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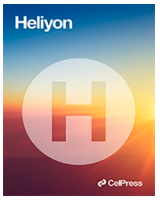

The oligodontia phenotype in a X-linked hypohidrotic ectodermal dysplasia patient with a novel *EVC2* variant

Yi Wu [a,1], Jing Sun [a,1], Caiqi Zhang [a,1], Siyuan Ma [a], Yiting Liu [a], Xiaoshan Wu [a,b,**], Qingping Gao [a,*]

[a] *The Stomatology Center of Xiangya Hospital, Academician Workstation for Oral & Maxillofacial Regenerative Medicine, Research Center of Oral and Maxillofacial Development and Regeneration, National Clinical Research Center for Geriatric Diseases, Central South Universtiy, Changsha, Hunan Province, China*

[b] *Hunan Key Laboratory of Aging Biology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China*

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ABSTRACT

Objectives: To analyse the pathogenic genes in a patient with hypohidrotic ectodermal dysplasia (HED) and explore the relationship between pathogenic genes and the oligodontia phenotype.
Methods: Clinical data and peripheral blood were collected from a patient with HED. Pathogenic genes were analysed by whole-exon sequencing (WES) and verified by Singer sequencing. The secondary and tertiary structures of the variant proteins were predicted to analyse their toxicity.
Results: The patient exhibited a severe oligodontia phenotype, wherein only two deciduous canines were left in the upper jaw. WES revealed a hemizygous *EDA* variant c.466C > T p.(Arg156Cys) and a novel heterozygous *EVC2* variant c.1772T > C p.(Leu591Ser). Prediction of the secondary and tertiary structures of the EDA variant p.(Arg156Cys) and EVC2 variant p.(Leu591Ser) indicated impaired function of both molecules.
Conclusion: The patient demonstrated a more severe oligodontia phenotype when compared with the other patients caused by the *EDA* variant c.466C > T. Since Evc2 is a positive regulator of the Sonic Hedgehog (Shh) signal pathway, we speculated that the EVC2 variant p.(Leu591Ser) may play a synergistic role in the oligodontia phenotype of HED, thereby exacerbating the oligodontia phenotype. Knowledge of oligodontia caused by multiple gene variants is of great significance for understanding individual differences in oligodontia phenotypes.

1. Introduction

Hypohidrotic ectodermal dysplasia (HED) is an inherited heterogeneous disorder characterised by the abnormal development of ectodermal derivatives, such as hair, sweat glands, and teeth [1]. More than 90 % of HED cases can be elucidated by heterozygous or biallelic variants of *EDA* (OMIM 300451), *EDAR* (OMIM 604095), *EDARADD* (OMIM 606603), and *WNT10A* (OMIM 606268) [2]. *EDA*

* Corresponding author. The Stomatology Center of Xiangya Hospital, Central South University, Xiangya Road 87#, Changsha, Hunan Province, 410008, China.
** Corresponding author. The Stomatology Center of Xiangya Hospital, Central South Universtiy, Xiangya Road 87#, Changsha, Hunan Province, 410008, China.
E-mail addresses: drwuxiaoshan@csu.edu.cn (X. Wu), dentgao@163.com (Q. Gao).
[1] These authors contributed equally to the manuscript.

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variants are the most common and lead to X-linked hypohidrotic ectodermal dysplasia (XLHED, OMIM 305100). Abnormal dentition morphology with severe tooth loss is one of the major clinical phenotypes of XLHED [3]. The number and position of the missing teeth varied significantly among patients with XLHED. Almost all male XLHED patients had oligodontia, with an average number of missing permanent teeth of 22.4 (range:10–28), while at least 73 % of female patients had hypodontia or oligodontia, with an average of 3.4 (range: 0–22) [4]. However, the mechanisms underlying individual differences in the number and position of missing teeth remain predominantly unknown.

EDA, located on Xq13.1, encodes transmembrane ectodysplasin-A (EDA). The EDA protein includes four major functional domains: the transmembrane domain, the furin domain with EDA precursor protein cleavage recognition sites, the collagen domain that maintains the normal folded conformation of the tumour necrosis factor (TNF) domain, and the TNF domain that binds to the downstream molecule EDAR [5]. Variants in different domains may lead to different oligodontia phenotypes. Zeng et al. have computed the incidence of variants and average number (*mean* ± *s.d.*) of missing permanent teeth in different domains: variants affecting the transmembrane (2.2 %, 23.7 ± 2.1), furin (14.9 %, 23.6 ± 3.6), collagen (1.5 %, 25.5 ± 5.0), and TNF domain (64.2 %, 15.1 ± 7.9) [6]. This study may partly elucidate the vast differences in oligodontia phenotypes among XLHED patients. However, the oligodontia phenotype was not the same in patients with the *EDA* variant at the same site.

EVC2 (OMIM 607261) and *EVC* (OMIM 604831) are two adjacent genes located on 4p16 that are arranged head-to-head and regulated by the same promoter [7]. Variants in *EVC2* or *EVC* are responsible for the autosomal recessive disease Ellis-van Creveld syndrome (EvC, OMIM 225500) or the autosomal dominant genetic disease Weyers acrofacial dysostosis (WAD, OMIM 193530). The clinical phenotypes of EvC include short stature, postaxial polydactyly, hypoplastic nails, tooth dysplasia, oligodontia, cardiac septal defects, etc [8]. WAD is usually caused by variants in the last exon of *EVC2* and has phenotypes similar to EvC; however, the symptoms of short ribs and cardiac septal defects are mild [9,10]. The study has demonstrated that a heterozygous variant in exon 11 of *EVC2* can also lead to non-syndromic tooth agenesis [11].

Multiple gene variants are becoming increasingly common and may account for individual differences in oligodontia phenotypes. In this study, we report the case of a five-year-old male XLHED patient with a novel heterozygous *EVC2* variant and find that the oligodontia phenotype was more severe than that of patients with the *EDA* variant at the same site.

2. Materials and methods

2.1. Clinical case

A five-year-old boy was referred to the Stomatology Center of Xiangya Hospital, Central South University, complaining of tooth eruption abnormalities. Physical examination revealed oligodontia, sparse hair, eyebrows, and thin and dry skin, and a primary diagnosis of HED was established.

The number and location of missing teeth were recorded. Cone-beam computed tomography (CBCT) was used to analyse permanent tooth germs inside the jaw. By asking about his family history, it was learned that none of the patient's relatives, including his parents and twin sister, had similar symptoms. With informed consent from his mother, we took oral and facial photographs and obtained venous blood samples from him, his mother, and his sister. The mother and twin sister of the patient underwent an oral examination and CBCT. All study procedures were approved by the Medical Ethics Committee of Xiangya Hospital of Central South University (Approval Number:202203062).

2.2. Whole-Exome sequencing (WES) to screen for pathogenic variants

Genergy Bio-Technology Co., Ltd. (Shanghai, China) conducted DNA extraction and WES of qualified DNA samples. Double-terminal sequencing (2 × 150bp) Illumina HiSeq sequencing platform was used for high-throughput sequencing of the samples. Skewer (version.0.2.2) (https://sourceforge.net/projects/skewer) was used to dynamically remove splices and low-quality sequences from the 3′ end of the sequencing data. The quality of the pre-processed data was evaluated using FastQC (v.0.11.9) (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The effective sequencing data were compared with the reference genome using Sentieon (v.202112.01) Burrows-Wheeler Alignment mem (https://www.insvast.com/sentieon), and the variants were detected in samples using Sentieon (v.202112.01). The loci of the variants were labelled using ANNOVAR (v.2017.07.17) (https://annovar.openbioinformatics.org/en/latest/user-guide/download/). The major filtering steps for the pathogenic variants are depicted in Table S1 in the Supplementary Materials.

2.3. Sanger sequencing to verify the pathogenic variants

Genomic DNA was extracted from the peripheral blood of the proband, his mother, and sister using a TIANamp Blood DNA Midi Kit (Tiangen, Beijing, China). The upstream and downstream primers were designed, respectively, according to the upstream and downstream sequences of *EDA* and *EVC2* variant sites (Table S2). The *EDA* and *EVC2* coding sequences were amplified by polymerase chain reaction, and the polymerase chain reaction products were commissioned by Tsingke Biotechnology Co., Ltd. (Beijing) for Sanger sequencing. The results were compared with the reference sequences for each gene (*EDA*, NM_001399.5; *EVC2*, NM_001166136.2) to verify the WES results.

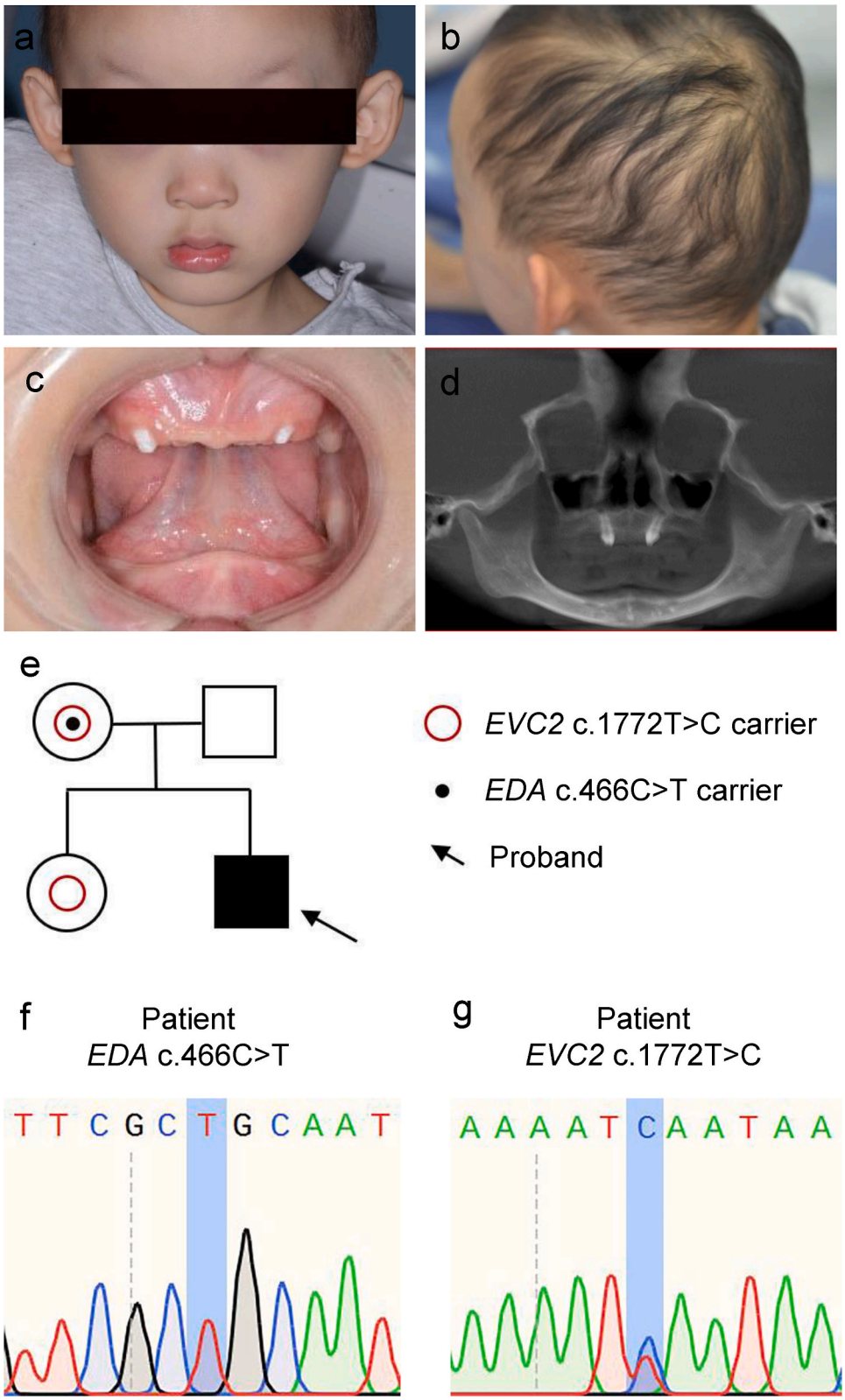

Fig. 1. Clinical data and pedigree of the patient and Sanger sequencing results. a–b sparse hair and eyebrows, thin and dry skin; c severe oligodontia. Only two conical cusps in the upper jaw; d CBCT: only two deciduous teeth in the upper jaw; e pedigree of the patient; f–g Sanger sequencing results.

2.4. Prediction of secondary and tertiary structures of the variant proteins

UCSC (http://genome.ucsc.edu/) was used to query the original exon sequences, and the original amino acid sequences were obtained post translation. PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) was used to predict the secondary structures of the wild-type and variants. The tertiary structure of wild-type EDA was queried using UniProt (https://www.uniprot.org/). AlphaFold2 (https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb) was used to predict the tertiary structures of EDA variant, wild-type EVC2, and EVC2 variant. HDOCK (http://hdock.phys.hust.edu.cn/) was used to simulate the molecular connection between EVC2 and EVC. Structural figures were prepared using PyMol (PyMol Molecular Graphics System v.2.0, Education-Use-Only PyMol Builds).

2.5. Permanent dentition of children (aged 3–5 years) with CBCT

To determine the number of missing teeth in the patient, we studied the CBCT images of the permanent dentition in normal children

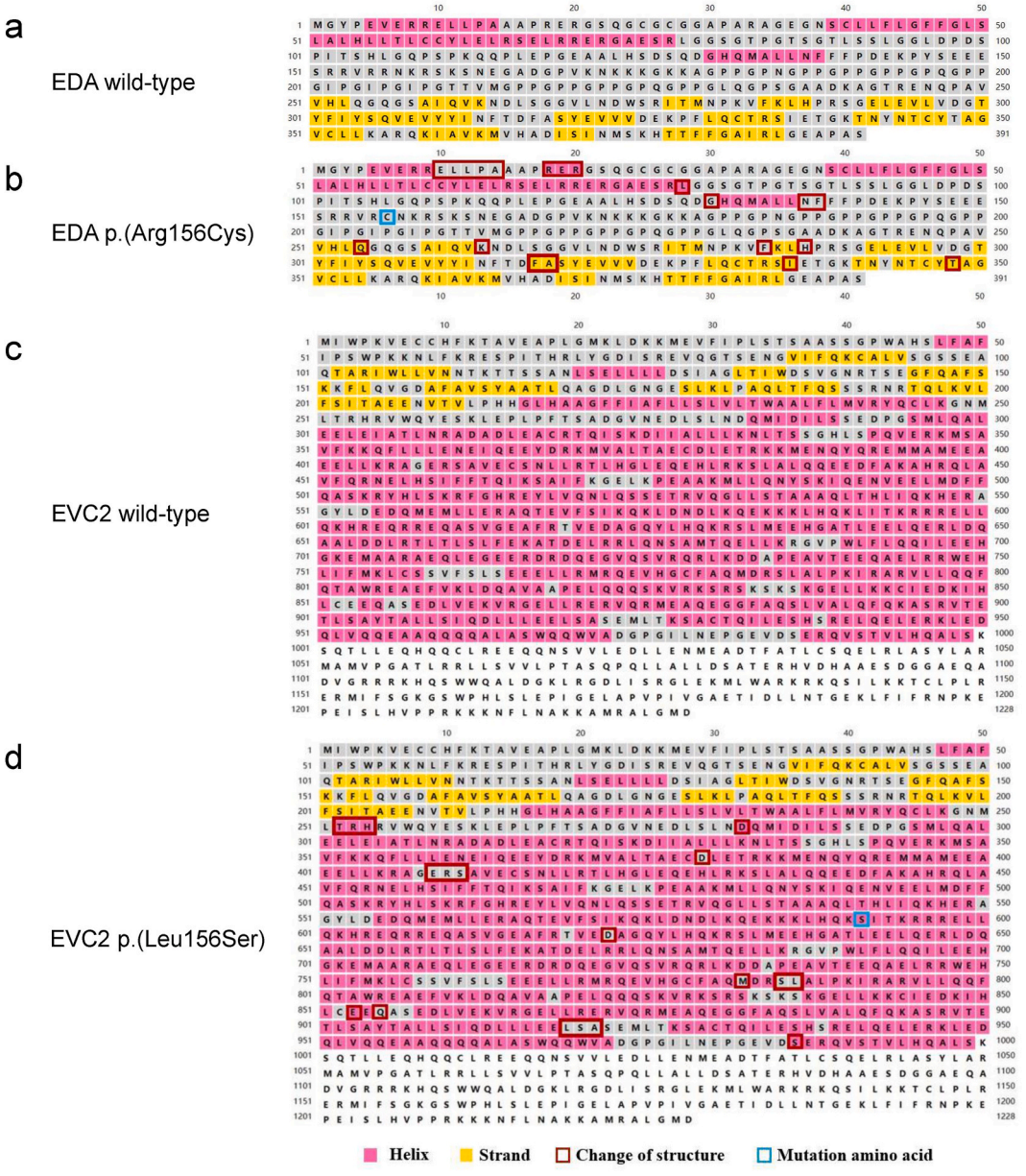

Fig. 2. Prediction of secondary structure of proteins. a–b secondary structure prediction of EDA wild-type and EDA p.(Arg156Cys); c–d secondary structure prediction of EVC2 wild-type and EVC2 p.(Leu591Ser).

aged 3–5 years. In addition to the CBCT of the normal twin sister of the patient when she was 3 years and 4 months old, we also randomly selected CBCT images of three children in clinical practice. The inclusion criteria were as follows:1) 3–5-year-old children; 2) no oral and maxillofacial dysplasia; 3) no systemic diseases; 4) CBCT examination demonstrating no congenital tooth loss; and 5) seeking medical treatment for dental caries, dental trauma, uneven dentition, or oral examination.

3. Results

3.1. Clinical report

The patient exhibited typical HED symptoms, including sparse hair, dry skin, severe oligodontia, and prominent ears (Fig. 1 a–b). On oral examination, only two conical cusps were observed in the upper jaw, and the alveolar ridge was narrow (Fig. 1 c). Using CBCT, we found that the patient had only two deciduous canines in the upper jaw (Fig. 1 d). The oral examination and CBCT findings of the mother and twin sister of the patient were normal. According to the mother of the patient, the father did not have any congenital tooth loss.

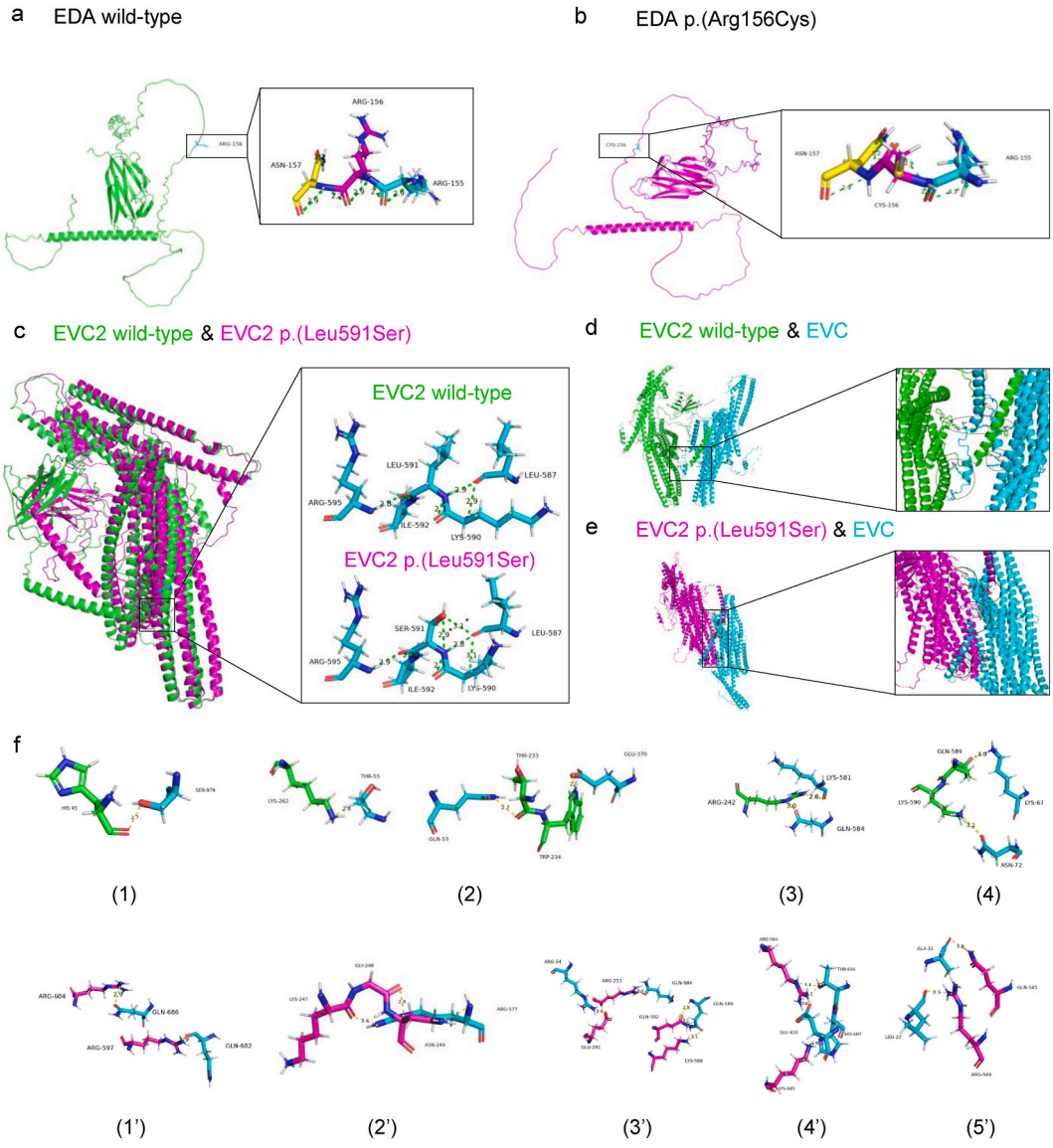

Fig. 3. Prediction of tertiary structure of proteins. a–b tertiary structure prediction and hydrogen bond analysis of EDA wild-type and EDA p.(Arg156Cys); c overlap plot and hydrogen bond analysis of tertiary structure prediction of EVC2 wild-type and EVC2 p.(Leu591Ser); d, f.(1)–(4) simulation of molecular docking between EVC2 wild-type and EVC, and four binding sites between them; e, f.(1′)–(5′) simulation of molecular docking between EVC2 p.(Leu591Ser) and EVC, and five binding sites between them.

Table 1
Statistical analysis of clinical phenotypes in patients with *EDA* variant c.466C > T.

Patient	Sex	Age	Variants		Deciduous teeth										Permanent teeth													Other clinical features	Reference
					right					left					right							left								
				Max	V	IV	III	II	I	I	II	III	IV	V	7	6	5	4	3	2	1	1	2	3	4	5	6	7		
				Mand	V	IV	III	II	I	I	II	III	IV	V	7	6	5	4	3	2	1	1	2	3	4	5	6	7		
P1	M	5	*EDA* c.466C > T		■	■	□	■	■	■	■	□	■	■	?	■	■	■	■	■	■	■	■	■	■	■	■	?	Sparse hair, dry skin, hypohidrosis, conical teeth, prominent ears	This study
			EVC2 c.1772T > C		■	■	■	■	■	■	■	■	■	■	?	■	■	■	■	■	■	■	■	■	■	■	■	?		
P2	M	5	*EDA* c.466C > T		4 teeth remain										3 teeth remain														Abnormal crown morphology of permanent teeth	[18]
P3	F	14	*EDA* c.466C > T		Unknown										■	□	■	■	■	■	■	■	■	■	■	■	■	■	Sparse hair, dry skin, hypohidrosis, eczema	[17]
			WNT10A c.637G > A												■	□	■	■	■	■	■	■	■	■	■	■	■	■		
P4	M	3	*EDA* c.466C > T		4 teeth remain, but the positions were unknown																								Sparse hair, dry skin, hypohidrosis, conical teeth, prominent forehead, wide nasal bridge, thick and everted lips, prominent ears, fever episodes, recurrent respiratory infections	[19]
F5	M	Unknown	*EDA* c.466C > T		Unknown										The number of missing permanent teeth does not exceed 27, without specific description														Sparse hair, dry skin, hypohidrosis, conical teeth, facial dysmorphism, hypoplastic nails	[20]
P6	M	6	*EDA* c.466C > T		Unknown										Oral examination revealed only two conical central incisors in the upper jaw, lacking imaging verification														Sparse hair, conical teeth, prominent forehead, wide nasal bridge, thick and everted lips, fever episodes	[21]
P7	M	7	*EDA* c.466C > T		Unknown										□	■	■	■	■	■	■	■	■	■	■	■	■	■	Sparse hair, dry skin, hypohidrosis	[22]
															■	■	■	■	■	■	■	■	■	■	■	■	■	□		

Max, maxillary; Mand, mandibular; F5, family 5, including 6 patients; M, male; F, female; ■, the tooth in this position is missing; □, the tooth in this position is not missing; ?, it is uncertain whether the patient had a congenital absence of the second molars; Unknown, not mentioned in the literature.

The first permanent tooth to erupt in humans is the first molar at the age of six. However, it is uncertain whether most permanent tooth germs can be observed within the jaw by age five through X-ray examination [12,13]. To determine whether the absence of all permanent tooth germs in this patient was due to congenital oligodontia or immaturity, we first studied the permanent dentition of children (aged 3–5 years) using CBCT. The CBCT scans of the normal twin sister of the patient and three other children were selected. The results demonstrated that the tooth germ of the second permanent molar with the dental follicle could be seen in the jaw as young as three years of age (Fig. S1). This finding led us to conclude that all permanent tooth germs in this patient were lost due to congenital oligodontia.

3.2. A hemizygous EDA variant and a novel heterozygous EVC2 variant were detected

WES results demonstrated that the proband carried a hemizygous *EDA* variant c.466C > T p.(Arg156Cys) and a heterozygous *EVC2* variant c.1772T > C p.(Leu591Ser), and these two missense variants were both from his mother. The unaffected mother carried a heterozygous *EDA* variant, c.466C > T p.(Arg156Cys) and a heterozygous *EVC2* variant, c.1772T > C p.(Leu591Ser). His unaffected twin sister carried only one heterozygous *EVC2* variant, c.1772T > C p.(Leu591Ser) (Fig. 1 e). The pathogenicity scores of the variants are listed in Table S3.

3.3. The variant sites were verified by Sanger sequencing

Sanger sequencing was used to verify the WES results. Sanger sequencing results demonstrated that the 466 base of the *EDA* exon sequence was replaced by T, and the 1772 base of the *EVC2* exon sequence was a heterozygous variant, where T was partially replaced by C (Fig. 1 f–g). The novel *EVC2* variant was submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/).

3.4. The configurations of the variant proteins were altered

Secondary structures of the EDA variant p.(Arg156Cys) and the EVC2 variant p.(Leu591Ser) were predicted. The variant EDA protein demonstrated changes in multiple α-helices and β-sheet folds in its secondary structure (Fig. 2 a–b), and the EVC2 variant demonstrated changes in multiple α-helices (Fig. 2 c–d). Tertiary structure prediction of the EDA variant p.(Arg156Cys) demonstrated that a hydrogen bond was formed between amino acids 156 and 155, which turned the polypeptide chain, resulting in the β-sheet of the protein structure. This alteration can significantly affect protein function (Fig. 3 a–b). In the EVC2 variant p.(Leu591Ser), serine replaces leucine, resulting in the amino acid 591 forming two additional hydrogen bonds with amino acids 590 and 587. Therefore, the overall protein structure was affected (Fig. 3 c). Evc2 can combine with Evc to form a dimer and then combine with Smoothened to form a complex. H-Dock was used to simulate the molecular docking between EVC2 and EVC and revealed that EVC2 wild-type and EVC had four binding sites, forming a mirror-like dimer structure, whereas the EVC2 variant p.(Leu591Ser) and EVC formed a tightly bound dimer structure with five binding sites (Fig. 3 d–f). The structural phase of the EVC2-EVC complex changed significantly.

4. Discussion

Tooth agenesis is one of the most common congenital craniofacial abnormalities that not only affect the masticatory and linguistic functions of the patient but also lead to cranial facial growth and developmental disorders, which may be harmful to their appearance and psychology. Tooth agenesis can be classified as syndromic (with ectodermal appendage abnormalities) or non-syndromic (with no systemic symptoms) [14]. The genes that have been clearly associated with human tooth agenesis include *PAX9* (OMIM 167416), *MSX1* (OMIM 142983), *AXIN2* (OMIM 604025), *EDA, EDAR, EDARADD, WNT10A, LRP6* (OMIM 603507), etc [15]. The majority of studies on the genetic diagnosis of tooth agenesis have been exclusively based on the isolated sequencing of individual genes. However, tooth agenesis caused by polygenic variants has rarely been discussed. Mu et al. found that a combined reduction in *MSX1* and *PAX9* sequence doses exaggerated the risk of oligodontia in two Mexican families [16]. He et al. reported for the first time simultaneous *EDA* and *WNT10A* variants in patients with HED and non-syndromic tooth agenesis, suggesting that *WNT10A* and *EDA* dual variants could lead to tooth agenesis in the Chinese population [17]. The patient harboured a hemizygous *EDA* and a novel heterozygous *EVC2* variant in this study. The *EDA* variant c.466C > T p.(Arg156Cys) had been reported in other studies, and we statistically analysed the clinical phenotypes of these patients (Table 1) [17–22], focusing on their oligodontia phenotype. In these studies, the number of missing deciduous teeth was often low, and the number of missing permanent teeth was less than 27 [20]. Only two deciduous teeth were retained in this study, and all permanent teeth were missing; therefore, this patient had a more severe oligodontia phenotype. Consequently, the novel heterozygous *EVC2* variant may synergistically affect the oligodontia phenotype.

HED is one of the most common forms of ectodermal dysplasia and is characterised by sparse hair, hypohidrosis, oligodontia, and typical facial features [23]. The major pathogenic genes for HED include *EDA, EDAR, EDARADD, WNT10A*, and *EDA* variants, which accounted for most HED cases, followed by *WNT10A*, *EDAR,* and *EDARADD* [2,24]. All four genes could cause severe oligodontia phenotypes, but some differences existed. In the *EDA*-induced XLHED patients, the mean number of missing permanent teeth was 22.4 (range 14–28) in males and 3.4 (range 0–22) in females [3,4]. *WNT10A*-induced tooth agenesis demonstrates great variability, ranging from isolation to syndromes. In *WNT10A*-induced HED patients, the number of missing permanent teeth ranges from 5 to 22, and the other signs of ectodermal dysplasia are mild [25]. *EDAR*-induced HED can result in the loss of multiple permanent teeth (range 3–16) [26]. One study reported that the *EDARADD* variant could lead to the loss of six permanent teeth [27], while the oligodontia phenotype of the *EDARADD* variant is not completely known and lacks statistics owing to its low frequency.

Sex is also an important factor when analysing differences in oligodontia phenotypes. In patients with XLHED, the mean number of missing permanent teeth in hemizygous males was more than five times that in heterozygous females, and heterozygous females demonstrated varying degrees of severity due to X chromosome inactivation [28]. In patients with *WNT10A* heterozygous variants, the incidence of tooth agenesis was also significantly higher in men than that in females [29]. Additionally, different variants and environmental factors may lead to differences in oligodontia phenotypes. Zeng et al. revealed that truncating mutations in *EDA* are associated with more missing permanent teeth, and missense mutations are correlated with fewer missing permanent teeth [6]. A previous study suggested that maternal smoking during pregnancy was associated with hypodontia [30].

In addition to the above factors, variants in different domains could elucidate most of the differences in oligodontia phenotypes. EDA is a type II membrane protein with four important functional domains: a transmembrane, an extracellular domain containing a recognition sequence for furin protease, TNF, and a collagen-like domain. When the furin site splits, EDA precursor protein can dissociate from the cell membrane to form a trimer and activate EDAR on the cell membrane with its TNF domain, thus initiating the EDAR/EDARADD/NF-κB signal pathway in the cell and regulating the development of ectoderm and its derived tissues [31]. In our study, the *EDA* variant c.466C > T p.(Arg156Cys) was located in the domain of the furin recognition sequence, which could make the polypeptide chain turn, according to the tertiary structure prediction of the EDA variant p.(Arg156Cys), which might result in the failure to cleave the EDA precursor protein. Variants that lead to varying degrees of functional loss in different domains can also lead to differences in oligodontia phenotypes. Studies have demonstrated that variants occurring in the furin domain lead to a more severe oligodontia phenotype than those occurring in the TNF domain, with a mean number of missing permanent teeth of 23.6 and 15.1, respectively.

Table 2
Summary of clinical characteristics of patients with variants in the *EVC2* gene.

EVC2 variants	Location	Variant type	Clinical features	Oligodontia	Reference
c.198insGGCGG	Exon 1	Homozygous	atrial septal defect, short limbs, genu valgum, postaxial polydactyly, multiple oral frenulae, oligodontia, teeth dysplasia	Yes	[7]
c.475C > T	Exon 4	Compound	short limbs, short ribs, postaxial polydactyly, partial atrioventricular	No	[10]
c.1145+1G > A	Intron 9	heterozygous	canal, hypoplastic lungs, club feet		
c.848T > G	Exon 7	Homozygous	atrial septal defect, short limbs, postaxial polydactyly, hypoplastic nails, teeth dysplasia	Unknown	[7]
c.848T > G	Exon 7	Homozygous	short stature, postaxial polydactyly, short ribs, narrow palate	No	[10]
c.1024A > T	Exon 9	Compound	postaxial polydactyly, hypoplastic nails, atrial septal defect, patent ductus	No	[10]
c.1155delT	Exon 10	heterozygous	arteriosus, multiple oral frenula		
c.1195C > T	Exon 10	Homozygous	ventricular septal defect, short limbs, postaxial polydactyly	No	[7]
c.1195C > T	Exon 10	Compound	short stature, short limbs, genu valgum, postaxial polydactyly, hypoplastic	Unknown	[33]
c.2484G > A	Exon 14	heterozygous	nails, teeth dysplasia, coxa vara deformity, defects in lateral epiphyses		
c.1467_1468dupGA	Exon 10	Homozygous	short stature, short limbs, postaxial polydactyly, hypoplastic nails, multiple oral frenula, patent ductus arteriosus, aortic valve stenosis, congenital thoracolumbar scoliosis	No	[10]
c.1472C > T	Exon 11	Heterozygous	oligodontia	Yes	[11]
c.1855C > T	Exon 12	Homozygous	short stature, short limbs, short ribs, postaxial polydactyly, hypoplastic nails, multiple oral frenulae, oligodontia, teeth dysplasia, fusion of the hamate and capitate	Yes	[7]
c.2056insC	Exon 14	Homozygous	short limbs, short ribs, postaxial polydactyly, hypoplastic nails, teeth dysplasia, multiple oral frenulae, hyperplasia of the alveolar ridges, patent ductus arteriosus	Unknown	[7]
c.2653C > T	Exon 15	Compound	short stature, short limbs, genu valgum, postaxial polydactyly, hypoplastic	Yes	[34]
IVS5-2A > G	Intron 5	heterozygous	nails, teeth dysplasia, oligodontia		
c.3337C > T	Exon 17	Compound	retained primary tooth, displaced mature teeth, polydactyly, hypoplastic	No	[35]
c.3754C > T	Exon 18	homozygous	nails, heart murmur		
c.3265C > T	Exon 18	Compound	Short stature, postaxial polydactyly, short ribs, anteriorly placed anus	No	[10]
c.3659+2T > C	Exon 21	heterozygous			
c.3660delC	Exon 22	Homozygous	atrioventricular septal defect, genu valgum, polydactyly, hypoplastic nails, multiple oral frenulae, teeth dysplasia	Unknown	[7]
c.3660delC	Exon 22	Heterozygous	postaxial polydactyly, hypoplastic nails, sparse hair, multiple oral frenula, abnormally shaped teeth, ventricular septal defect, aortic coarctation	No	[10]
c.3660delC	Exon 22	Homozygous	Short stature, postaxial polydactyly, hypoplastic nails, sparse hair, Shone's complex, ventricular septal defect, bilateral deafness, multiple oral frenula	No	[10]
c.3793delC	Exon 22	Heterozygote	short stature, postaxial polydactyly, hypoplastic nails, teeth abnormalities	No	[10]
c.3793delC	Exon 22	Heterozygote	short stature, postaxial polydactyly, hypoplastic nails, teeth dysplasia	No	[9]
c.3793delC	Exon 22	Homozygous	polydactyly, hypoplastic nails, multiple oral frenulae, delayed teeth eruption, oligodontia	Yes	[36]
c.3797T > A	Exon 22	Heterozygous	postaxial polydactyly, hypoplastic nails, multiple oral frenulae, teeth dysplasia, oligodontia	Yes	[9]
c.3797T > G	Exon22	Heterozygous	postaxial polydactyly, hypoplastic nails, teeth dysplasia, oligodontia, osteopenia, mental delay	Yes	[9]
c.3805G > T	Exon 22	Heterozygous	postaxial polydactyly, hypoplastic nails, enamel hypoplasia, oligodontia	Yes	[10]

Unknown, the literature mentioned that the patient had teeth dysplasia, but did not explicitly mention whether the patient had an oligodontia phenotype.

EVC2 and *EVC* are the pathogenic genes of EvC and WAD. Most patients with EvC or WAD have tooth dysplasia, including oligodontia, delayed eruption, conical teeth, abnormal enamel formation, and so on [32]. The *EVC2* variant can also lead to non-syndromic tooth agenesis [11]. We summarised the clinical characteristics of patients with *EVC2* variants (Table 2) [7,9–11,33–36] and found that approximately 35 % of the patients had oligodontia, which could lead to 8–15 teeth missing [11,34,36].

Haushalter et al. studied the expression patterns of genes related to tooth development in mice and found that *Evc2* was expressed in the oral ectoderm and ectomesenchymal compartments of tooth germs, which proved that *Evc2* was related to tooth development [37]. Some studies have suggested that Evc2 may affect tooth development by influencing the Sonic Hedgehog (Shh) signal pathway [38,39]. Shh signal pathway is one of the major pathways regulating tooth development and is important for the occurrence of tooth crowns and roots [40–42]. When the secreted protein Hedgehog initiates the cascade activation of the Shh signal pathway, the activated Smoothened translocates to the primary cilium. Evc-Evc2 and Smoothened then form a protein complex in a specific compartment (the EVC zone) of the cilia. This complex mediates SuFu inhibition and Gli activation. Activated Gli enters the nucleus to regulate the expression of related genes [38,43]. The complex formed by Evc2, Smoothened, and Evc must be in the EVC zone of cilia to function. Dorn et al. found that in recessive EvC, the Evc2 variant was not located in the cilia, thereby preventing the formation of active complexes. In WAD, the Evc2 variant could interfere with the Shh signal pathway because the formed complex is inactive and incorrectly located in the cilia [39]. Heterozygous variants in *EVC2* may also cause the complex to be incorrectly located in the cilia, thereby interfering with the SHH signal pathway. Kangas et al. constructed transgenic mice with increased Eda signal pathway activity and found that the Shh signal pathway expression increased in time and space [44]. Furthermore, Horakova et al. found that a lack of Eda could delay the expression of the Shh pathway and decrease the expression region in the primary enamel node of functional teeth [45]. These studies indicate that the Eda and Shh signal pathways are correlated. In this study, the protein structure prediction of EVC2 p.(Leu591Ser) implied an injured EVC2 protein, resulting in tighter binding of EVC2-EVC dimers. This alteration might affect protein binding to Smoothened or lead to abnormal localisation of the complex, thereby interfering with the SHH pathway, which might result in a more severe oligodontia phenotype.

Thus, we speculate that, in our case, the novel *EVC2* variant c.1772T > C p.(Leu591Ser) may aggravate the oligodontia phenotype, predominantly caused by the *EDA* variant c.466C > T p.(Arg156Cys). Polygenic variants play an important role in analysing individual differences in tooth agenesis. In patients with tooth agenesis, a combination of polygenic variants may lead to changes in gene structure, gene expression, and protein-protein interactions, resulting in diverse tooth agenesis phenotypes. However, the oligodontia phenotype of *EDA* variants, combined with other gene variants, needs to be studied in more cases. Additionally, there is a lack of direct evidence for the association between *EDA* and *EVC/EVC2*, and the relationship between *EVC2* and the EDA pathway requires further study. In the future, we will explore the synergistic pathogenesis of *EDA* and *EVC2* during tooth agenesis.

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Data availability statement

Data will be made available on request.

Ethics statement

We conducted the study with the informed consent of the patient and his guardian and in accordance with the Declaration of Helsinki. All procedures performed in studies were approved by the Medical Ethic Committee of Xiangya Hospital of Central South University (Approval Number: 202203062). The patient's mother (his legal guardian) provided informed consent to participate in the study and agreed to disclose his anonymised case details and images.

CRediT authorship contribution statement

Yi Wu: Writing - original draft, Methodology. **Jing Sun:** Writing - review & editing, Methodology. **Caiqi Zhang:** Visualization, Methodology. **Siyuan Ma:** Formal analysis, Data curation. **Yiting Liu:** Formal analysis, Data curation. **Xiaoshan Wu:** Writing - review & editing, Resources, Conceptualization. **Qingping Gao:** Writing - review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23056.

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