H
EDA1-F03	M, 5	Tunisia	++	+++	+++	Eczema, dry skin	c.730C>T (Vincent et al., 2001)	p.R244X
EDA1-F04	F, 4	France	+	+(C)	++	Dry skin, facial dysmorphism	c.764G>A (Pääkkönen et al., 2001)	p.G255D
EDA1-F05	M, 8	France	+++	++	+++	Facial dysmorphim	c.871G>C (Bayes et al., 1998)	p.G291R
EDA1-F06	M, 14	France	++	+++	+++	Eczema, facial dysmorphism	c.871G>A (Bayes et al., 1998)	p.G291R
EDA1-S01	M, 16	Spo, Yemen and Djibouti	+++	+++	++	Facial dysmorphism	c.871G>A (Bayes et al., 1998)	p.G291R
EDA1-F07	M, 28	France	++	++	+		c.871G>A (Bayes et al., 1998)	p.G291R
EDA1-F08	M, 13	France	+	+++	++	Dry skin, eczema, facial dysmorphism	c.892G>C (Bayes et al., 1998)	p.D298H
EDA1-S02	M, 4	Spo, Morocco, CS	+	+++	++	Nipples hypoplasia	c.895G>A (Monreal et al., 1998)	p.G299S
EDA1-F09	F, 28	France	++	++	+	Hypoplasia of the right nipple	c.895G>A (Monreal et al., 1998)	p.G299S
EDA1-F10	M, 3	France	+	+++	++	Eczema	c.1133C>T (Vincent et al., 2001)	p.T378M
EDA1-F11	M, 8	France	++	+++	++	Eczema	c.1141G>A (Gunadi et al. 2009, G>C)	p.G381R
EDA1-F12	M, 36	France	+++	+++	+++	Dry skin	c.789_825del (Bayes et al., 1998)	p.K263DfsX5
EDA1-F13	M, 10	France	+	++	+	Eczema	c.-181?_396+?del (Kere et al., 1996 ; Pääkkönen et al., 2001)	p.?

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Proband's number	Sex, age (year)	Transmission, origin	Hair	Teeth	Sweating	Other signs	Nucleotide changes	Predicted proteic changes
<i>EDA1 (novel mutations)</i>								
EDA1-F14	F, 29	Algeria	+++	++	-	Eczema	c.2T>G	p.M1R
EDA1-F15	M, 31	France	++	+++	++		c.358G>T	p.E120X
EDA1-F16	F, 6	France	+	++	-	Dry skin, eczema	c.466C>G	p.R156G
EDA1-F17	M, 3	France	++	+++	+++		c.620G>T	p.G207V
EDA1-F18	M, 5	France	++	+ (C)	+	Dry mouth, facial dysmorphism	c.632C>G	p.T211R
EDA1-S03	M, 9	Spo, France/India	+++	+++	++	Eczema, facial dysmorphism	c.694C>T	p.Q232X
EDA1-F19	F, 10	France	++	++	+		c.797T>G	p.L266R
EDA1-F20	M, 14	France	+	+++	+	Dry skin, eczema	c.820T>A	p.W274R
EDA1-S04	M, 2	Spo, Congo	+++	+++	+++		c.878T>C	p.L293P
EDA1-F21	M, 6	France	+	++	-	Eczema	c.886C>G	p.L296V
EDA1-S05	M, 11	Spo, France	++	+++	++		c.896G>A	p.G299D
EDA1-S06	M, 33	Spo, France	++	+++	+	Palmar keratoderma, facial dysmorphism	c.968T>G	p.V323G
EDA1-S07	M, 16	Spo, France	+++	+++	++	Dry skin, eczema	c.1037G>A	p.C346Y
EDA1-S08	M, 6	Spo, France	+	+	+		c.1067C>T	p.A356V
EDA1-F22	M, 34	France	+++	+++	+++		c.397-1G>A	p.?
EDA1-F23	M, 6	Japan	++	+++	++	Dry skin, facial dysmorphism	c.925-1G>C	p.?
EDA1-F24	F, 43	France	++	++	++		c.361delG	p.A121PfsX16
EDA1-F25	M, 3	Japan	+	+++	++	Eczema, no lacrimation	c.640dupA	p.M214NfsX26
EDA1-F26	M, 5	France	+	+++	+	Eczema	c.573_590del	p.G192_Q197del
EDA1-F27	M, 11	Portugal	++	+++	+	Dry skin, facial dysmorphism	c.503-?_1176+?del	p.?

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Proband's number	Sex, age (year)	Transmission, origin	Hair	Teeth	Sweating	Other signs	Nucleotide changes	Predicted proteic changes
EDAR								
EDAR-S01	M, 7	Spo, AR, Portugal	++	+++	++	Eczema	c.[266G>A]+[442+1G>A] (Monreal et al. 1999; Chassaing et al. 2006)	p.R89H + ?
EDAR-F01	M, 3	AR, Pakistan, CS	+	+++	++		c.[1073G>A]+[1073G>A] (Shimomura et al., 2009)	p.R358Q + p.R358Q
EDAR-F02	M, 11	AR, Kuwait, CS	++	++	++		c.[1208C>T]+[1208C>T] (Chassaing et al. 2006)	p.T403M + p.T403M
EDAR-F03	F, 8	AD, France	++	++	+	Facial dysmorphism	c.1222A>T	p.I408F
EDAR-F04	M, 17	AD, Sefarad	+	+	+	Facial dysmorphism	c.1259G>A (Monreal et al. 1999)	p.R420Q
EDAR-F05	M, 34	AD, Algeria, CS	++	+	+	Dry skin	c.1259G>A (Monreal et al. 1999)	p.R420Q
EDAR-S02	M, 3	Spo, AR, Turkey, CS	+++	+++	+++	Facial dysmorphism, diffuse palmoplantar hyperkeratosis	c.[156_157delinsC]+[156_157delinsC]	p.G53EfsX50
EDAR-S03	F, 37	Spo, AD, Portugal	+	++	+		c.875_876del	p.P292RfsX52
EDAR-F06	F, 10	AD, Sefarad	++	+++	+	Eczema, dry skin	c.1049_1052del	p.D351AfsX20
EDAR-F07	M, 6	AD, Portugal, CS	+	+	+		c.1186_1188dup	p.Q396dup
EDARADD								
EDARADD-F01	F, 11	AD, France	+	+	+	Dry skin, abnormal nails, palmo-plantar keratoderma	c.328G>T	p.D110Y

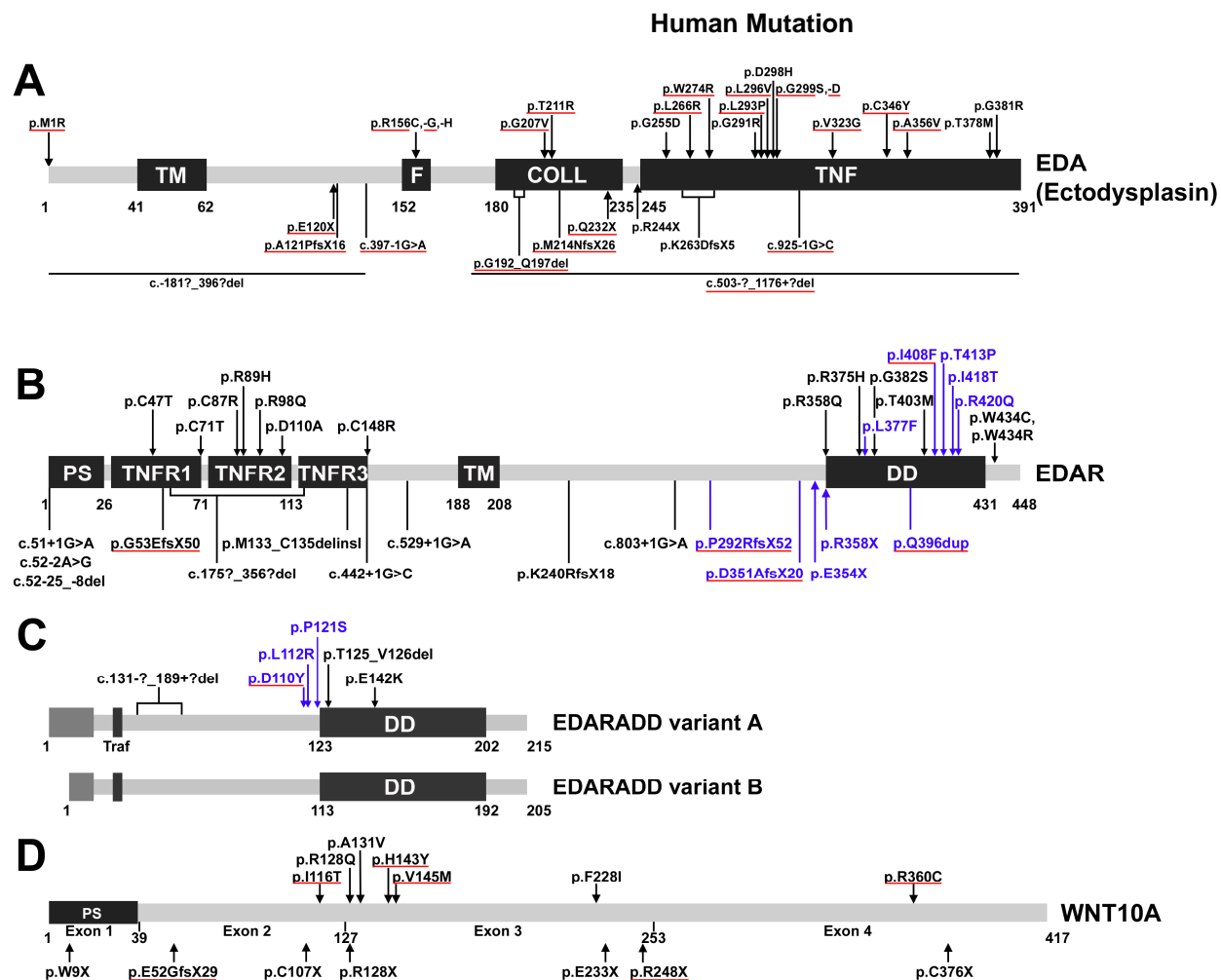
Mutations are annotated with +1 corresponding to the A of the ATG translation initiation codon, or with the initiation codon as codon 1 in the GenBank reference sequences (NM_001399.4, and NP_001390.1 for *EDA1*; NM_022336.3, and NP_071731.1 for *EDAR*; NM_080738.3 and NP_080738.3 for *EDARADD*). For the three genes: M male; F female; Spo sporadic (all other cases are familial), CS consanguinity, AR autosomal recessive, AD autosomal dominant; +, ++, and +++ degree of severity of present feature, - within normal clinical limits; C conical teeth.

Table 2: Clinical description of patients with mutations in *WNT10A* gene.

Proband's number	Patient	Sex, Age (year)	Origin	Hair	Teeth	Sweating	Other signs	Nucleic changes	Predicted proteic changes
HED/EDA phenotype									
WNT10A-F01	II.1	M, 46	France	-	-	-		c.682T>A	p.F228I
	II.3	F, 44	France	-	A	-		c.321C>A	p.C107X
	III.1	M, 17	France	-	A+	Hypo		c.[321C>A]+[682T>A]	p.C107X + p.F228I
WNT10A-F02	I.1	F, 70	France	-	A	Hypo		c.[682T>A](+)[682T>A]	p.F228I + p.F228I
	II.1	M, 49	France	-	D	-		c.682T>A	p.F228I
	II.2	F, 45	France	-	-	-		c.321C>A	p.C107X
	II.3	M, 46	France	-	-	-		c.321C>A	p.C107X
	II.4	F, 44	France	-	Ed	Hyper		c.682T>A	p.F228I
	II.5	F, 46	France	-	Ed, Sh	Hyper	Dry skin	c.682T>A	p.F228I
	II.6	M, 50	France	-	-	-		c.321C>A	p.C107X
	III.1	M, 18	France	-	A	-	Palmo-plantar keratoderma	c.[321C>A]+[682T>A]	p.C107X + p.F228I
WNT10A-F03	III.4	F, 16	France	-	A	-		c.[321C>A]+[682T>A]	p.C107X + p.F228I
	III.5	M, 24	France	-	A, D	-		c.[321C>A]+[682T>A]	p.C107X + p.F228I
	I.2	F, 63	France	-	D	Hypo		c.[321C>A]+[347T>C]	p.C107X + p.I116T
WNT10A-F07	II.2	F, 41	France	-	D	Hypo		c.321C>A	p.C107X
	III.1	M, 18	France	T	A+	Anh	Dry eyes, otitis	c.[321C>A]+[682T>A]	p.C107X + p.F228I
WNT10A-F08	III.2	M, 14	France	-	-	-		c.321C>A	p.C107X
	II.3	M, 44	France	Eb	A	Hypo		c.682T>A	p.F228I
WNT10A-F09	II.4	M, 44	France	Eb	A	Hypo		c.682T>A	p.F228I
	I.1	M, 42	France	-	C, Ed	-		c.682T>A	p.F228I
WNT10A-F09	II.2	F, 13	France	T	A	Ih		c.682T>A	p.F228I
	I.2	M, 77	France	-	-	Ih		c.682T>A	p.F228I
	II.2	F, 42	France	-	-	-		c.682T>A	p.F228I
	III.1	F, 16	France	-	M, A+	-		c.682T>A	p.F228I
	III.2	M, 12	France	-	M, A+	Ih	Inverted nipples, periorbital pigmentation	c.682T>A	p.F228I
WNT10A-S01	III.3	F, 9	France	-	M, A+	-		c.682T>A	p.F228I
	F, 29	Turkey, CS	T	M, A	Anh	Follicular keratosis		c.[433G>A](+)[433G>A]	p.V145M + p.V145M
	F, 18	China, CS	T	M, A	Hypo	Palmoplantar keratoderma		c.[742C>T](+)[742C>T]	p.R248X + p.R248X
	F, 9	France	Eb	M, A	Hyperthermia upon infections	Dry skin		c.427C>T	p.H143Y
WNT10A-S04	M, 6	France	T	M, A, Sh	Hypo		c.682T>A	p.F228I	

Proband's number	Patient	Sex, Age (year)	Origin	Hair	Teeth	Sweating	Other signs	Nucleic changes	Predicted proteic changes
OODD syndrome									
WNT10A-S05		M, 35	France	Eb	A	-	Slight onychodysplasia, palmoplantar keratoderma	c.146dupT	p.E52GfsX29
OODD-like syndrome									
WNT10A-F04	I.2	M, 89	France	Sp	?	?	Thick nails	c.682T>A	p.F228I
	II.1	F, 57	France	?	?	?		c.682T>A	p.F228I
	II.2	M, 48	France	-	S	-		c.682T>A	p.F228I
	III.2	F, 24	France	-	M,A+, C, D	-	Split nails, eczema	c.[682T>A]+[682T>A]	p.F228I + p.F228I
WNT10A-F05	I.2	F, 66	France	-	-	-		c.682T>A	p.F228I
	I.6	F, 58	France	-	Ed	-		c.682T>A	p.F228I
	II.1	F, 29	France	-	PT	-		c.682T>A	p.F228I
	II.2	F, 25	France	T	M,A+	-	Nail dystrophy	c.[682T>A](+)[682T>A]	p.F228I + p.F228I
Unclassified ED									
WNT10A-F06	I.1	F, 50	France	T	-	-	Eczema	c.1078C>T	p.R360C
	I.2	M, 47	France	-	Ed	Hyperhidrosis	Delayed puberty	c.682T>A	p.F228I
	II.1	F, 20	France	T	A	-		c.[682T>A]+[1078C>T]	p.F228I + p.R360C
	II.2	M, 17	France	T	A+, Ed	Hyperhidrosis upon infections	Soft nails, eczema, short stature (-2DS), delayed puberty	c.[682T>A]+[1078C>T]	p.F228I + p.R360C
	II.3	M, 13	France	-	A-	Hyperhidrosis upon infections	Eczema	c.[682T>A]+[1078C>T]	p.F228I + p.R360C

Mutations are annotated with +1 corresponding to the A of the ATG translation initiation codon, or with the initiation codon as codon 1 in the GenBank reference sequences (NM_025216.2, and NP_079492.2 for *WNT10A*). Proband (in bold) and their relatives figure in the table, all numbered from their pedigree presented in Figure 2. M male; F female; WNT10A-F01 to F09 are familial cases, WNT10A-S01 to S05 are sporadic; CS consanguinity; - within normal clinical limits, ? unknown information; A agenesis, A+ severe agenesis, M microdontia, Ed enamel dysplasia, D persistent deciduous teeth, C conical teeth, Sh abnormal tooth shape, PT palatal tooth; T thin and fragile hair, Eb rare eyebrows, Sp sparse hair; Ih intolerance to heat, Hypo hypohidrosis and Anh anhidrosis.



Supp. Figure S1: Distribution of previously known and newly identified mutations regarding to functional domains of EDA1 (A), EDAR (B), EDARADD (C) and WNT10A proteins (D). Missense mutations are indicated above the schematic representation of each protein, whereas nonsense, splice and truncating mutations are positioned underneath. Mutations with a dominant mode of inheritance in EDAR and EDARADD figure in blue; newly identified mutations are underlined in red. Ectodysplasin is mainly mutated in its furin cleavage site, its collagenous and its TNF domains. Mutations in EDAR are located either in the N-terminal ligand binding domain, or in the C-terminal Death Domain (DD). Interestingly, all dominant EDAR mutations are located in its C-terminal region. All EDARADD missense mutations are located in or very close to its Death Domain. TM: transmembrane domain; F: furin cleavage site; COLL: collagen domain; TNF: TNF-like domain; PS: peptide signal; TNFR1 to -3: TNF Receptor like domain; DD: Death domain. Reference sequences used to annotate all mutations are: NP_001390.1 (EDA1), NP_071731.1 (EDAR), NP_080738.3 (EDARADD), NP_079492.2 (WNT10A).

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A. EDA1

	G207	T211	L266	W274	L293	L296	G299	V323	C346	A356
Homo sapiens	GIPGIPGTTVM	KNDLSGG	LNDWSRI	SGELEVLVDGTYF	YEVVVDE	YNTCYTA	LLKARQK			
Pan troglodytes	GIPGIPGTTVM	KNDLSGG	LNDWSRI	SGELEVLVDGTYF	YEVVVDE	YNTCYTA	LLKARQK			
Macaca mulatta	GIPGIPGTTVM	KNDLSGG	LNDWSRI	SGELEVLVDGTYF	YEVVVDE	YNTCYTA	LLKARQK			
Mus musculus	GIPGIPGTTVM	KNDLSGG	LNDWSRI	SGELEVLVDGTYF	YEVVVDE	YNTCYTA	LLKARQK			
Rattus norvegicus	GIPGIPGTTVM	KNDLSGG	LNDWSRI	SGELEVLVDGTYF	YEVVVDE	YNTCYTA	LLKARQK			
Bos taurus	GIPGIPGTTVM	KNDLSGG	LNDWSRI	SGELEVLVDGTYF	YEVVVDE	YNTCYTA	LLKARQK			
Canis familiaris	GIPGIPGTTVM	KNDLSGG	LNDWSRI	SGELEVLVDGTYF	YEVVVDE	YNTCYTA	LLKARQK			
Gallus gallus	GIPGIPGTTVM	K---NGG	LNDWSRI	SGELEVLVDGTYF	PILVFQW	-----	-----			
Danio rerio	GIPGIPGSNAM	KEDLSEG	LKNWRMI	SGELEVLVDGTYF	YEVVMVDK	FNTCYTA	LLRARQR			
	*****:..*	* . *	*::*	*	*****:*****	:::				

B. EDAR

	I408
Homo sapiens	TAGYSIPELLT
Pan troglodytes	TAGYSIPELLT
Macaca mulatta	TAGYSIPELLT
Mus musculus	TAGYSIPELLT
Rattus norvegicus	TAGYSIPELLT
Canis familiaris	TAGYSIPELLT
Gallus gallus	TAGYSIPELLT
Xenopus laevis	TAGYSIPDLLT
Danio rerio	TAGYSIPDLLA
	*****:*:*:

C. EDARADD

	D110
Homo sapiens	LNDQDLLDV
Pan troglodytes	LNDQDLLDV
Macaca mulatta	LNDQDLLDV
Mus musculus	LNDQDLLDT
Rattus norvegicus	LNDQDLLDV
Bos taurus	LNDQDLLDV
Canis familiaris	LNDQDLLDV
Gallus gallus	LDDEDLLYT
Danio rerio	MNDEDLLYS
	::*:***

D. WNT10A

	I116	H143	V145	R360
Homo sapiens	TRNKIPYES	AGVVHAVSNAC	GTVGRLCNK	
Pan troglodytes	TRNKIPYES	AGVVHAVSNAC	GTVGRLCNK	
Macaca mulatta	TRNKIPYES	AGVVHAVSNAC	GTVGRLCNK	
Mus musculus	TRNKVPYES	AGVVHAVSNAC	GTVGRLCNK	
Rattus norvegicus	TRNKVPYES	AGVVHAVSNAC	GTVGRLCNK	
Bos taurus	TRNKIPYES	AGVVHAVSNAC	-----	
Canis familiaris	TRNKIPYES	AGVVHAVSNAC	GTVGRLCNK	
Gallus gallus	TKNKIPYES	AGVVHAVSNAC	GTQGRLCNK	
Xenopus laevis	TKNKIPYDS	AGVVHAVSNAC	GTQGRICNK	
Danio rerio	TRNKIPYES	AGVVHAVSNAC	GTQGRICNK	
Drosophila melan.	TKSRNPHAS	AGVAHSVARAC	GTVGRKCNR	
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Supp. Figure S2: Evolutionary conservation of residues with novel missense mutations in EDA1, EDAR, EDARADD and WNT10A proteins.

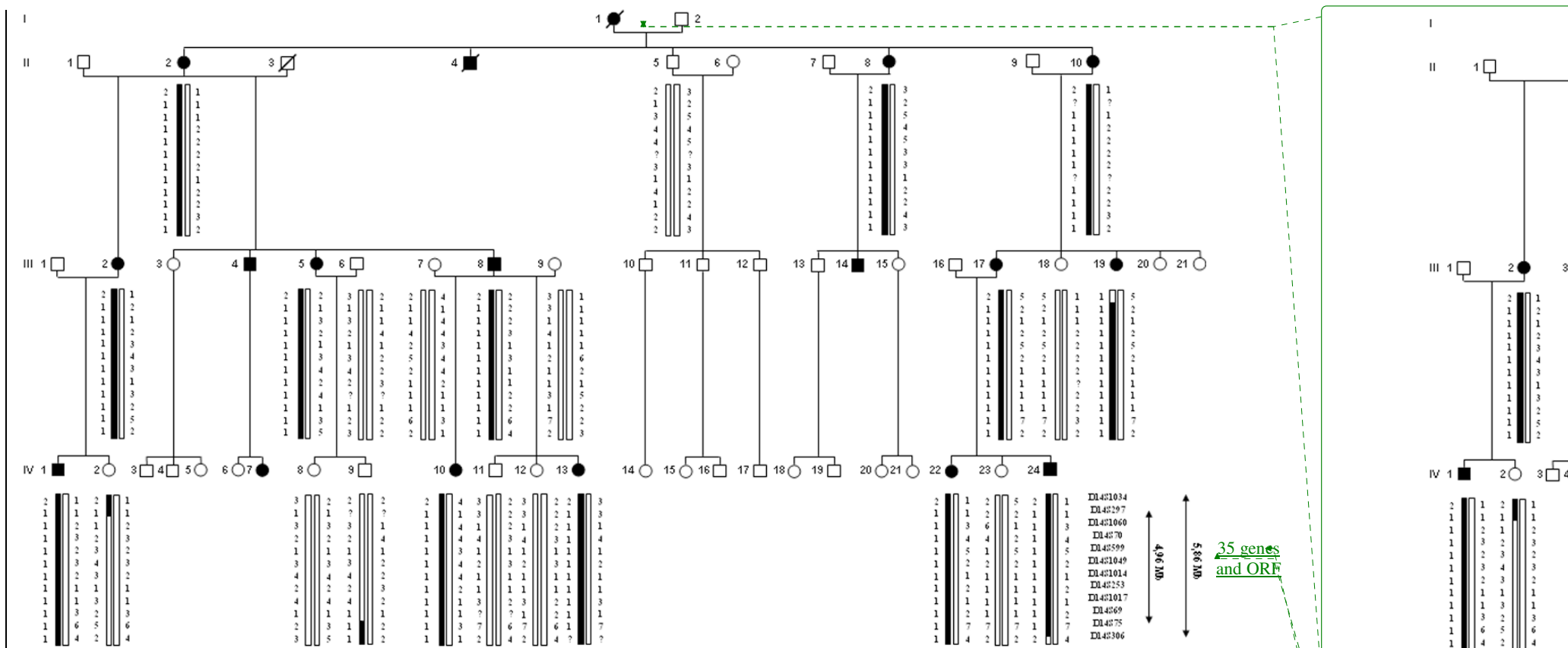
Alignments were performed using Clustalw program, using a BLOSUM matrix.

(A) Conservation of the 10 novel missense mutations in EDA1: sequences used for the alignment are NP_001390.1 (Homo sapiens), XP_529025.2 (Pan troglodytes), XP_001082424.1 (Macaca mulatta), NP_034229.1 (Mus musculus), XP_228582.5 (Rattus norvegicus), NP_001075212.1 (Bos taurus), NP_001014770.1 (Canis lupus familiaris), XP_420158.1 (Gallus gallus), NP_001108537.1 (Danio rerio).

(B) Conservation of the novel missense mutation on residue I408 in EDAR: sequences used for the alignment are NP_071731.1 (Homo sapiens), XP_001139583.1 (Pan troglodytes), XP_001084259.1 (Macaca mulatta), NP_034230.1 (Mus musculus), XP_345110.4 (Rattus norvegicus), XP_538426.2 (Canis familiaris), NP_001012629.1 (Gallus gallus), NP_001080516.1 (Xenopus laevis), NP_001108536.1 (Danio rerio).

(C) Conservation of the novel missense mutation on residue D110 in EDARADD: sequences used for the alignment are NP_542776.1 (Homo sapiens), XP_514291.2 (Pan troglodytes), XP_001099801.1 (Macaca mulatta), NP_598398.3 (Mus musculus), XP_574055.3 (Rattus norvegicus), XP_596273.2 (Bos taurus), XP_849101.1 (Canis familiaris), NP_001012405.2 (Gallus gallus), XP_695138.3 (Danio rerio).

(D) Conservation of the four novel missense mutations in WNT10A: sequences used for the alignment are NP_079492.2 (Homo sapiens), XP_516098.2 (Pan troglodytes), XP_001095740.1 (Macaca mulatta), NP_033544.1 (Mus musculus), NP_001101697.1 (Rattus norvegicus), NP_001092548.1 (Bos taurus), XP_545648.2 (Canis familiaris), NP_001006590.1 (Gallus gallus), ABG49498.1 (Xenopus laevis), NP_571055.1 (Danio rerio), NP_609109.2 (Drosophila melanogaster).



Supp. Figure S3: Haplotype analysis of the HED family for polymorphic markers on chromosome 14q12-13.1. Four recombinant events occurred in individuals III.19, IV.2, IV.9 and IV.24. Filled symbols represent affected individuals. The common disease-associated haplotype is shown in black.

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Supp. Table S1: Sequences of the primers used for direct sequencing of *EDAI*, *EDAR*, *EDARADD* and *WNT10A* genes, and QMPSF analysis for *WNT10A* gene.

Genes	Exons	Forward primers (5'-3')	Reverse primers (5'-3')	
<i>EDAI</i>	1	CGGAGTAGAGCTGCACATGCG	CCAGGGCAGGTTGTCTTCGGT	
	3	GGAGGGGAAGATGGGCTCAG	TGGTGGCTCACGCCTGTAAT	
	4	AGGAGTCAGAAGACAGAAATGG	AAGGGCAGGGAGAAGAACAAG	
	5	AGATCGTGCCACTGAACTCC	GCTCTCAGGATCACCCACTC	
	6	CCACTGAAGATGAAGGTCAGG	GCAAGACACCCTTTCCTTAGC	
	7	GGTCACATAGCTAGGAAGCGG	CTTTCAGCTCCGTCATCAGTG	
	8	CAGGCCTGGCAGCTGCTTTAC	TGGCCCCCTCTCTCTTTCCTC	
	9	GAACAATGCCTGTACCTGTCT	AAGTCAAGCAGGCCTTGTCAC	
	<i>EDAR</i>	2	CAGAGTCAGCCCAAGTGGCAT	GCTGTGTGTATCACACCACAAAC
3		CCAGGTGATCAACCAGGAGCC	ATGAATGCCTTAGCTGGTGAGTGC	
4		AGACAGCTGGCACGTCCTACT	ACAGGGGTTCATGGATACTGC	
5		CTGAGTGGACAGAGCAGGTG	AAGGCTCAGATGTGGCAAAC	
6		CAATAACGATGACTCTTTAGGG	GAGTTGATCCCTCTATGGGTG	
7-8		CCAGCGCGGAGGATTTGGTTC	CAGTATGGTTCAGCATGTGAGAG	
9		TGCTTGTGCTCTCACATGCT	CCAGTCAGCAAAGAGGTGGT	
10		GTGCCACAAGGTGCCAGT	CCCGTCTTGCAGGAGAGCTGA	
11		AGTCTGACCACCCAGCTGAGC	ATGCCTCCGATATCTGGGAAC	
12		CCTTCTATTGACTGTGACTTGCAACA	AGCTCCAGAGCCCTCGTTGG	
<i>EDARADD</i>		1a	GAAAGAACCACAAACCAAAACC	TGCCTTCACACATAAGAACAG
		1b	AGGTACCGAGGGACGCGC	GTTTGCAGGACGTGTCTCAAC
	2	AGTAAGGTTTTTCTTCAGCCTAAG	CCAGGGAAGTGGGTAAAGCC	
	3	CCTTGATTTTCATTCTGTGCGA	GTCACGAGCTAATCTATGGGC	
	4	ATCCTTAAGAGCAGAGTTTGG	CTGTTTATGATCTAGAAAATCCTG	
	5	GCGCTCAAGGTGCTCGTATTCT	TTACAGGCGCCCACCACAACC	
	6-1	TATGATGCTTTTGACAATTCAGCA	ACGAGCACAATTCGTCATAGGACA	
6-2	CGTGTACCCCAACGGTGAAAA	CCCCTCCACAAAACCTGCCAGC		
<i>WNT10A</i>	1	GAGTCGGAGCTGTGTGTCG	GAGCTCACTGCCTTTGGTTC	
	2	CTGGGCAGGATGATTGTGAG	CTGAGATCAGAAAAGAGGAAGG	
	3	TGATTTCTGCCCTTCTTTGAC	TGCACAGTGCATACTCAGTG	
	4	GGTACAGAAGTTCTTCTGACTG	AGAAGTGAGTGGTGGGGTTC	
<i>WNT10A-QMPSF</i>	1	GAGTCGGAGCTGTGTGTCG	FAM-AGCGCTGGCCGCGGCTGG	
	2	FAM-CTATGAGAGTCCCATCTTCAGC	CTGAGATCAGAAAAGAGGAAGG	
	3	TGATTTCTGCCCTTCTTTGAC	FAM-CGATGGCGTAGGCAAAAGCG	
	4	GGTACAGAAGTTCTTCTGACTG	FAM-CCACGAAACAGCACCAGTGG	
<i>GFAP-QMPSF</i>	3	GAGGAAAGGATTGATGGCCA	FAM-AAGAACCGGATCTCCTCCTC	
<i>MLH1-QMPSF</i>	18	FAM-GTAGTCTGTGATCTCCGTTT	ATGTATGAGGTCCTGTCCTA	

Supp. Table S2: Clinical description of non-mutated patients.

<u>Proband's number</u>	<u>Gender, age (year)</u>	<u>Ethnical origin</u>	<u>Hair</u>	<u>Teeth</u>	<u>Sweating</u>	<u>Other signs</u>
HED-F01	F, 18	France	++	+++	+++	Onychodystrophy
HED-F02	M, 11	Portugal	++	±	+++	Facial dysmorphism
HED-F03	M, 19	France	+++	++	+++	Telangiectasia on the face
HED-S01	M, 10	Spo, France	-	+++	++	
HED-F04	M, 10	France	++	++	++	

M male; F female; Spo sporadic (all other cases are familial); +, ++, and +++ degree of severity of present feature, - within normal clinical limits.

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