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RESEARCH ARTICLE

Comparative analysis of rare *EDAR* mutations and tooth agenesis pattern in *EDAR*- and *EDA*-associated nonsyndromic oligodontia

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Abstract

Nonsyndromic oligodontia is a rare congenital anomaly. Mutations in the *ectodysplasin A receptor* (*EDAR*) gene are the primary cause of hypohidrotic ectodermal dysplasia but are rarely reported in nonsyndromic oligodontia. This study investigated *EDAR* mutations in multiplex nonsyndromic oligodontia and comparatively analyzed the *EDAR*- and *EDA*-related tooth agenesis patterns. Mutation screening was carried out using whole-exome sequencing and familial segregation. Evolutionary conservation and conformational analyses were used to evaluate the potential pathogenic influence of *EDAR* mutants. *EDAR* mutations were found to occur in 10.7% of nonsyndromic oligodontia cases. We reported seven heterozygous mutations of *EDAR*, including five novel mutations (c.404G>A, c.871G>A, c.43G>A, c.1072C>T, and c.1109T>C) and two known mutations (c.319A>G and c.1138A>C). Genotype–phenotype correlation analysis demonstrated that the *EDAR*-related tooth agenesis pattern was markedly different from *EDA*. The mandibular second premolars were most frequently missing (57.69%) in *EDAR*-mutated patients. Our results provide new evidence for the genotypic study of nonsyndromic oligodontia and suggest that EDAR haploinsufficiency results in nonsyndromic tooth agenesis. Furthermore, the distinct pattern between *EDAR*- and *EDA*-related tooth agenesis can be used as a guide for mutation screening during the clinical genetic diagnosis of this genetic disorder.

KEYWORDS

ectodysplasin A, *ectodysplasin A receptor*, EDAR haploinsufficiency, genotype–phenotype analysis, nonsyndromic oligodontia

Liutao Zhang and Miao Yu contributed equally to this study.

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1 | INTRODUCTION

Oligodontia, a severe form of tooth agenesis, is a relatively rare congenital anomaly distinguished by the developmental failure of six or more teeth (excluding the third molars; Nieminen, 2009). The prevalence rate of oligodontia ranges from 0.08% to 0.14% according to studies on different populations (Dhamo et al., 2018; Schalk-van der Weide, Beemer, Faber, & Bosman, 1994). In line with other types of tooth agenesis, namely hypodontia (less than six missing teeth) and anodontia (total agenesis of teeth), oligodontia is presented either as an isolated trait that only affects the dentition (nonsyndromic oligodontia), or as an oral manifestation of multiple clinical syndromes (syndromic oligodontia), such as hypohidrotic ectodermal dysplasia (HED; Chhabra, Goswami, & Chhabra, 2014; De Coster, Marks, Martens, & Huysseune, 2009; Visinoni, Lisboa-Costa, Pagnan, & Chautard-Freire-Maia, 2009).

Genetic factors implicated in tooth development play a major role in both nonsyndromic and syndromic oligodontia (M. Yu, Wong, Han, & Cai, 2019). Compared to a majority of syndromic oligodontia which exhibit explicit etiologies (M. Yu et al., 2019), the pathogenic genes in nonsyndromic oligodontia have not yet been fully identified (Wong et al., 2018). To the best of our knowledge, only 11 genes have been associated with nonsyndromic oligodontia thus far (Massink et al., 2015; Ruf, Klimas, Honemann, & Jabir, 2013). Among them, *PAX9*, *MSX1*, *EDA*, *AXIN2*, and *WNT10A* are the most frequent causal genes, while the six other genes, namely *EDAR*, *EDARADD*, *KRT17*, *NEMO*, *LRP6*, and *WNT10B*, have been rarely associated with nonsyndromic oligodontia (Massink et al., 2015; Sun et al., 2019; M. Yu et al., 2019; P. Yu et al., 2016). Therefore, there is a need to identify novel candidate genes or mutations associated with nonsyndromic oligodontia.

Mutations in members of the ectodysplasin A (EDA)/ectodysplasin A receptor (EDAR)/nuclear factor-κB (NF-κB) pathway, such as EDA, EDAR, and EDAR-associated death domain (EDARADD), are associated with HED-related tooth agenesis (Bashyam et al., 2012; Feng et al., 2018; Wohlfart, Soder, Smahi, & Schneider, 2016). The EDA/EDAR/NF-κB pathway has been previously found to be required for normal embryogenesis, particularly in the development of tooth, hair, skin, and other ectodermal organs (Sadier, Viriot, Pantalacci, & Laudet, 2014). In this pathway, EDAR functions as a core member, interacting with its ligand, EDA, and its adaptor, EDARADD, which results in the downstream activation of NF-κB signaling (Okita, Asano, Yasuno, & Shimomura, 2019). EDAR contains an extracellular ligand-binding domain (LBD), a single transmembrane region, and an intracellular death domain (DD), enabling it to function as a transmembrane factor (Sadier et al., 2014).

Although approximately 70 *EDAR* mutations have been identified in autosomal dominant or recessive HED patients (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/), 10 mutations have been identified in nonsyndromic tooth agenesis (Eisenberg et al., 2013; Yamaguchi et al., 2017; Zeng et al., 2017). Among these, only four *EDAR* mutations were found to be associated with nonsyndromic oligodontia, including one nonsense mutation (c.73C>T; p. Arg25*) and three missense mutations (c.973C>T; p. Arg325Trp, c.1135G>A; p. Glu379Lys, c.1172T>A; p. Met391Lys). Previously, we showed an association between EDAR polymorphisms and nonsyndromic oligodontia in a Chinese population (Chen, Liu, Han, Liu, & Feng, 2017). Therefore, we hypothesize that *EDAR* is a reliable causative gene for nonsyndromic oligodontia, and the *EDAR* mutation spectrum and the phenotypic variability of *EDAR*-associated nonsyndromic oligodontia need to be further explored.

In this study, we performed mutation screening in a cohort of 112 unrelated patients with nonsyndromic oligodontia, and identified five novel mutations (c.404G>A, c.871G>A, c.43G>A, c.1072C>T, and c.1109T>C) and two known mutations in *EDAR*. Tertiary structural prediction suggested that the conformational changes observed in the mutants might impair EDAR function. Furthermore, comparative analysis of the characteristics of tooth agenesis in *EDAR*- and *EDA*-related nonsyndromic oligodontia indicated that *EDAR* can be a candidate gene for the genetic screening of nonsyndromic oligodontia. This study is the first to report the phenotypic comparison between *EDAR*- and *EDA*-related tooth agenesis.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

Informed consent was obtained from the participants or the parents or guardians of the patients for the mutational analyses and clinical photographs. This study was approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-201736082).

2.2 | Subjects

A cohort of 112 unrelated patients with nonsyndromic oligodontia (61 males and 51 females between 3 and 42 years of age) from 2008 to 2019, were recruited to participate in this study from the Department of Prosthodontics, Peking University School of Stomatology. The patients all confirmed that their missing permanent teeth were not due to extraction or injuries. The diagnosis of permanent tooth agenesis was confirmed using panoramic radiographs. For accessing patients' deciduous dentitions, we backtracked their pediatric dental records, and inquired the patients' parents about the status of the primary dentition. During dental comprehensive examinations, the dental specialist also visually inspected patients' ectodermal organs, such as hair, skin, and nails, and asked if the patient had normal sweating.

2.3 | Whole-exome sequencing and mutation screening

Genomic DNA was isolated from peripheral blood lymphocytes using the Universal Genomic DNA Kit (ComWin Biotech) according to the

manufacturer's protocols and was delivered to the ANGEN Gene Medicine Technology Company (ANGEN) for whole-exome sequencing analysis. The selection criteria of the pathogenic gene mutations were as follows: (1) orodental development-related genes were included in screening (Prasad et al., 2016); (2) both *inDels* (short insertions or deletions) and single-nucleotide polymorphisms (nonsense, missense, and splicing mutations) with minor allele frequency ≤ 0.01 in Exome Aggregation Consortium (http://exac.broadinstitute.org), 1000 Genomes (http://www.1000genomes.org), and Genome Aggregation database (http://gnomad.broadinstitute.org) were selected; (3) the functional impact was preliminarily predicted using MutationTaster (http://www.mutationtaster.org), Sorting Intolerant from Tolerant-SIFT (http://sift.jcvi.org/), and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) to confirm the pathogenicity.

2.4 | Mutation analysis and familial cosegregation analysis

In total, seven predicted causative mutations of *EDAR* (RefSeq NM_022336.4) were identified based on the selection criteria. The probands were subjected to targeted Sanger sequencing. Cosegregation analysis was conducted in attainable nuclear family members to validate candidate mutations. In total, 11 coding exons and intron–exon boundaries of *EDAR* were amplified by polymerase chain reaction (PCR). The PCR products were sent to RuiBiotech Company for direct sequencing. PCR primers and conditions (Table S1) were designed using Primer-Blast tools (National Center for Biotechnology Information).

2.5 | Conservation analysis

The amino acid sequences of EDAR among different species, from zebrafish to humans, were obtained from UniProt (https://www.uniprot.org). Evolutionary conservation analysis of the missense mutations was performed on the Multiple Sequence Alignment Server (T-coffee, http://tcoffee.crg.cat).

2.6 | Three-dimensional structural analysis

Three-dimensional (3D) homo structures of the LBD (from amino acid 1 to 151) and DD domains (from amino acid 343 to 426) of wild-type EDAR were separately established via homology modeling using the SWISS-MODEL (https://swiss-model.expasy.org). The two best templates for the homology modeling of the LBD and DD were used, as previously described (Parveen et al., 2019). Subsequently, the conformational changes of the EDAR mutations were visualized using the PyMOL software (PyMOL Molecular Graphics System; DeLano Scientific).

2.7 | Analysis of tooth agenesis pattern in nonsyndromic individuals with EDAR or EDA mutations

To study the tooth agenesis pattern caused by *EDAR* mutations, we obtained data on 17 patients (10 males and 7 females) with defined *EDAR* mutations from our medical record database and 9 patients with detailed tooth agenesis sites reported in 2 previous studies (Eisenberg et al., 2013; Zeng et al., 2017). We also included data on 84 patients with *EDA*-mutations (69 males and 15 females) with detailed documentation of tooth agenesis sites (or with panoramic film) from 14 studies. All the patients included were nonsyndromic. The positions of congenital tooth agenesis were compiled in the upper and lower arches, combined with the left and right sides (upper right quadrant, upper left quadrant, lower left quadrant, and lower right quadrant). The total number of missing teeth in the four quadrants were counted to compare the differences between the average rate of tooth agenesis of the upper and lower arches, as well as the left and right sides. Moreover, the number of missing teeth at each tooth position among the patients was counted to determine the prevalence of tooth agenesis in different positions. Statistical analysis was performed using the χ^2 test using SPSS 24.0 and Prism 8. A $p < .05$ was considered statistically significant.

3 | RESULTS

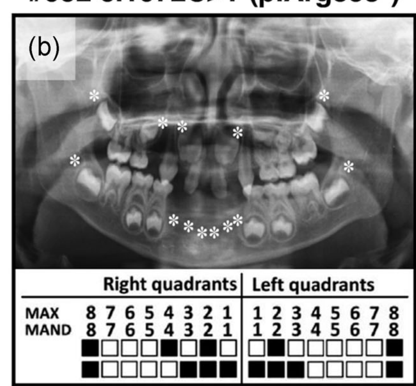
3.1 | Mutational analysis and tooth agenesis phenotypic findings

All affected patients with oligodontia appeared to have normal facial features, hair, skin, sweat glands, and nails according to our patient database and through the follow-up examinations. Therefore, patients with syndromic tooth agenesis were excluded from this study. In total, four distinct heterozygous *EDAR* mutations (10.7%) not found in healthy controls ($n = 100$) were detected in 12 of the 112 unrelated patients with nonsyndromic oligodontia. All 12 patients had agenesis of six or more teeth (excluding the third molars) and thus were diagnosed with nonsyndromic oligodontia (Figure 1). These four mutations, consisting of one heterozygous nonsense mutation (c.1072C>T; p. Arg358*) and three heterozygous missense mutations (c.404G>A; p. Cys135Tyr, c.1109T>C; p. Val370Ala, and c.319A>G; p. Met107Val) are presented in Figures S1a–c and S1g). In addition, three heterozygous missense mutations (c.871A>G; p. Ala291Thr, c.43G>A; p. Val15Ile, and c.1138A>C; p. Ser380Arg), which were identified in our previous study, were included in the genotype–phenotype correlation analysis, resulting in seven mutations (Figure S1d–f). Notably, five of these seven mutations (c.1072C>T, c.404G>A, c.1109T>C, c.871A>G, and c.43G>A) were previously unreported. A summary of the location and potential pathogenicity of these mutations is provided in Figure 2a.

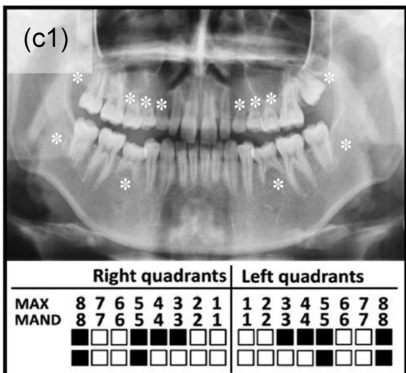
#945 c.404G>A (p.Cys135Tyr)

(a)
Right quadrants Left quadrants
MAX 8 7 6 5 4 3 2 1 1 2 3 4 5 6 7 8
MAND 8 7 6 5 4 3 2 1 1 2 3 4 5 6 7 8

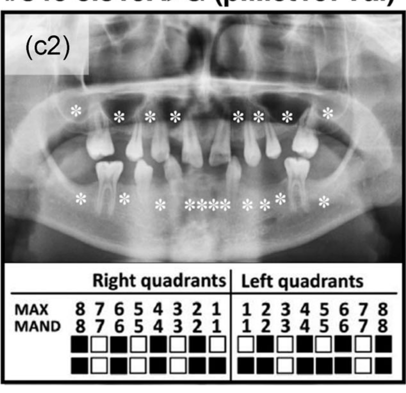
#952 c.1072C>T (p.Arg358*)



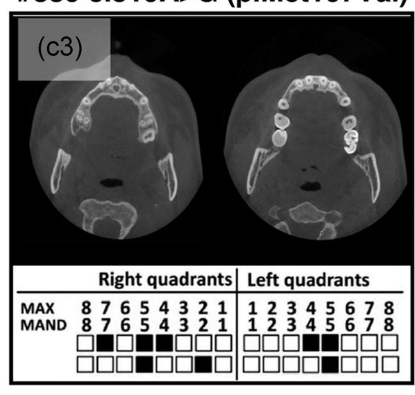
#892 c.319A>G (p.Met107Val)



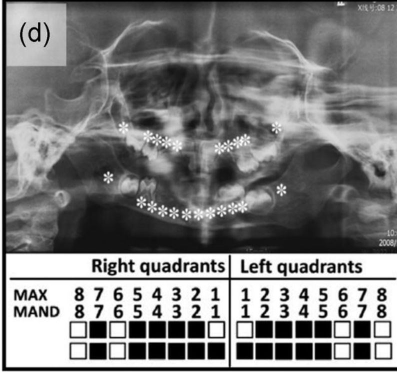
#846 c.319A>G (p.Met107Val)



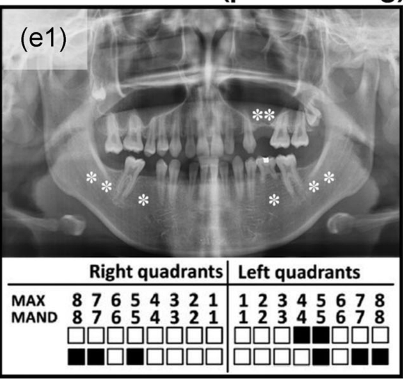
#889 c.319A>G (p.Met107Val)



#629 c.871G>A (p.Aal291Thr)



#667 c.1138A>C (p.Ser380Arg)



#002 c.1138A>C (p.Ser380Arg)

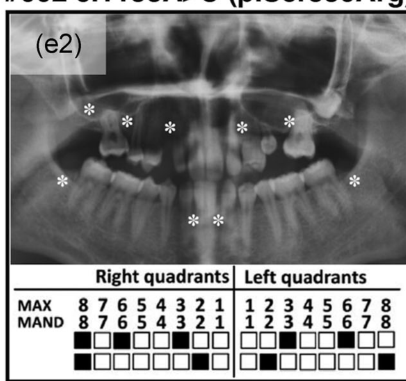


FIGURE 1 Dental characteristics of probands with distinct *EDAR* mutations. (a,b, $c_{1–3}$, d, $e_{1–2}$) Panoramic radiographs or cone-beam computed tomography of #945 proband (II:1), #952 proband (II:1), #892 proband (II:1), #846 proband (II:1), #889 proband (II:1), #629 proband (II:1), #667 proband (II:1), and #002 proband (II:1). Asterisks denote the position of missing teeth. Missing teeth are marked with black squares. *EDAR*, ectodysplasin A receptor; Max, maxillary; Mand, mandibular

3.2 | Conservation analysis

Bioinformatics analysis was performed to predict the functional impact of these seven *EDAR* mutations. The distribution of the 5 novel and 10 reported *EDAR* heterozygous mutations involved in nonsyndromic tooth agenesis was annotated on the human EDAR schematic structure (Figure 2b,c). Specifically, two missense mutations (c.G404A; p. Cys135Tyr, c.A319G; p. Met107Val) were located in the LBD and one missense mutations (c.T1109C; p. Val370Ala) and one nonsense mutation (c.C1072T; p. Arg358*) were located in the DD. The results of evolutionary conservation analysis revealed that the EDAR amino acid residues Val15, Cys135, Ala291, Cys352, Arg358, Val370, and Ser380 were highly conserved across several species (Figure 2d).

3.3 | Conformational alterations of EDAR mutants

Homology modeling and 3D structural analysis were used to compare the 3D conformational alterations between the wild-type and

(a)

Proband No.	Exon	Domain	Nucleotide /Protein Change	Mutation Type	SIFT	PolyPhen-2	ExAC (MAF)	Data
#945	5	LBD[1]	c.404G>A p.Cys135Tyr	Missense	0.02 Damaging	0.998 Probably damaging	Not present	Newly found
#952	12	DD[2]	c.1072C>T p.Arg358*	Nonsense	-	-	Not present	Newly found
#892 #846 #880 #889 #955	4	LBD[1]	c.319A>G p.Met107Val	Missense	0.82 Tolerated	0.00 Benign	0.007668	Newly found
#915 #944 #918	12	DD[2]	c.1109T>C p.Val370Ala	Missense	0.00 Damaging	0.658 Probably damaging	0.1300	Newly found
#629	10	-	c.871G>A p.Aal291Thr	Missense	0.91 Tolerated	0.714 Probably damaging	0.00006685	Previous work
#667 #002	12	DD[2]	c.1138A>C p.Ser380Arg	Missense	0.00 Damaging	1.000 Probably damaging	0.001927	Previous work
#690	2	LBD[1]	c.43G>A p.Val15Ile	Missense	0.63 Tolerated	0.091 Benign	0.0008666	Previous work

[1]LBD: ligand binding domain, [2]DD: death domain.

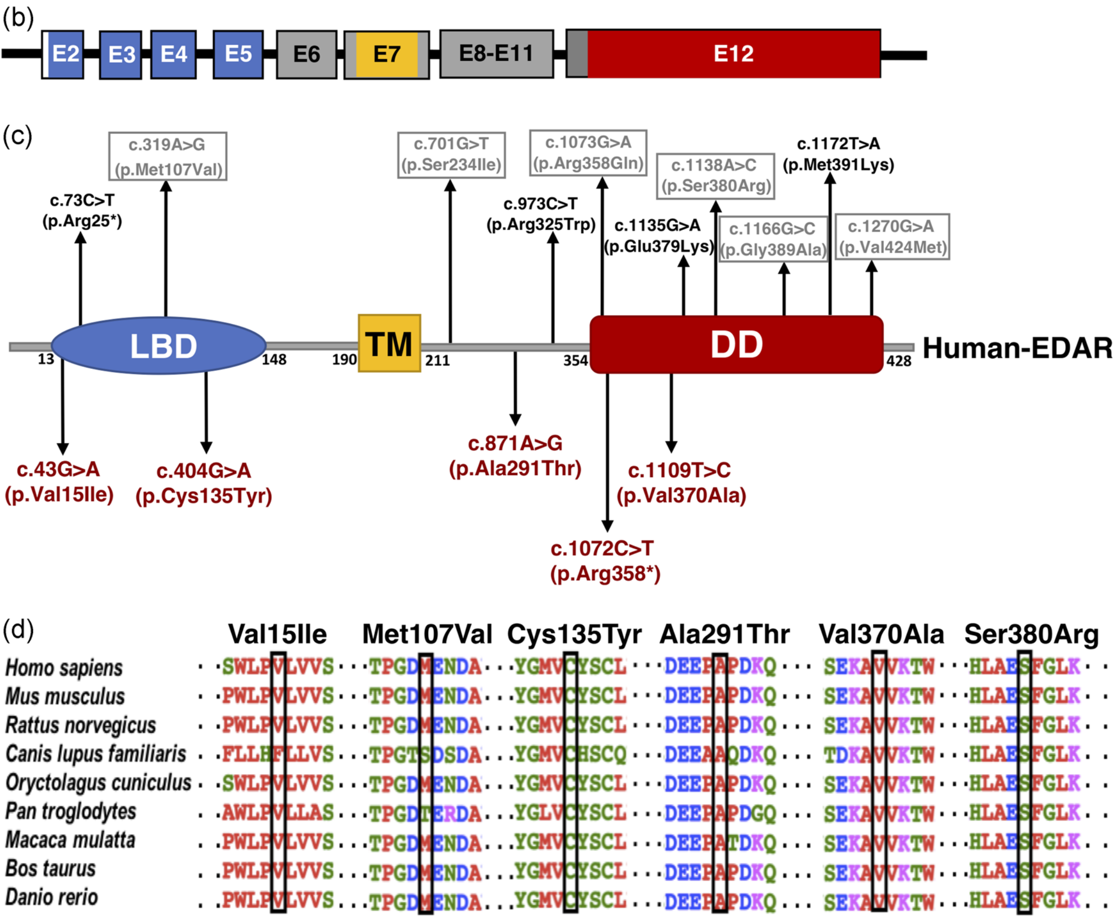


FIGURE 2 Functional impact prediction, location, and conservation analysis of *EDAR* mutations in nonsyndromic tooth agenesis. (a) Functional impact prediction of four newly and three previously detected *EDAR* mutations. (b) Schematic diagram of the human *EDAR* gene. (c) Distribution of mutations in the different domains of human EDAR protein. Mutations previously reported in nonsyndromic hypodontia and nonsyndromic oligodontia and novel mutations are denoted in gray, black, and red, respectively. (d) Conservation analysis of affected amino acids in EDAR missense mutations. DD, death domain; EDAR, ectodysplasin A receptor; LBD, ligand-binding domain; TM, transmembrane

mutant EDAR proteins to evaluate the potential functional impact of the *EDAR* mutations. The functional domains of wild-type EDAR consisted of LBD (Figures 3a and 3c) and DD (Figures 3e, 3g, and 3i). Structural analysis revealed that, in the LBD, the p. Cys135Tyr mutation resulted in the hydrophobic residue Cys135 being substituted with a Tyr, a polar amino acid with an aromatic ring, and a longer side-chain than Cys, resulting in a significant conformational change in the β-sheets near the 135th residue (Figure 3a,a',b,b'). The p. Met107Val mutation resulted in the residue Met107 being substituted with Val, an amino acid with a longer side-chain than Met, which may affect the interaction of 107th residue with the surrounding residues (Figure 3c,c',d,d').

In the DD, the residue Val370 was found at the outer surface of the loop (Figure 3e,e'). A substitution of Val with Ala, an amino acid with a shorter side-chain than Val (Figure 3f,f'), may result in a conformational change. The p. Ser380Arg mutation resulted in the residue Ser380 being substituted with Arg, an amino acid with a longer positively charged side-chain than Ser, which may affect the interaction of the 380th residue with the surrounding residues (Figure 3g,g',h,h'). In addition, the truncated p. Arg358* mutation resulted in the complete disappearance of the conformation of the 358th residue, possibly abolishing its affinity to downstream adaptor EDARADD (Figure 3i,i',j,j').

3.4 | Patterns of EDAR-/EDA-associated nonsyndromic tooth agenesis

Although *EDAR* mutations have been previously demonstrated to be associated with autosomal dominant nonsyndromic tooth agenesis (Table S2), the pattern of tooth agenesis caused by *EDAR* mutations has not yet been elucidated. In this study, statistical analysis was used to find a unique tooth agenesis pattern in the permanent dentition associated with *EDAR* mutations: the mandibular second premolars (mandibular PM2) were the most affected (57.69%), while the maxillary central incisor (maxillary CI) was the least affected

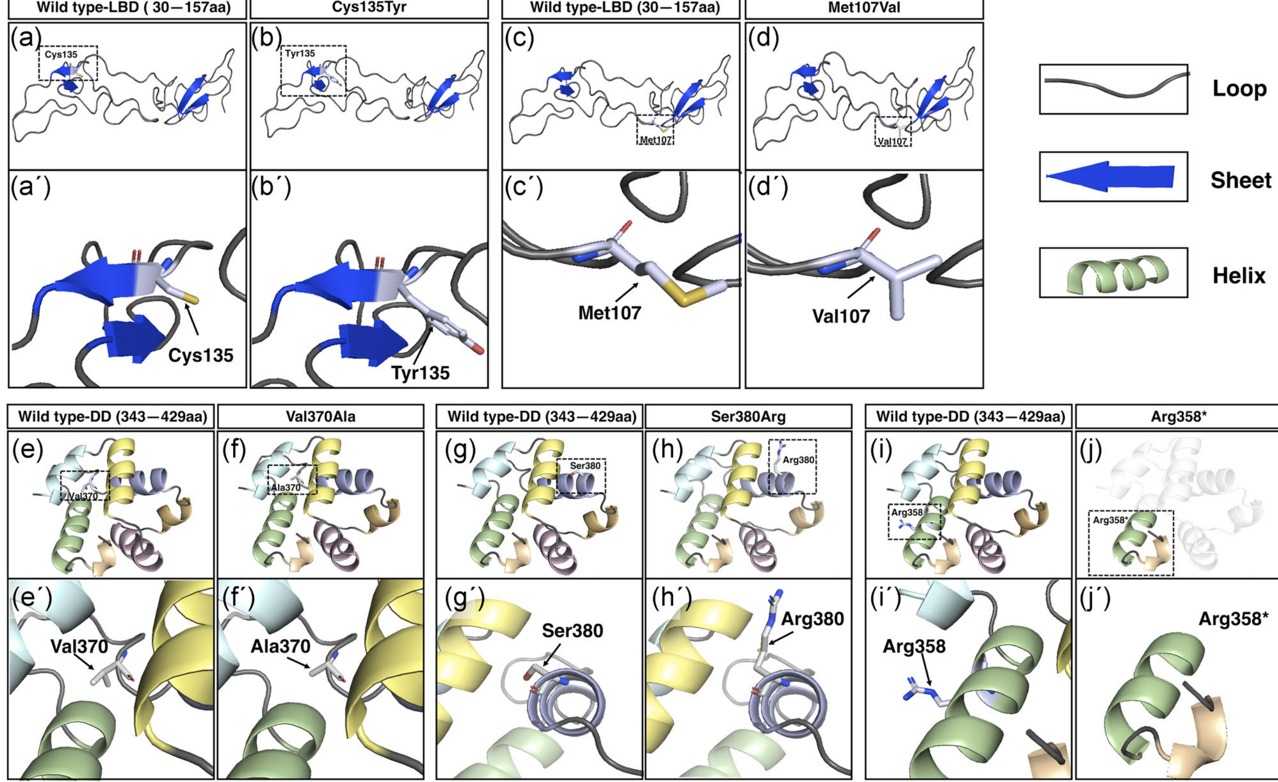


FIGURE 3 Structural modeling of the wild-type and mutated functional domains of EDAR protein. (a–d) Structural changes of the p. Cys135Try and p. Met107Val mutants compared with the wild-type LBD domain (from 30 to 157 aa). Dashed boxes denote the location of the (a) Cys135, (b) Tyr135, (c) Met107, and (d) Val107 residues. (a'–d') Higher magnifications of the boxed region surrounding the residues in the above images. Arrows indicate the hydrophobic residue Cys135 changed into the polar residue Tyr135 with an aromatic ring and a long side-chain (a',b'). Arrows indicate the residue Met107 substituted with Val with a longer side-chain (c',d'). (e–j) Structural changes of the p. Val370Ala, p. Ser380Arg, and p. Arg358* mutants compared with the wild-type DD domain (from 343 to 429 aa). Dashed boxes indicate the location of the (e) Val370, (f) Ala370, (g) Ser380, (hArg380, and (i, j) Arg358 residues. (e'–j') Higher magnifications of the boxed region surrounding the residues in the above images. Arrows indicate the residue Val370 substituted with Ala with a shorter side-chain (e',f'). Arrows indicate the residue Ser380 substituted with Arg with a positively charged side-chain (g',h'). Arrow indicates the structures of helices and loops that disappeared after the 358th residue (i', j'). aa, amino acid; DD, death domain; EDAR, ectodysplasin A receptor; LBD, ligand-binding domain

(0%; Figure 4a–c). Compared with other areas, the average missing rate of mandibular molar areas (7.69% and 11.54%) and maxillary molar areas (7.69% and 15.38%) were markedly lower. Interestingly, the average missing rate of maxillary CI (0%) was significantly lower than that of the mandibular CI (28.85%). However, there was no significant difference (p > .05) in the average rates of tooth agenesis between the maxillary dentition (30.22%) and the mandibular dentition (27.20%). Moreover, the rate of tooth agenesis on the left side (29.95%) was comparable to that on the right (27.74%; p > .05). More importantly, based on the patients' pediatric dental records and the information acquired from the parents, the primary dentition appeared unaffected.

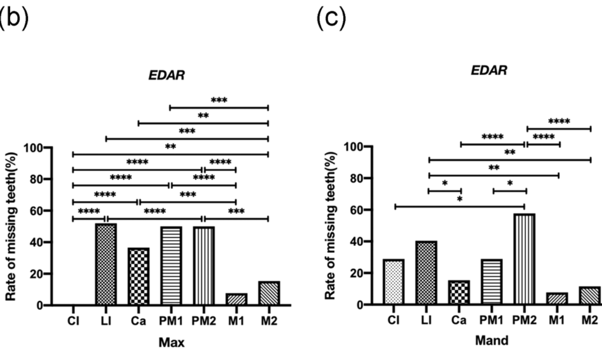
Since EDA, the ligand of EDAR in the EDA/EDAR/NF-κB pathway is one of the most common pathogenic genes of nonsyndromic oligodontia (Han et al., 2008; Song et al., 2009), we investigated whether there was a distinction between *EDAR*- and *EDA*-related tooth agenesis patterns. To this end, we summarized the permanent tooth agenesis positions from a total of 84 reported *EDA*-mutated patients. The male–female ratio was 4.6:1 (69:15), and the female patients showed fewer teeth lost than affected males or only loss of the upper lateral incisors (LIs; Table S3). Subsequently, statistical analysis (Figure 4d–f and Table S4) revealed that maxillary LI, mandibular LI, and mandibular CI had the highest missing rate (73.81%, 75.00%, and 76.79%), with no statistically significant difference between them (p > .05). Moreover, maxillary first molars (maxillary M1) had the lowest missing rate (7.14%). Similar to the *EDAR*-related pattern, the rate of tooth agenesis on the left (35.29%) and right (36.05%) sides, and in the maxillary (33.50%) or mandibular (37.84%) dentition showed no significant differences (p > .05) in *EDA*-related nonsyndromic oligodontia. Concerning the deciduous dentition, about 30 *EDA*-mutated patients with detailed records on deciduous teeth lacked 2–14

(a)

	Quadrant	Tooth position							Total missing number
		CI	LI	Ca	PM1	PM2	M1	M2	
EDAR	Max R	0/26 (0.00)	12/26 (46.15)	10/26 (38.46)	13/26 (50.00)	12/26 (46.15)	2/26 (7.69)	4/26 (15.38)	53/182 (29.12)
	Max L	0/26 (0.00)	15/26 (57.69)	9/26 (34.62)	13/26 (50.00)	14/26 (53.85)	2/26 (7.69)	4/26 (15.38)	57/182 (31.32)
	Mand R	8/26 (30.77)	11/26 (42.31)	4/26 (15.38)	6/26 (23.08)	13/26 (50.00)	2/26 (7.69)	3/26 (11.54)	47/182 (25.82)
	Mand L	7/26 (26.92)	10/26 (38.46)	4/26 (15.38)	9/26 (34.62)	17/26 (65.38)	2/26 (7.69)	3/26 (11.54)	52/182 (28.57)
	Max	0/52 (0.00)	27/52 (51.92)	19/52 (36.54)	26/52 (50.00)	26/52 (50.00)	4/52 (7.69)	8/52 (15.38)	110/364 (30.22)
	Mand	15/52 (28.85)	21/52 (40.38)	8/52 (15.38)	15/52 (28.85)	30/52 (57.69)	4/52 (7.69)	6/52 (11.54)	99/364 (27.20)
	R	8/52 (15.38)	23/52 (44.23)	14/52 (26.92)	19/52 (36.54)	25/52 (48.08)	4/52 (7.69)	7/52 (13.46)	100/364 (27.47)
	L	7/52 (13.46)	25/52 (48.08)	13/52 (25.00)	22/52 (42.31)	31/52 (59.62)	4/52 (7.69)	7/52 (13.46)	109/364 (29.95)

(d)

	Quadrant	Tooth position							Total missing number
		CI	LI	Ca	PM1	PM2	M1	M2	
EDA	Max R	27/84 (32.14)	62/84 (73.81)	33/84 (39.29)	32/84 (38.09)	24/84 (28.57)	5/84 (5.95)	16/84 (19.05)	199/588 (33.84)
	Max L	27/84 (32.14)	62/84 (73.81)	30/84 (35.71)	32/84 (38.09)	23/84 (27.38)	7/84 (8.33)	14/84 (16.67)	195/588 (33.16)
	Mand R	67/84 (79.76)	65/84 (77.38)	24/84 (28.57)	23/84 (27.38)	22/84 (26.19)	11/84 (13.10)	13/84 (15.48)	225/588 (38.27)
	Mand L	62/84 (73.81)	61/84 (72.62)	25/84 (29.76)	23/84 (27.38)	27/84 (32.14)	9/84 (10.71)	13/84 (15.48)	220/588 (37.41)
	Max	54/168 (32.14)	124/168 (73.81)	63/168 (37.50)	64/168 (38.10)	47/168 (27.98)	12/168 (7.14)	30/168 (17.86)	394/1176 (33.50)
	Mand	129/168 (76.79)	126/168 (75.00)	49/168 (29.17)	46/168 (27.38)	49/168 (29.17)	20/168 (11.90)	26/168 (15.48)	445/1176 (37.84)
	R	94/168 (55.95)	127/168 (75.60)	57/168 (33.93)	55/168 (32.74)	46/168 (27.38)	16/168 (9.52)	29/168 (17.26)	424/1176 (36.05)
	L	89/168 (52.98)	123/168 (73.21)	55/168 (32.74)	55/168 (32.74)	50/168 (29.76)	16/168 (9.52)	27/168 (16.07)	415/1176 (35.29)



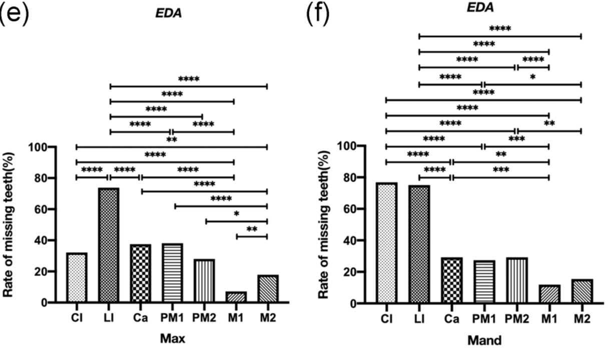


FIGURE 4 Patterns of *EDAR*- and *EDA*-related nonsyndromic tooth agenesis. (a) Number of missing teeth among 26 nonsyndromic tooth agenesis patients with *EDAR* mutations at each tooth position of their permanent dentition (excluding the third molars) based on our database and previous reports. The numerator denotes the number of missing teeth in each tooth position and the denominator denotes the number of patients with *EDAR* mutations. The number in brackets denotes the rate of missing teeth. (b,c) Percentage of missing tooth positions at each maxillary and mandibular dentition in all nonsyndromic tooth agenesis patients with *EDAR* mutations (n = 26). (d) Number of missing teeth among 84 nonsyndromic tooth agenesis patients with *EDA* mutations at each tooth position of their permanent dentition (excluding the third molars) based on previous reports. The numerator denotes the number of missing teeth in each tooth position and the denominator denotes the number of patients with *EDA* mutations. The number in brackets denotes the rate of missing teeth. (e, f) Percentage of missing tooth positions at each maxillary and mandibular dentition in all nonsyndromic tooth agenesis patients with *EDA* mutations (n = 84). Ca, canine; CI, central incisor; *EDA*, ectodysplasin A; *EDAR*, ectodysplasin A receptor; L, left; LI, lateral incisor; Max, maxillary; Mand, mandibular; Mo1, first molar; Mo2, second molar; PM1, first premolar; PM2, second premolar; R, right. Statistical significance p-value is marked with *<.05, **<.01, ***<.001, and ****<.0001

deciduous teeth, with the majority being deciduous mandibular incisors and maxillary LIs (our unpublished data).

4 | DISCUSSION

In the literature, the etiopathogenesis of EDAR in autosomal dominant or recessive HED is undoubted (Cluzeau et al., 2011; van der Hout et al., 2008; Trzeciak & Koczorowski, 2016). However, only 10 mutations in the *EDAR* gene have been associated with nonsyndromic tooth agenesis (Eisenberg et al., 2013; Yamaguchi et al., 2017; Zeng et al., 2017). Hence, the importance and functional role of *EDAR* mutations in the pathogenesis of nonsyndromic tooth agenesis are yet to be fully characterized. In this study, we reported six missense and one nonsense *EDAR* mutations in nonsyndromic oligodontia, five of which were novel. Our findings greatly expand the spectrum of *EDAR* mutations and provide strong evidence for the contribution of *EDAR* mutations to nonsyndromic oligodontia.

In the EDA/EDAR/NF-κB pathway, EDAR is a receptor of EDA, as well as an adaptor of EDARADD (Sadier et al., 2014). The cysteine-rich LBD in the extracellular region and the potential DD in the intracellular region of EDAR play different roles in this pathway (Mikkola, 2008). EDAR utilizes the LBD to combine with EDA, subsequently associating with EDARADD via the DD, ultimately activating downstream NF-κB signaling (Mikkola & Thesleff, 2003; Nieminen, 2009). Interestingly, we found that 53% of the *EDAR* mutations that cause nonsyndromic tooth agenesis occurred in the DD. However, only 27% of mutations were distributed in the LBD, confirming that the DD is a hotspot for germline mutations. Consistent with this, our previous studies on HED-related tooth agenesis also reported 70% of *EDAR* mutations within the DD (our unpublished data). These results suggest that the DD, encoded by exon 12, is the main functional domain of EDAR. Therefore, genetic alterations in the DD severely affected the development of tooth and other ectodermal organs in humans.

In this study, protein structure analysis revealed that the p. Cys135Tyr and p. Met107Val mutations in the LBD and the p. Val370Ala, p. Ser380Arg, and p. Arg358* mutations in the DD resulted in various conformational changes in the mutant proteins, suggesting that alterations in the EDAR structure reduce its affinity to EDA or EDARADD and further affect the interrelations between EADR and EDA or EDARADD, potentially disrupting the activity of the downstream NF-κB signaling pathway, and subsequently resulting in the failure of tooth formation. Therefore, these results suggest that heterozygous loss-of-function mutations in EDAR may contribute to the nonsyndromic tooth agenesis through haploinsufficiency. However, further functional analyses of EDAR mutations will be required to fully elucidate these pathogenic mechanisms.

It has been widely confirmed that the EDA/EDAR/NF-κB pathway contributes to the clinical homogeneity and genetic heterogeneity of HED (Fournier et al., 2018). However, the nonsyndromic tooth agenesis profile resulting from *EDAR* mutations was incomprehensive due to its low detection rate. In our study, we elucidated the *EDAR*-related tooth agenesis pattern, which was characterized by the mandibular second premolars being the most affected tooth position (57.69%), while the maxillary CIs were the least affected (0%). Our results suggest that the development of the second premolars, rather than the maxillary CIs, is more dose-sensitive to EDAR. Importantly, we found that heterozygous *EDAR* mutations tended to result in nonsyndromic tooth agenesis, while homozygous *EDAR* mutations were inclined to result in the occurrence of HED. These findings imply that EDAR acts in a concentration-dependent manner during ectodermal development, where relatively mild mutations result in isolated tooth agenesis, while more severe mutations are manifested as dysplasia across all ectodermal organs.

Murine studies have previously demonstrated that the precise tuning of the EDAR–EDA interaction is responsible for the formation of tooth and cusp number (Ohazama & Sharpe, 2004; Pispa et al., 2004; Tucker, Headon, Courtney, Overbeek, & Sharpe, 2004). We confirmed a 10.7% (12/112) detection rate of *EDAR* mutations in nonsyndromic oligodontia, which was much lower than the *EDA* mutation detection rate (27%; Han et al., 2008; Song et al., 2009). This indicates that the importance of EDAR during tooth development is relatively lower than that of EDA. Subsequently, the correlation and distinction between *EDAR*- and *EDA*-related nonsyndromic tooth agenesis was investigated. As a result, we found that the mandibular incisors and maxillary LIs were the most commonly affected tooth positions (76.79%, 75.00%, and 73.81%), while the maxillary first molars were the least affected (7.14%) in patients with *EDA* mutations, indicating that the development of the anterior region of the dentition is more sensitive to *EDA* mutations (Fournier et al., 2018; Han et al., 2008). Therefore, the comparative analysis of tooth agenesis patterns in the permanent dentition demonstrates that the mandibular premolars are more sensitive to *EDAR* mutations, while the anterior teeth are more sensitive to *EDA* mutations. Furthermore, the molars appear to be less susceptible to both *EDA* and *EDAR* mutations, with the lowest missing rate at these positions. Interestingly, we observed a notable phenotypic variation within the *EDAR*- and *EDA*-related nonsyndromic tooth agenesis families. For example, the dental phenotypes of *EDA* c.947A>G mutation (p. Asp316Gly, NM_001399.5) range from anodontia in three males to missing one tooth or a few teeth in three females (Table S3), suggesting that this allele is linked to an X-linked recessive-related phenotype. Other possible factors, such as genetic and epigenetic modifiers, may also be implicated in the pathogenesis of tooth agenesis.

It is worth noting that few studies in the literature have focused on the tooth agenesis of the deciduous dentition. In the present study, *EDAR* mutations were found to have little effect on the deciduous teeth, while *EDA* mutations resulted in varying degrees of deciduous tooth agenesis. Our results suggest that the development of deciduous teeth may more rely on *EDA*, rather than *EDAR*. It is possible that when comparing to *EDAR*, dysfunctional *EDA* might severely impair the NF-κB activations in various stages during tooth development, thus leading to abnormalities in the permanent dentition and even in the primary dentition.

5 | CONCLUSIONS

In this study, five novel *EDAR* mutations responsible for nonsyndromic oligodontia were identified, enriching the literature on the *EDAR* mutation spectrum. Furthermore, this study is the first to characterize the *EDAR*-related tooth agenesis pattern, systemically comparing the similarities and differences of the *EDAR*- and *EDA*-related tooth agenesis patterns in nonsyndromic tooth agenesis. Our findings provide new evidence for the genotypic study of nonsyndromic oligodontia and facilitate the diagnosis, treatment, genetic counseling, and prenatal diagnosis of this rare congenital anomaly by health providers.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The mutations identified in this study were submitted to the database of ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), and the submission ID is SUB7330934.

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REFERENCES

Bashyam, M. D., Chaudhary, A. K., Reddy, E. C., Reddy, V., Acharya, V., Nagarajaram, H. A., … Hariharan, S. V. (2012). A founder ectodysplasin A receptor (EDAR) mutation results in a high frequency of the autosomal recessive form of hypohidrotic ectodermal dysplasia in India. *British Journal of Dermatology*, *166*(4), 819–829. https://doi.org/10.1111/j.1365-2133.2011.10707.x

Chen, Y. T., Liu, H. C., Han, D., Liu, Y., & Feng, H. L. (2017). Association between EDAR polymorphisms and non-syndromic tooth agenesis in the Chinese Han population. *Chinese Journal of Dental Research*, *20*(3), 153–159. https://doi.org/10.3290/j.cjdr.a38770

Chhabra, N., Goswami, M., & Chhabra, A. (2014). Genetic basis of dental agenesis—Molecular genetics patterning clinical dentistry. *Medicina oral, patología oral y cirugía bucal*, *19*(2), e112–e119. https://doi.org/10.4317/medoral.19158

Cluzeau, C., Hadj-Rabia, S., Jambou, M., Mansour, S., Guigue, P., Masmoudi, S., & Smahi, A. (2011). Only four genes (EDA1, EDAR, EDARADD, and WNT10A) account for 90% of hypohidrotic/anhidrotic ectodermal dysplasia cases. *Human Mutation*, *32*(1), 70–72. https://doi.org/10.1002/humu.21384

De Coster, P. J., Marks, L. A., Martens, L. C., & Huysseune, A. (2009). Dental agenesis: Genetic and clinical perspectives. *Journal of Oral Pathology and Medicine*, *38*(1), 1–17. https://doi.org/10.1111/j.1600-0714.2008.00699.x

Dhamo, B., Kuijpers, M. A. R., Balk-Leurs, I., Boxum, C., Wolvius, E. B., & Ongkosuwito, E. M. (2018). Disturbances of dental development distinguish patients with oligodontia-ectodermal dysplasia from isolated oligodontia. *Orthodontics & Craniofacial Research*, *21*(1), 48–56. https://doi.org/10.1111/ocr.12214

Eisenberg, L., Arte, S., Parmanen, S., Pirinen, S., Alaluusua, S., & Nieminen, P. (2013). Candidate gene analysis of tooth agenesis identifies novel mutations in six genes and suggests significant role for WNT and EDA signaling and allele combinations. *PLoS One*, *8*(8), 73705. https://doi.org/10.1371/journal.pone.0073705

Feng, X., Weng, C., Wei, T., Sun, J., Huang, F., Yu, P., & Qi, M. (2018). Two EDA gene mutations in Chinese patients with hypohidrotic ectodermal dysplasia. *Journal of the European Academy of Dermatology and Venereology*, *32*(8), e324–e326. https://doi.org/10.1111/jdv.14874

Fournier, B. P., Bruneau, M. H., Toupenay, S., Kerner, S., Berdal, A., Cormier-Daire, V., … Dure-Molla, M. (2018). Patterns of dental agenesis highlight the nature of the causative mutated genes. *Journal of Dental Research*, *97*(12), 1306–1316. https://doi.org/10.1177/0022034518777460

Han, D., Gong, Y., Wu, H., Zhang, X., Yan, M., Wang, X., … Song, S. (2008). Novel EDA mutation resulting in X-linked non-syndromic hypodontia and the pattern of EDA-associated isolated tooth agenesis. *European Journal of Medical Genetics*, *51*(6), 536–546. https://doi.org/10.1016/j.ejmg.2008.06.002

van der Hout, A. H., Oudesluijs, G. G., Venema, A., Verheij, J. B. G. M., Mol, B. G. J., Rump, P., … van Essen, A. J. (2008). Mutation screening of the Ectodysplasin-A receptor gene EDAR in hypohidrotic ectodermal dysplasia. *European Journal of Human Genetics*, *16*(6), 673–679. https://doi.org/10.1038/sj.ejhg.5202012

Massink, M. P., Creton, M. A., Spanevello, F., Fennis, W. M., Cune, M. S., Savelberg, S. M., … van Haaften, G. (2015). Loss-of-function mutations in the WNT co-receptor LRP6 cause autosomal-dominant oligodontia. *American Journal of Human Genetics*, *97*(4), 621–626. https://doi.org/10.1016/j.ajhg.2015.08.014

Mikkola, M. L. (2008). TNF superfamily in skin appendage development. *Cytokine and Growth Factor Reviews*, *19*(3-4), 219–230. https://doi.org/10.1016/j.cytogfr.2008.04.008

Mikkola, M. L., & Thesleff, I. (2003). Ectodysplasin signaling in development. *Cytokine and Growth Factor Reviews*, *14*(3-4), 211–224. https://doi.org/10.1016/s1359-6101(03)00020-0

Nieminen, P. (2009). Genetic basis of tooth agenesis. *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution*, *312B*(4), 320–342. https://doi.org/10.1002/jez.b.21277

Ohazama, A., & Sharpe, P. T. (2004). TNF signalling in tooth development. *Current Opinion in Genetics & Development*, *14*(5), 513–519. https://doi.org/10.1016/j.gde.2004.07.008

Okita, T., Asano, N., Yasuno, S., & Shimomura, Y. (2019). Functional studies for a dominant mutation in the EDAR gene responsible for hypohidrotic ectodermal dysplasia. *Journal of Dermatology*, *46*(8), 710–715. https://doi.org/10.1111/1346-8138.14983

Parveen, A., Khan, S. A., Mirza, M. U., Bashir, H., Arshad, F., Iqbal, M.,… Wasif, N. (2019). Deleterious variants in WNT10A, EDAR, and EDA causing isolated and syndromic tooth agenesis: A structural perspective from molecular dynamics simulations. *International Journal of Molecular Sciences*, *20*(21). https://doi.org/10.3390/ijms20215282

Pispa, J., Mustonen, T., Mikkola, M. L., Kangas, A. T., Koppinen, P., Lukinmaa, P. L., … Thesleff, I. (2004). Tooth patterning and enamel formation can be manipulated by misexpression of TNF receptor Edar. *Developmental Dynamics*, *231*(2), 432–440. https://doi.org/10.1002/dvdy.20138

Prasad, M. K., Geoffroy, V., Vicaire, S., Jost, B., Dumas, M., Le Gras, S., & Bloch-Zupan, A. (2016). A targeted next-generation sequencing assay for the molecular diagnosis of genetic disorders with orodental involvement. *Journal of Medical Genetics*, *53*(2), 98–110. https://doi.org/10.1136/jmedgenet-2015-103302

Ruf, S., Klimas, D., Honemann, M., & Jabir, S. (2013). Genetic background of nonsyndromic oligodontia: A systematic review and meta-analysis. *Journal of Orofacial Orthopedics*, *74*(4), 295–308. https://doi.org/10.1007/s00056-013-0138-z

Sadier, A., Viriot, L., Pantalacci, S., & Laudet, V. (2014). The ectodysplasin pathway: From diseases to adaptations. *Trends in Genetics*, *30*(1), 24–31. https://doi.org/10.1016/j.tig.2013.08.006

Schalk-van der Weide, Y., Beemer, F. A., Faber, J. A., & Bosman, F. (1994). Symptomatology of patients with oligodontia. *Journal of Oral Rehabilitation*, *21*(3), 247–261. https://doi.org/10.1111/j.1365-2842.1994.tb01141.x

Song, S., Han, D., Qu, H., Gong, Y., Wu, H., Zhang, X., … Feng, H. (2009). EDA gene mutations underlie non-syndromic oligodontia. *Journal of Dental Research*, *88*(2), 126–131. https://doi.org/10.1177/0022034508328627

Sun, S., Li, F., Liu, Y., Qu, H., Wong, S. W., Zeng, L., … Han, D. (2019). A novel inhibitor of nuclear factor kappa-B kinase subunit gamma mutation identified in an incontinentia pigmenti patient with syndromic tooth agenesis. *Archives of Oral Biology*, *101*, 100–107. https://doi.org/10.1016/j.archoralbio.2019.03.013

Trzeciak, W. H., & Koczorowski, R. (2016). Molecular basis of hypohidrotic ectodermal dysplasia: An update. *Journal of Applied Genetics*, *57*(1), 51–61. https://doi.org/10.1007/s13353-015-0307-4

Tucker, A. S., Headon, D. J., Courtney, J. M., Overbeek, P., & Sharpe, P. T. (2004). The activation level of the TNF family receptor, Edar, determines cusp number and tooth number during tooth development. *Developmental Biology*, *268*(1), 185–194. https://doi.org/10.1016/j.ydbio.2003.12.019

Visinoni, A. F., Lisboa-Costa, T., Pagnan, N. A., & Chautard-Freire-Maia, E. A. (2009). Ectodermal dysplasias: Clinical and molecular review. *American Journal of Medical Genetics, Part A*, *149A*(9), 1980–2002. https://doi.org/10.1002/ajmg.a.32864

Wohlfart, S., Soder, S., Smahi, A., & Schneider, H. (2016). A novel missense mutation in the gene EDARADD associated with an unusual phenotype of hypohidrotic ectodermal dysplasia. *American Journal of Medical Genetics, Part A*, *170A*(1), 249–253. https://doi.org/10.1002/ajmg.a.37412

Wong, S. W., Han, D., Zhang, H., Liu, Y., Zhang, X., Miao, M. Z., … Feng, H. (2018). Nine novel PAX9 mutations and a distinct tooth agenesis genotype-phenotype. *Journal of Dental Research*, *97*(2), 155–162. https://doi.org/10.1177/0022034517729322

Yamaguchi, T., Hosomichi, K., Yano, K., Kim, Y. I., Nakaoka, H., Kimura, R., … Inoue, I. (2017). Comprehensive genetic exploration of selective tooth agenesis of mandibular incisors by exome sequencing. *Human Genome Variation*, *4*, 17005. https://doi.org/10.1038/hgv.2017.5

Yu, M., Wong, S. W., Han, D., & Cai, T. (2019). Genetic analysis: Wnt and other pathways in nonsyndromic tooth agenesis. *Oral Diseases*, *25*(3), 646–651. https://doi.org/10.1111/odi.12931

Yu, P., Yang, W., Han, D., Wang, X., Guo, S., Li, J., … Cai, T. (2016). Mutations in WNT10B are identified in individuals with oligodontia. *American Journal of Human Genetics*, *99*(1), 195–201. https://doi.org/10.1016/j.ajhg.2016.05.012

Zeng, B., Zhao, Q., Li, S., Lu, H., Lu, J., Ma, L., … Yu, D. (2017). Novel EDA or EDAR mutations identified in patients with X-linked hypohidrotic ectodermal dysplasia or non-syndromic tooth agenesis. *Genes*, *8*(10). https://doi.org/10.3390/genes8100259

SUPPORTING INFORMATION

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