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Short Report

X-linked and autosomal recessive Hypohidrotic Ectodermal Dysplasia: genotypic-dental phenotypic findings from a retrospective study of 24 families

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Hypohidrotic ectodermal dysplasia (HED) is characterized by abnormal development of ectodermal structures and its molecular etiology corresponds to mutations of *EDA-EDAR* genes. The aim of this study was first to investigate the genotype and dental phenotype associated with HED and second, to explore possible correlations between dental features and molecular defects. A total of 27 patients from 24 unrelated families exhibiting clinical signs of HED (22 XLHED males, 5 autosomal recessive forms) were retrospectively included. In the sample, 25 different mutations on *EDA* and *EDAR* genes were detected; 10 were not previously described. *EDA* and *EDAR* mutations corresponded respectively to 80.0% and 20.0% of the mutations. The dental phenotype analysis revealed a mean number of primary and permanent missing teeth ranging respectively from 14.5 (4–20) to 22.5 (10–28); the majority of the patients exhibited dysmorphic teeth. Overall, no differential expression in the degree of oligodontia according to either the mutated gene, the mutated functional sub-domains, or the mutation type, could be observed. Nevertheless, the furin group exhibited severe phenotypes unobserved in the TNF group. Significant differences in the number of some primary missing teeth (incisor and canine) related to *EDA-EDAR* genes defects were detected for the first time between XLHED and autosomal recessive HED, suggesting differential local effects of *EDA-EDAR* genes during odontogenesis. The present genotypic-phenotypic findings may add to the knowledge of the consequences of the molecular dysfunction of EDA-NF-κB in odontogenesis, and could be helpful in genetic counseling to distinguish autosomal forms from other HED syndromes.

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Key words: Dental phenotype – *EDA* – *EDAR* – hypohidrotic ectodermal dysplasia – X-linked HED

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Introduction

Hypohidrotic ectodermal dysplasia (HED) is a heterogeneous condition characterized by developmental defects of ectoderm-derived organs (1). Phenotype is complex and associates hypotrichosis, sweat gland dysfunctions, nail and tooth defects, skin lesions and craniofacial dysmorphies. Oral

phenotype includes multiple congenitally missing teeth, root and crown dysmorphies, mainly conical-shaped incisors, slightly abnormal molar crown shape, and reduced saliva flow (2–4). Besides X-linked HED (OMIM 305100), autosomal dominant and recessive forms have been described (OMIM 224900) (5–9). Molecular etiology of HED consists of mutations in genes coding for the Ectodysplasin (EDA)-Ectodysplasin Receptor (EDAR)-Ectodysplasin Receptor Associated Death-Domain (EDARADD) complex, which is implicated in epithelial-mesenchymal interactions during ectodermal morphogenesis and odontogenesis (10–11). *EDA* gene is mutated in XLHED; *EDAR* and *EDARADD* genes are mutated both in autosomal dominant and recessive forms of HED (12–13). However, precise genotype-dental phenotype correlations have not been identified in HED (14–15).

The aim of this study was first to investigate the dental phenotype associated with HED, and second to determine a potential correlation between the severity of the observed dental features and (i) the involved gene (*EDA* or *EDAR*), (ii) the *EDA* mutation type (missense or truncating), (iii) or the *EDA* mutation location (TNF homology subdomain or furin proteolytic cleavage site).

Materials and methods

Study group

The patients were recruited from the National French Reference Center for Dental Manifestations of Rare Diseases (University Hospital, Strasbourg) and the Reference Center for Genodermatosis (Department of Dermatology, Necker-Enfants Malades Hospital, AP-HP, Paris), and were examined in their corresponding center. A total of 27 patients from 24 unrelated families exhibiting the clinical signs of HED were retrospectively reviewed. Mutations were identified either in *EDA* or *EDAR* genes. Genotyping was performed by the Department of Medical Genetics (Purpan University Hospital) and the Laboratory of Genetics (Necker-Enfants Malades Hospital, AP-HP, Paris) using protocols previously published (6, 12, 14). The study was approved by the Ethical Committee of Necker-Enfants Malades Hospital.

Inclusion criteria

The age of the HED patients included in the study ranged from 3 to 51 years. A clinical and molecular diagnosis of XLHED or autosomal recessive HED was undertaken for each patient. An appropriate informed consent was obtained from all the patients or their parents when they were under the age of 18.

Dental phenotypic analysis

All HED patients were followed-up clinically and radiographically by the same examiner using panoramic radiographs, intra-oral photographs and plaster casts. In addition, dental history was recorded to exclude tooth loss as a consequence of extraction or dento-alveolar trauma. Such follow-ups allowed for a phenotypic analysis of primary and permanent dentition in patients starting at 3 years of age. The analysis of permanent dentition in young children was therefore performed from the dental germs of permanent teeth visible on the panoramic radiograph. The identification of the existing teeth was based on topographic and morphological criteria. The analysis of crown shape anomalies was particularly focused on the incisors and molars. Both first and second molars were pooled for further analyses because the clinical and radiographic diagnoses of first or second molar agenesis are difficult given the malpositions and dysmorphologies of these teeth in HED. The relationships between phenotypic severity and gene defects-*EDA* or *EDAR* mutations, truncating or non-truncating *EDA* mutations, and *EDA* furin or TNF domains mutation location–were evaluated.

Genotyping and *EDA-EDAR* genes mutation analysis

EDA gene

Genomic DNA was prepared from venous blood using standard protocols. The eight exons of the *EDA* gene and the flanking intronic sequences were amplified by PCR using previously published primers (5). For SSCA analysis, denatured PCR products were separated on a denaturing MDE gel and run at 4°C under 2 conditions: overnight at 3W and 4–5 hours at 12W. For sequencing, PCR fragments were purified using QIAquick Gel Extraction and sequenced using the Perkin-Elmer Dye-terminator cycle sequence kit (Applied Biosystems, UK). Reactions were analyzed in an ABI373Strecht sequencer and sequence analysis was carried out using Max Vector Programs. Some mutations were screened by restriction enzyme digestion of PCR amplified exons, as R156H mutation abolish the *Fnu*4HI restriction site.

EDAR gene

The 12 *EDAR* gene exons with the exon-intron borders were amplified by PCR, the PCR products were subsequently purified using QIAquick Gel Extraction kits and sequenced using a Big Dye DNA sequencing kit (Applied Biosystems, UK). Reactions were analyzed in an ABI3100 sequencer.

Statistical analysis

Statistical analyses were performed with the Statistica 7.0® software (Statsoft, Tulsa, OK, USA). For each mutation, the mean number of missing teeth and the corresponding range of variation (minimum-maximum) were calculated. Comparisons of mutation-related total number of missing teeth in the primary and permanent dentitions were performed using Mann-Whitney non parametric test; similar comparisons were then carried out separately for maxillary and mandibular dentitions. For each mutation, pairwise comparisons between maxillary and mandibular number of agenesis in primary and permanent dentition were performed using a Wilcoxon test. For each tooth-type in each dentition, differences in mutation-related agenesis were then detected through a Mann-Whitney test.

Results

Mutation analysis

The diagnosed genotypes are listed in Table 1. In the sample studied, 25 different mutations on *EDA* and *EDAR* genes were detected in the 24 families; 10 were not previously described. The number and proportions of mutation types and locations affecting our cohort of patients are listed in details in Table 2. A majority of *EDA* mutations corresponded to missense mutations (60.0%) whereas nonsense and frameshifting mutations represented 10.0% and 15.0% of *EDA* pathogenic mutations. Two inframe deletions (10.0%) localized in exon 2 and exon 8 and one large genic deletion (5.0%) were also identified in our XLHED study group.

EDAR gene mutations were identified in 5 patients affected by autosomal recessive HED. These genetic defects consisted in a splice site mutation of exon 6 (cytoplasmic death domain), and two missense mutations localized respectively on exons 3–4 (ligand binding domain) and exon 12 (cytoplasmic death domain). Polymorphism of exons 5, 7, 9 and 11 was also detected in one family. The genetic defects in both heterozygous patients linked respectively exon 3 and exon 4 missense mutations with the same exon 6 splice mutation (Table 1). The three homozygous patients had exon 12 missense mutations. No mutations of *EDARADD* gene were included.

Novel mutations were detected in 10 families; most of them (9/10) concerned the *EDA* gene: 4 missense, 2 frameshift and 2 nonsense mutations were identified. In autosomal HED, the novel *EDAR* mutation corresponded to a missense change affecting exon 12. Precise descriptions of the defects are listed in Table 1.

Dental phenotype analysis

Dental features of the HED patients included in this study are summarized in Tables 1 and 3. Considering all HED patients, the mean number of primary and permanent congenitally missing teeth was respectively of 14.5 (range 4–20) and 22.5 (range 10–28). The majority of the patients exhibited dysmorphic primary and permanent teeth.

The number of missing teeth in the *EDA* and *EDAR* groups of patients presented no significant statistical differences. Nevertheless the autosomal recessive HED patients tended to exhibit slightly more tooth agenesis in the primary dentition (respectively an average of 16.5 and 14.0 primary missing teeth); this is observed either in the maxillary teeth than in the mandibular teeth (Table 3). In contrast, the XLHED patients tended to present slightly more agenesis in the permanent dentition (respectively an average of 22.8 and 21.0 permanent missing teeth).

Considering the major functional domains of EDA, the dental phenotype related respectively to the mutations altering the TNF homology domain or the furin recognition site presented no significant statistical differences. An absence of significant phenotypic difference was also observed between the *EDA* truncating and non-truncating mutations (Table 3).

Maxillary-mandibular differences in the number of primary or permanent tooth agenesis were significant within *EDA* and *EDAR* groups of patients ($p < 0.01$); the phenotype being systematically more severe in the mandible. Such discrepancy was also encountered for the TNF and furin mutation groups ($p < 0.05$).

Considering tooth-type affected by agenesis, only significant differences between *EDA* and *EDAR* mutations groups were demonstrated in the primary dentition (Fig. 1a,b). Agenesis of maxillary central incisor and canine, as well as mandibular canine were more frequent in the *EDAR* group of patients ($p < 0.05$). No differences were found between the TNF and furin mutations groups.

Table 1. *EDA* and *EDAR* gene mutations and the corresponding dental phenotypic findings

Family	Patient	Age (years)	Gene involved	Nucleotide change	Amino Acid change	Gene location	Mutation type	Domain	Mode of inheritance	Number of missing teeth in the primary dentition	Number of missing teeth in the permanent dentition	Frequency of crown shape anomaly in the permanent dentition
1	1	7	*ED1*	c.465 C>T	p.Arg155Cys	exon 2	Missense	Furin	X-linked	14	22	100%
2	**2**	5	*ED1*	c.466 C>T	p.Arg156Cys	exon 2	Missense	Furin	X-linked	15	25	100%
3	**3**	4	*ED1*	c.467 G>A	p.Arg156His	exon 2	Missense	Furin	X-linked	12	22	100%
4	**4**	8	*ED1*	c.457 C>T	p.Arg153Cys	exon 2	Missense	Furin	X-linked	14	22	100%
5	**5**	33	*ED1*	c.466 C>G	p.Arg156Gly	exon 2	Missense	Furin	X-linked	nd	26	nd
6	**6**	5	*ED1*	c.467 G>A	Arg156His	exon 2	Missense	Furin	X-linked	16	26	100%
6	**7**	4	*ED1*	c.467 G>A	Arg156His	exon 2	Missense	Furin	X-linked	20	28	nd
7	**8**	7	*ED1*	c.756 T>G	p.His252Gln	exon 6	Missense	TNF	X-linked	nd	10	33%
8	**9**	51	*ED1*	c.754 C>A	p.His252Asn	exon 6	Missense	TNF	X-linked	nd	20	nd
9	**10**	4	*ED1*	c.896 G>A	p.Gly 299Asp	exon 7	Missense	TNF	X-linked	18	26	100%
10	**11**	5	*ED1*	c.891 G>C	p.Asp298His	exon 7	Missense	TNF	X-linked	12	24	100%
10	**12**	4	*ED1*	c.891 G>C	p.Asp298His	exon 7	Missense	TNF	X-linked	14	23	100%
11	**13**	15	*ED1*	c.1048 G>T	p.Gly350Trp	exon 8	Missense	TNF	X-linked	nd	18	100%
12	**14**	3	*ED1*	c.1067 C>T	p.Ala356Val	exon 8	Missense	TNF	X-linked	4	nd	nd
13	**15**	40	*ED1*	c.503_526+del	p.Gly168_Gly 176Del.	exon 2	Del in frame	Furin	X-linked	nd	21	100%
14	**16**	3	*ED1*	Mutation 9bp	9 bp Del.	exon 8	Del in frame	TNF	X-linked	19	28	nd
15	**17**	9	*ED1*	c.503- ?_ 1176+ ? del	p.Gly168_Ser 391Del.	exons 3-8	Large Del	TNF	X-linked	14	23	100%
16	**18**	7	*ED1*	c.149 C>A	p.Ser50X	exon 1	Nonsense	Transmb	X-linked	16	23	100%
17	**19**	3	*ED1*	c.694 C>T	p.Gln232X	exon 4	Nonsense	Collagen	X-linked	13	23	100%
18	**20**	5	*ED1*	c.579delT	p.Pro193Profs86X	exon 4	Frameshift	Collagen	X-linked	20	28	nd
19	**21**	3	*ED1*	c.572−590del	p.Pro191Argfs82X	exon 4	Frameshift	Collagen	X-linked	8	nd	nd
20	**22**	9	*ED1*	c.884delT	p.Val295Aspfs12X	exon 7	Frameshift	TNF	X-linked	20	23	nd
21	**23**	6	*EDAR*	c.140 G>A c.528+1G>A	p.Cys47Tyr n.d.	exon 3 exon 6	Missense Splice	LBD	AR	16	25	100%
22	**24**	5	*EDAR*	c.266 G>A c.528+1G>A	p.Arg89His n.d.	exon 4 exon 6	Missense Splice	LBD	AR	14	18	100%
23	**25**	4	*EDAR*	c.1208C>T c.1208C>T	p.Thr403Met p.Thr403Met	exon 12 exon 12	Missense	DD	AR	16	24	nd
23	**26**	2	*EDAR*	c.1208C>T c.1208C>T	p.Thr403Met p.Thr403Met	exon 12 exon 12	Missense	DD	AR	20	nd	nd
24	**27**	15	*EDAR*	c.1302G>T c.1302G>T	p.Trp434Cys p.Trp434Cys	exon 12 exon 12	Missense	DD	AR	nd	17	nd

nd: non defined; transmb.: transmembrane domain of Ectodysplasin; Del: deletion; AR: autosomal recessive mutation. LBD: Ligand Binding Domain, DD: Death-domain. The new mutations are highlighted in grey. *ED1* Reference Sequence NM_001399 and *EDAR* Reference Sequence NM_022336

Table 2. Number and proportions of HED patients recruited according to *EDA-EDAR* mutation types and locations.

Gene involved							
EDA							*EDAR*
Type of mutations							Type of mutations
Truncating mutations	Non-truncating mutations	Missense Furine	Missense TNF			All *EDA* mutations	Missense/Splice mutations
6 27.2%	16 72.7%*	7 31.8%*	7 31.8%*			22 81.5%	5 18.5%
		Exon 2 7 31.8%	Exon 6 2 9.1%	Exon 7 3 13.6%	Exon 8 2 9.1%		

*For the different types of *EDA* mutations, the corresponding proportions are calculated according to all *EDA* patients; for *EDA-EDAR* patients, the proportions are calculated according to the whole cohort of HED patients.

Table 3. Number of congenitally missing teeth in primary and permanent dentitions according to HED patient genotype

		Number of congenitally missing teeth according to genotype						
		Gene involved						
		EDA					*EDAR*	All mutations considered together
		Type of mutations					Type of mutations	
Type of dentition	Localization	Truncating mutations	Non-truncating mutations	Missense TNF	Missense Furine	All domains	Missense Splice	
Primary dentition	Maxilla	6.0 (4–10)	6.0 (2–10)	5.0 (2–8)	6.3 (4–10)	6.1 (2–10)	7.5 (6–10)	6.4 (2–10)
	Mandible	8.2 (4–10)	8.2 (2–10)	7.4 (2–10)	8.8 (8–10)	7.8 (2–10)	9.5 (8–10)	8.1 (2–10)
	Full mouth	14.2 (8–20)	14.0 (4–20)	10.0 (4–14)	15.1 (12–20)	14.0 (4–20)	16.5 (14–20)	14.5 (4–20)
Permanent dentition	Maxilla	11.0 (9–14)	10.5 (3–14)	8.6 (3–12)	11.8 (10–14)	10.5 (3–14)	9.5 (7–12)	10.3 (3–14)
	Mandible	13.0 (12–14)	12.3 (7–14)	11.8 (7–14)	12.5 (12–14)	12.2 (7–14)	11.0 (10–14)	12.0 (7–14)
	Full mouth	24.0 (23–28)	22.5 (10–28)	19.0 (10–24)	24.4 (22–28)	22.8 (10–28)	21.0 (17–25)	22.5 (10–28)

For each type of mutations, the mean number of congenitally missing teeth and the corresponding range of variation (min-max) are respectively given.

Discussion

Genetic epidemiology

This study reports genetic mutations linked to HED in a group of 27 patients from 24 unrelated families. Most of the mutations are *EDA* mutations (80.0 %), *EDAR* gene defects being reported in 20.0 % of the sample. Surprisingly, our cohort of HED patients includes an exceptional number of individuals affected by *EDAR* mutations (12). This may be due to the inclusion protocol of the National French Reference Center for Genodermatosis (Department of Dermatology, Necker-Enfants Malades Hospital, AP-HP, Paris) which recruits patients with this type of syndrome from all over French territory.

Of the *EDA* mutations encountered in the present study, a large proportion (60.0%) corresponds to missense mutations whereas 15.0% are frameshift mutations, 10.0% are nonsense, 10.0% are inframe deletions and 5.0% represent large deletion. This result confirms the high prevalence of missense mutations (14, 16–19). The high frequency of defects located in exon 2 (30.0%) emphasizes the existence of a mutational hot spot in the *EDA* gene (14).

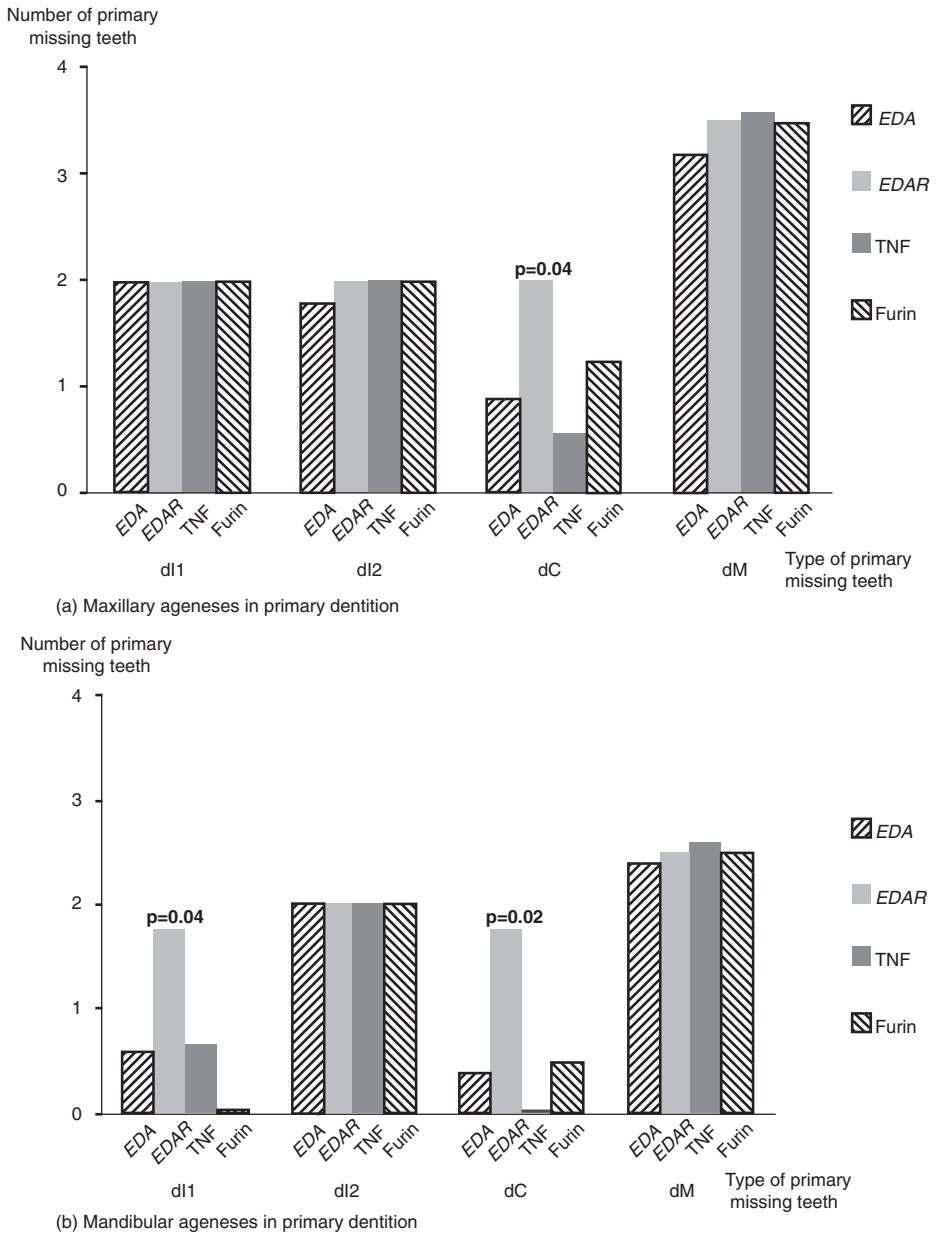

Fig. 1. Topographic distribution of dental agenesis in primary dentition for HED patients. a) Maxillary agenesis in individuals exhibiting mutations of *EDA* gene ($n = 14$), *EDAR* gene ($n = 4$), EDA-A1 TNF sub-domain ($n = 4$) and EDA-A1 furin recognition site ($n = 4$). b) Mandibular agenesis in individuals exhibiting mutations of *EDA* gene ($n = 17$), *EDAR* gene ($n = 4$), EDA-A1 TNF sub-domain ($n = 6$) and EDA-A1 furin recognition site ($n = 4$). dI1: deciduous central incisor; dI2: deciduous lateral incisor; dC: deciduous canine; dM: deciduous molars. In bold, significant differences in tooth-type agenesis number between *EDA* and *EDAR* mutations groups (results of the Mann-Whitney test).

The novel mutations identified in the present study correspond to 40.0 % of the *EDA-EDAR* genes mutations. This finding paralells the 41.1% detection rate of novel mutations observed by Vincent et al. (2001) (14).

The missense mutations (4/10 novel mutations) correspond to the majority of these novel mutations (13, 18, 20–24). These genetic defects involve systematically exons 2 or the coding regions of exons 6–8, as previously reported (3, 13, 14, 17, 20). The two nonsense mutations of *EDA* gene exons 1 and 4 identified in our study are both novel variants (Table 1). Novel frameshift mutations are detected on *EDA* gene exon 4 and for the first time on exon 7 (Table 1). Concerning *EDA* gene inframe deletions, the prevalence observed in our study (10.0 %) appears relatively lower than those previously described, *i.e.* 21.0% and 28.2% (14,

23). The exons 2 and 8 inframe deletions identified in our group of patients remain relatively unfrequent (14), exons 1 and 4 being predominantly affected (14, 23). The large deletion encompassing exons 3–8 is unique. Most of them are known to involve indeed between 1 and 3 exons (5, 18, 25). Interestingly, Vincent et al. (2001) (14) described a large deletion involving all the *EDA* gene exons. However, these genetic defects exhibit low prevalences, *i.e.* 5.5% and 5.9% as respectively reported in the literature (14, 18).

Concerning *EDAR* gene, most of the mutations are missense mutations (4/5) (Table 1). This finding is in accordance with the proportion of 72.2% of missense mutations reported by Chassaing *et al.* (2006) (12). Compound heterozygous mutations of *EDAR* gene are rare (12, 26, 27). The allelic association–exon 3 and exon 4 missense mutations with the same exon 6 splicing alteration–was not previously described. Moreover, a novel missense mutation–c.1302 G>T of *EDAR* gene exon 12–was identified in a family affected by an autosomal recessive form of HED (Table 1). A very uncommon clinical situation concerns the familly 23 with two brothers affected by an autosomal recessive form of HED. They are homozygous with the same *EDAR* gene exon 12 c.1208 C>T missense mutation (Table 1). Interestingly, the heterozygous mother exhibited moderate dental phenotypic manifestations. Such original observation underlines the necessity of clinical investigation in all the relatives of families affected by an autosomal recessive HED form.

Dental phenotypic manifestations in X-linked and autosomal recessive HED.

Oligodontia in both primary and permanent dentitions is frequently encountered in HED (28). Nevertheless, the degree of oligodontia affecting our patients appears more severe than that previously reported in the literature. An average of 14.5 primary missing teeth was found in our HED sample compared to the 8.8 dental ageneses reported by Barberia et al. (2006) (29). In permanent dentition, a severe oligodontia is observed (an average of 22.5 missing teeth in our group); the degree of oligodontia seems also to be more severe than previously reported in the literature, *i.e.* Prager et al. (2006) (2) reported an average of 13.8 absent teeth. A hypothesis for interpreting this relative phenotypic discrepancy could be the possible inclusion in previous studies of other forms of HED as those associating *EDARADD* or *NEMO* genes mutation for example.

A phenotypic similarity in the degree of oligodontia between X-linked HED and autosomal recessive HED is observed, as suggested by the absence of statistical differences in the corresponding average number of missing teeth (Table 3). Our observation confirms previous dental findings supporting a similar severity of oligodontia in both autosomal and XLHED forms (9, 12). Moreover, the severity of the dental manifestations seems to parallel the general clinical picture found in these two HED forms.

According to the functional domains of EDA-A1 mutated, no differential dental phenotypic expression was observed between the furin recognition site and TNF domain mutations in the primary or the permanent dentitions. Nevertheless, the dental agenesis range of variation seems to be more important in the TNF group than in the furin group, especially regarding the mandible in primary dentition and both jaws in permanent dentition. Besides, the TNF group exhibits moderate phenotypes, remaining unobserved in the furin group. No considerations about these between-group variations have been mentioned anteriorly in the literature. A molecular hypothesis could be that mutations in the TNF homology sub-domain can lead to partial or total inhibition of the EDA-A1-EDAR interaction, regarding the mutation location or the molecular tridimensional alterations of the binding site (18, 30). The most severe degrees of oligodontia in the TNF group involve exon 7 missense mutations, suggesting a major role of the proteic sequence in the binding to EDAR or in the TNF domain folding. In furin group, the most severe oligodontia seem to be associated with *EDA* exon 2 missense mutations affecting residue 156 (Table 1); this functionally important amino-acid is indeed located on the two overlapping furin recognition sequences (RVRR and RNKR spanning aa 153–156 and 156–159, respectively) (24). Mutations in these sequences lead to important alterations in the initiation process of odontogenesis (31–32) and thus to severe oligodontia.

Concerning the autosomal recessive form of HED, no apparent discrepancy in the dental phenotype could be detected between the two functional domains of EDAR mutated–Ligand Binding Domain and Death-Domain. Since similar observations are reported in Chassaing et al. (2006) (12), it could be hypothesized that both of these molecular domains appear to be characterized by the same functional importance in the dental morphogenetic functions of the EDA-EDAR-NF-κB pathway.

Non-truncating mutations and truncating mutations exhibit no phenotypic differences. This is also reported by Lexner et al. (2008) (4), patients with truncating and non-truncating *EDA* mutations exhibiting all severe oligodontia. As truncating mutations lead to non-functional EDA-A1 isoform,

the consequence of these molecular defects could be severe dental phenotypes. Interestingly, such dental phenotypes are also encountered in the non-truncating mutations (Table 1). This last type of mutation encoding for a functional EDA-A1 isoform affects indeed important functional domains as proteolytic cleavage and EDA-A1-EDAR binding sites known to be involved in early steps of odontogenesis (14, 24).

A more severe oligodontia in the mandible than in the maxilla is systematically found irrespective of the type of dentition (primary or permanent dentitions), the gene impaired (*EDA-EDAR*) and the functional domain affected (furin or TNF domains) (Fig. 1). This discrepancy in maxillary-mandibular ageneses mirrors a preferential alteration of mandibular odontogenesis and confirms the exitence of a differential molecular regulation in maxillary and mandibular odontogenesis, each of these morphogenetic process being related to a specific odontogenic homeobox code (33). Indeed, some homeotic genes, as *Alx3*, *Dlx5/6* or *Msx1/2* are expressed preferentially in the mandibular ectomesenchyme and their expression could be particularly altered by the molecular dysfunction of the EDA-NF-κB signaling pathway (34).

Primary dentition exhibits significant differences in tooth-type agenesis number according to *EDA-EDAR* gene defects (Fig. 1). The maxillary central incisors and canines as well as the mandibular canines seem to be more frequently absent in autosomal recessive HED. This finding suggests differential local effects of *EDA-EDAR* genes in odontogenesis of the primary dentition. Such an original phenotypic observation could provide an interesting phenotypic marker of the *EDAR* mutations.

In novel *EDA-EDAR* mutations, it is interesting to note that close genetic defects affecting the same amino-acid are responsible of very distinct dental phenotypic manifestations. Exon 6 mutations c.754 C>A and c. 756 T>G lead indeed respectively to minor and moderate oligodontia; 10 congenitally missing teeth are observed in the c.756 T>G mutation and 20 missing teeth are linked to the c.754 C>A mutation (Table 1). Such data emphasize the strong phenotypic variability in XLHED even in a context of close molecular status, and a potential role of epigenetic factors might be evoked for interpreting the previous differential phenotypic expression (35).

The large *EDA* gene deletion involving exons 3–8 is responsible for severe dental phenotypic manifestations in primary and permanent dentitions (Table 1). Moreover, this defect leads also to severe sweat glands dysfunction, hypotrichosis and facial dysmorphies. A nearly identical clinical picture has been described in an individual affected by an *EDA* gene complete deletion (14). The exons 3-8 deletion leads to the loss of the EDA-A1 collagen and TNF sub-domains interfering with the trimerization and EDAR binding. The resulting severe molecular anomalies seem therefore to induce marked dental phenotypic manifestations, emphasizing thus the major role of these different proteic domains in odontogenesis.

In conclusion, this detailed analysis of dental anomalies in our cohort of HED patients screened for *EDA-EDAR* mutations allows for a better understanding of the phenotypic variations associated with these genetic defects. Novel mutations are also depicted and their corresponding dental and general phenotypic expression described. Overall, no phenotypic difference could be put in an obvious place as well between X-linked and autosomal recessive HED, as between the two functional sub-domains of EDA-A1 mutated or else between the different types of mutations. Nevertheless, subtle differences seem to exist: the TNF group exhibits some moderate phenotypes that remain unobserved in the furine group. A more severe oligodontia in the mandible than in the maxilla is systematically found irrespective of the type of dentition, the gene impaired and the functional domain affected. Besides, primary dentition exhibits significant differences in tooth-type agenesis number related to *EDA-EDAR* genes defects, suggesting differential local effects of *EDA-EDAR* genes in odontogenesis of the primary dentition. This original phenotypic observation could provide an interesting phenotypic marker of the autosomal form of HED and more generally, it may add to the knowledge of the consequences of the molecular dysfuntion of EDA-NF-κB signaling pathway in the odontogenetic processes.

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Conflict of interest

All authors state that there are no actual or potential conflicts of interest in the present study,

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