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Novel *DLX3* variant identified in a family with tricho-dento-osseous syndrome

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ABSTRACT

Objectives: To identify *DLX3* variants in a Chinese family with typical clinical manifestations of tricho-dento-osseous syndrome (TDO).
Design: Sanger sequencing was performed to detect *DLX3* variants in the TDO family. Three-dimensional laser scanning microscopy, bioinformatic and conformational analyses were employed to explore the phenotypic characterization and the functional impact.
Results: We identified a novel heterozygous variant in the *DLX3* gene (c.534G>C; p.Gln178His). Familial co-segregation verified an autosomal dominant inheritance pattern. Bioinformatic prediction demonstrated the deleterious effects of the variant, and DLX3 structure changes suggested the corresponding functional impairments.
Conclusions: We identified a variant in the *DLX3* gene in an integrated family of Han nationality for the first time. This study expands the variant spectrum of *DLX3* and phenotype spectrum of TDO syndrome.

1. Introduction

Tricho-dento-osseous (TDO; OMIM #190320) syndrome, a rare ectodermal dysplasia with an autosomal dominant mode of inheritance, is mainly characterized by developmental disorders of the hair, teeth, and bones. The most common clinical manifestations are kinky, curly hair at birth (some cases report the straightening of hair with age) (Price, 1998), brittle nails, enamel hypoplasia, taurodontism (elongation of the dental pulp chamber) (Wright et al., 1997), and increased thickness and/or density of intramembranous and endochondral bones (Haldeman et al., 2004; Kula et al., 1996). The diagnosis of TDO is made based on clinical manifestations and can be further confirmed by DNA sequencing. Ten variants identified in the homeobox gene distal-less 3 (*DLX3*) gene have been closely related to the pathogenesis of TDO (Jain et al., 2017; Lee et al., 2008; Li et al., 2015; Mayer et al., 2010; Nieminen et al., 2011; Price, 1998; Whitehouse et al., 2019; Wright et al., 2008). Taurodontism and enamel hypoplasia are the most common phenotypes, whereas other phenotypes such as nail defects and bone sclerosis are variable among affected individuals.

DLX3 is a member of the distal-less family, which contains six *DLX* genes and encodes a protein that acts as a transcriptional activator (Feledy et al., 1999). *DLX3* is expressed in multiple tissues including hair follicles, the oral epithelium and mesenchyme derived from neural crest cells, and keratinocytes in mice (Morasso et al., 1996). A number of in vitro studies have elucidated the role of *DLX3* in osteogenic and osteoclastic processes in various cell lines (Choi et al., 2008; Hassan et al., 2004). Moreover, in mouse ameloblast LS8 cells, *DLX3* was shown to regulate the expression of enamel matrix proteins by directly binding to their enhancer regions (Zhang et al., 2015). *In vivo* studies further confirmed the important role of *DLX3* on bone mass. The 4 bp deletion (c.571_574delGGGG) of *DLX3* transgenic mice caused increased bone volume and mineral density that resulted from weakened bone resorption activity (Choi et al., 2009). Another in vivo study reported that the trabecular bones of *DLX3* conditional knockout mice have increased

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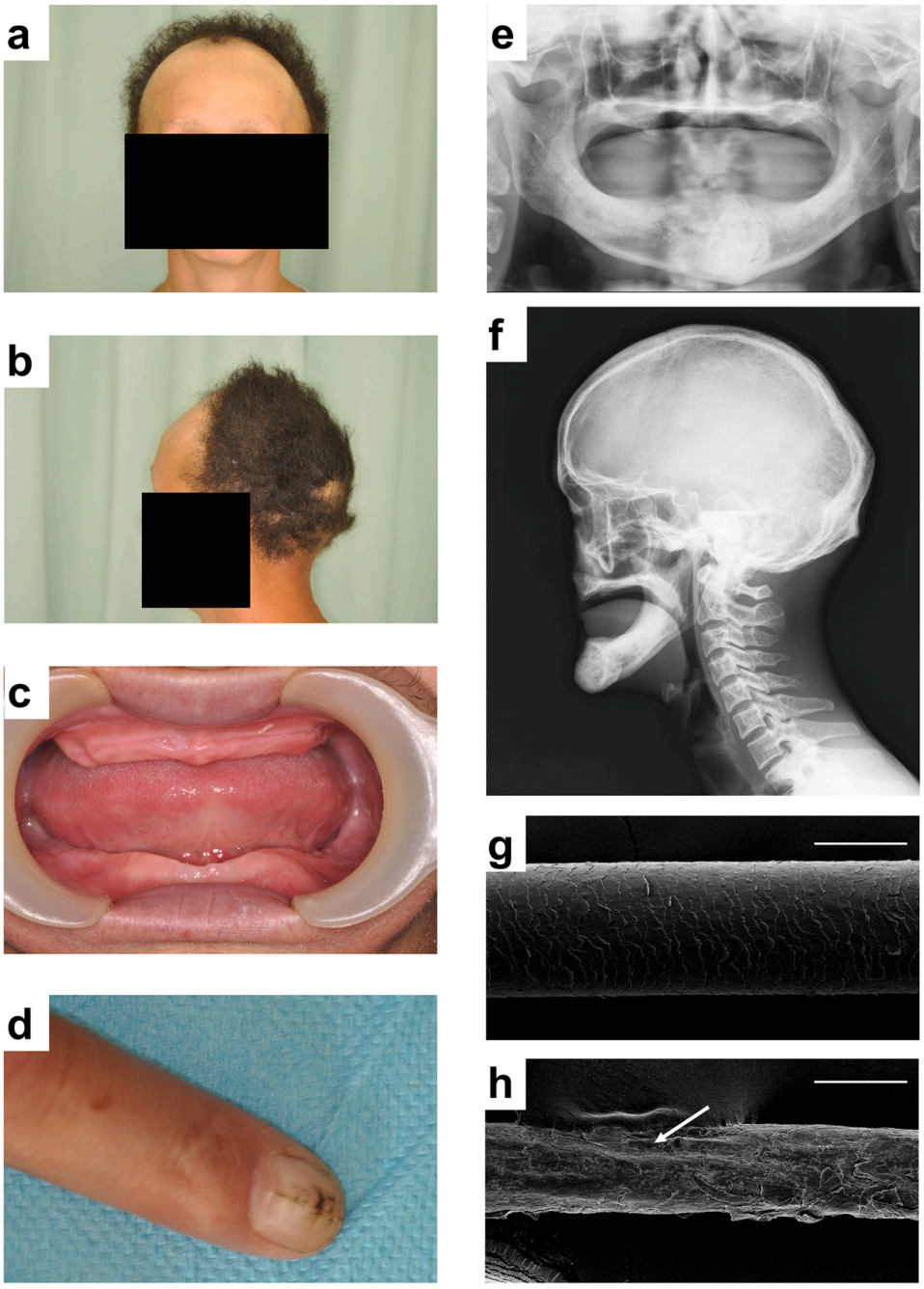

Fig. 1. Clinical features and radiographs of proband (IV:1) with TDO syndrome. (a, b) Frontal and lateral views. Kinky, curly hair and a lack of eyebrows can be seen. (c) Edentulous jaws. (d) Splitting on the superficial layer of the index finger can be seen. (e) Panoramic radiograph. There are no teeth in the mandible or maxilla. (f) Cephalometric radiograph. No increase in the thickness or density of the craniofacial bone is observed. (g) Hair from a normal individual shows normal hair morphology. (h) Hair from the proband. The white arrow indicates a longitudinal groove along the hair shaft. Scale bar: 50 μm.

bone mass due to the upregulation of bone matrix genes when *DLX3* is absent in mesenchymal cells and osteoblasts (Isaac et al., 2014). Furthermore, in mice, the selective ablation of *DLX3* caused complete alopecia due to failure of formation of the hair shaft (Hwang et al., 2008). Thus, *DLX3* plays a significant role in regulating the development of teeth and bone. Furthermore, *DLX3* can balance bone homeostasis by regulating both osteogenic and osteoclastic processes (Zhao et al., 2016).

In this study, we describe a Chinese family with several TDO phenotypes. A novel missense *DLX3* variant was identified in the family. Then we used bioinformatic and conservation analyses to predict the pathogenicity of the identified mutant. This study broadens the variant spectrum of *DLX3*.

2. Materials and methods

2.1. Subjects

A five-generation family was recruited form the Department of Prosthodontics, Peking University School of Stomatology (Beijing, China). Detailed intraoral examinations were performed by an experienced dentist. Panoramic radiographs were taken to observe the thickness and density of the craniofacial bones. Hair samples were randomly obtained from two patients and an unaffected family member. Saliva samples were contributed by four patients in addition to four unaffected family members. Authors had access to information that could identify individual participants during or after data collection. Informed consent was obtained from all the participants and the proband had provided consent for the publication of the clinical photographs. This study was

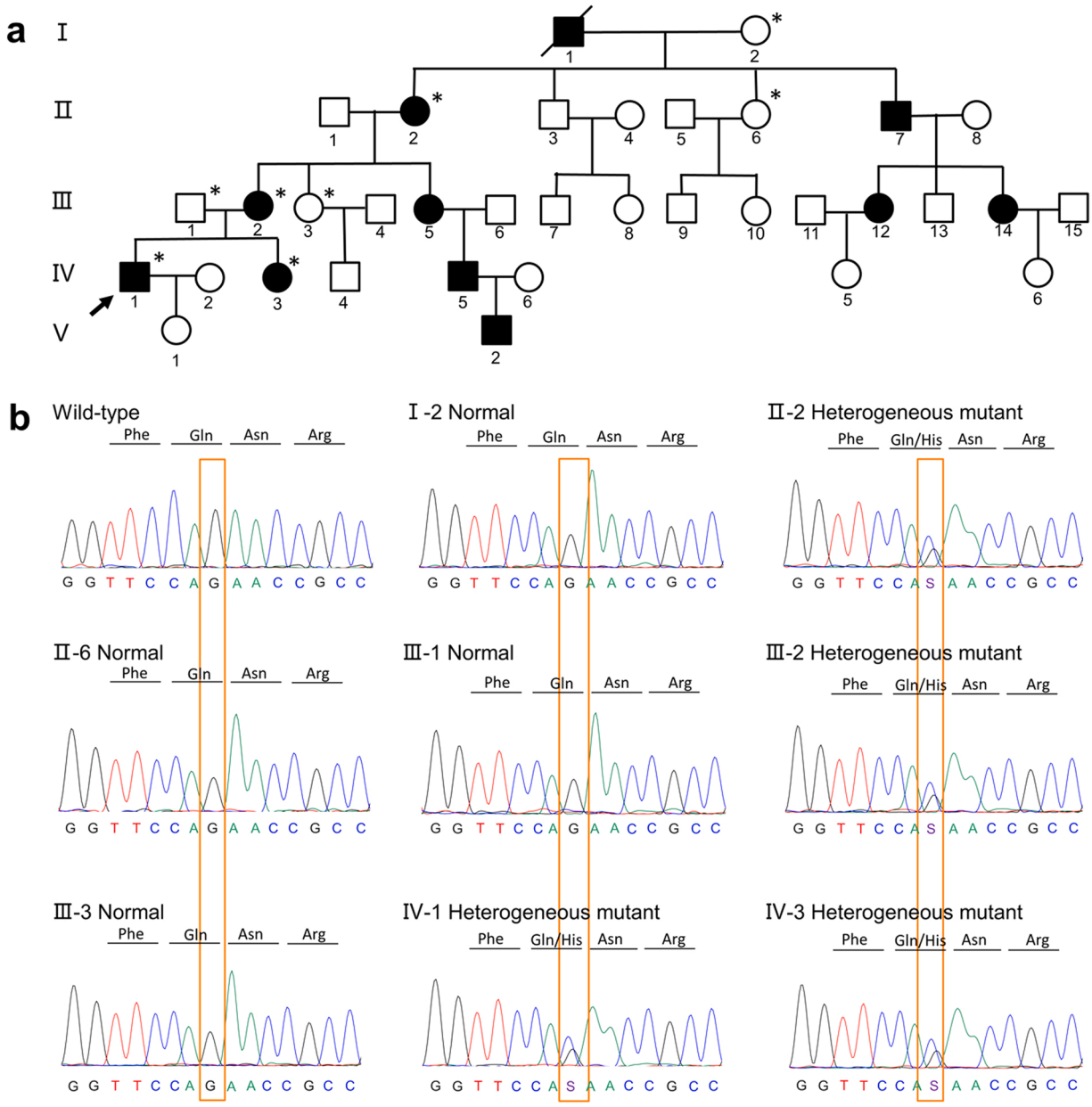

Fig. 2. Pedigree of the TDO family and variant analysis of the DLX3. (a) Pedigree of the family. The arrow indicates the proband. Black squares and circles represent TDO patients. Squares and circles with a slash represent individuals who have passed away. Asterisks represent participating family members. (b) Sequencing chromatograms of available DNA in the family present a heterozygous variant (c.534G>C)in the proband (IV-1) and the other three affected family members (II-2, III-2, and IV-3). Sequencing chromatograms of available DNA of unaffected family members showing wild-type genotype (I−2, II−6, III−1, and III−3).S represent the double peak (G or C).

conducted with permission from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-201736082).

2.2. Three-dimensional laser scanning microscopy

Hair samples obtained from the patients and unaffected family members were observed on a laser microscope (VK-X100/X200; KEYENCE, Osaka, Osakafu, Japan).

2.3. Variant detection

Genomic DNA of the patients and other family members was isolated from saliva samples using prepIT®•L2P (DNA Genotek Inc., Ontario, Canada). Polymerase chain reaction (PCR) was employed to amplify three exons and exon-intron boundaries of the *DLX3* gene. Primers and PCR conditions was according to the methods of Kim et al. (2006). The PCR products were sequenced by Sangon Biotech Company (Beijing, China).

2.4. Bioinformatics and conservation analysis

We excluded the missense variant with a minor allele frequency (MAF)≥ 0.01 in the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org), the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org) and the 1000 Genomes Project data in Ensembl (http://asia.ensembl.org/Homo_sapiens/Info/Index). Online bioinformatics analysis software, such as MutationTaster (a software tool which evaluates disease-causing potential of sequence alterations; https://www.mutationtaster.org/), SIFT (a tool that uses sequence homology to predict whether a substitution affects protein

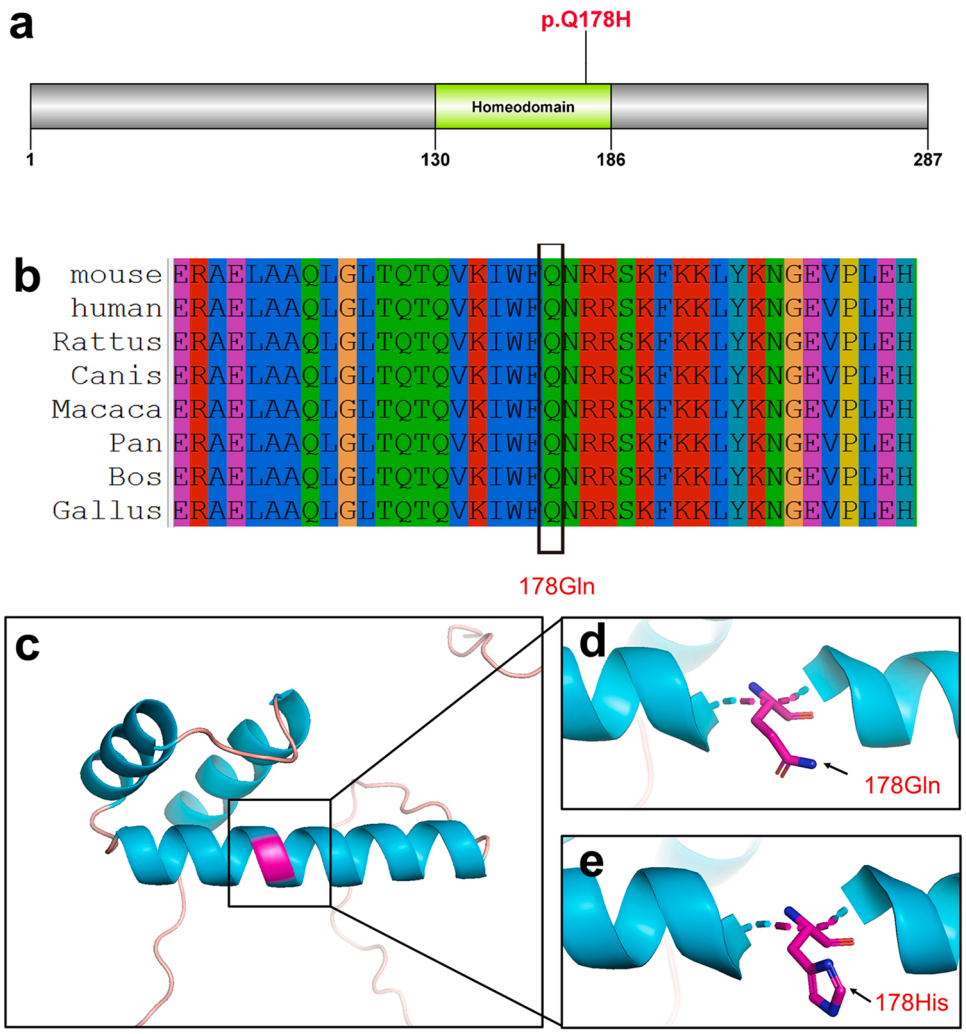

Fig. 3. Variant, conservation, and bioinformatics analyses. (a) Schematic diagram of the DLX3 protein. (b) Conservation analysis of eight different species shows that Gln178 in the DLX3 protein is highly conserved. (c) The predicted three-dimensional models of DLX3. (d) The three-dimensional structural of wild-type178 residue (Gln). (e) The three-dimensional structural of mutated 178 residue (His).

function; http://provean.jcvi.org/index.php), PROVEAN (Protein Variation Effect Analyzer; a software tool which predicts whether an amino acid substitution or indel has an impact on the biological function of a protein; http://provean.jcvi.org/index.php), PolyPhen-2 (Polymorphism Phenotyping v2; a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations; http://genetics.bwh.harvard.edu/pph2/) and fathmm (Functional Analysis through Hidden Markov Models; a high-throughput web-server capable of predicting the functional consequences of both coding variant; http://fathmm.biocompute.org.uk/) were used to predict the functional impact of the mutation. The DLX3 amino acid sequences of different species were obtained from ENSEMBL database. These sequences were aligned using ClustalX 2.1.

2.5. Three-dimensional structural analysis

The modeling of human DLX3 (UniProt 060479) was obtained from the AlphaFold Protein Structure Database (https://www.alphafold.ebi.ac.uk/). The three-dimensional structural analysis was performed using PyMOL2.3 software (DeLano Scientific LLC, San Carlos, CA, USA).

3. Results

3.1. Clinical findings

The proband (IV:1) was a 30-year-old male. His hair was sparse and curly at birth, and he lacked eyebrows (Fig. 1a and b). He was edentulous at a very young age because of the severe infection of his primary and permanent teeth (Fig. 1c). His right index finger had splitting of the superficial layers (Fig. 1d) and his nails had been brittle since childhood. There was no increased bone thickness or density observed in the radiographs of the proband (Fig. 1e and f); however, something like a dental osteoma was noted in the left mandible. The other affected individuals exhibited the penetrant features of kinky, curly hair at birth, a lack of eyebrows, edentulous jaws, and fragile nails. Unaffected members had normal dentition, hair, and nails. Laser scanning microscopy revealed longitudinal grooves along the hair shaft of the proband, and the hair shaft diameter was reduced in the affected individuals compared to the hair sample from the control (Fig. 1g and h). Moreover, the hair shaft lost its normal morphology and showed a flattened manifestation.

3.2. Variant detection

The pedigree of this family showed an autosomal dominant mode of

Table 1
Functional change prediction of the variant (c.534G>C; p.Gln178His) in *DLX3*.

Variant	c.534G>C
Type	Missense
Exon/Domain	Exon 3/Homeodomain
MutationTaster	Prediction: **Deleterious** (MutationTaster predicts a variant as deleterious or benign)
SIFT	Score: 0 Prediction: **Damaging** (Scores <0.05 are predicted to be deleterious; those ≥0.05 are predicted to be tolerated.)
PROVEAN	Score: − 4.46 Prediction: **Deleterious** (Scores <−2.5 are predicted to be deleterious; those ≥−2.5 are predicted to be neutral.)
PolyPhen-2	Score: 1 Prediction: **Probably damaging** (The threshold for the prediction score is between 0 and 1, and the closer to 1, the more harmful it is.)
FATHMM	Score: − 4.49 Prediction: **Damaging** (Scores <−3.0 are predicted to be damaging; those ≥−1.5 are predicted to be neutral.)
ACMG classification (evidence of pathogenicity)	**Likely Pathogenic** (PM1+PM2+ PP1+ PP2+PP3+ PP4)

PM1, Located in a mutational hot spot and/or critical and well-established functional domain; PM2, Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project,1000 Genomes Project, or Exome Aggregation Consortium; PP1, Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease; PP2, Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease; PP3, Multiple lines of computational evidence support a deleterious effect on the gene or gene product; PP4, Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

inheritance (Fig. 2a). A novel heterozygous variant in *DLX3* (NG_023063.1:g.8378G>C; NM_005220.3:c.534G>C) was identified in the proband (IV-1) and the other three participating affected family members (II-2, III-2, and IV-3) (Fig. 2b). This variant was a missense mutation, which results in the substitution of glutamine at residue 178 to histidine (NP_005211.1: p.Gln178His). This variant was not detected in the unaffected family samples (I−2, II−6, III−1, and III−3) (Fig. 2b).

3.3. Bioinformatics findings

The variant was not identified in the 1000 Genomes, ExAC, or gnomAD databases. To further analyze the pathogenicity of the *DLX3* variant (c.534G>C; p.Gln178His), we found that the variant located in the Homedomain (Fig. 3a). Based on online evidence from MutationTaster, Sorting Intolerant from Tolerant (SIFT), Protein Variation Effect Analyzer (PROVEAN), polymorphism phenotyping (PolyPhen-2) and fathmm, the variant was damaging (Table 1). According to the standards of 2015 American College of Medical Genetics and Genomics (ACMG), the variant (c.534G>C; p.Gln178His) was predicted to be likely pathogenic (Table 1). Comparisons of the human wild-type DLX3 protein sequence with orthologs from a series of animals indicated that Gln178 of the DLX3 protein is highly conserved across several species (Fig. 3b). Three-dimensional structural analysis showed that the residue at sequence position 178 in this protein is a glutamine which has a neutral side chain and the variant residue is a histidine which has a positively charged side chain, making it hydrophilic (Fig. 3c,d and e).

4. Discussion

Tissues derived from the ectoderm give rise to a variety of organs such as hair follicles, teeth and nails (Pispa & Thesleff, 2003). Ectodermal dysplasia is a condition that encompasses a series of disorders of the ectodermal appendages (Pinheiro & Freire-Maia, 1994). TDO syndrome is considered one of the ectodermal dysplasia disorders of the teeth, hair, and nails with an unclear incidence rate (Wright et al., 1997). TDO was first described as a distinct syndrome by Lichtenstein et al. in 1971 (Lichtenstein et al., 1972). The clinical manifestations of TDO are variable; the most common phenotypes of TDO include taurodontism and enamel defects (Jain et al., 2017; Jorgenson & Warson, 1973; Kula et al., 1996; Lee et al., 2008; Li et al., 2015; Mayer et al., 2010; Nieminen et al., 2011; Seow, 1993; Shapiro et al., 1983; Whitehouse et al., 2019; Wright et al., 1997, 2008). As a result of enamel defects, dental abscesses are common, which can lead to edentulous in some affected adults. Kinky, curly hair at birth is the second most common phenotype, which in approximately half of patients, straightens with age (Price, 1998). However, even within the same family, the hair defects may differ among affected members (Seow, 1993). Other ectodermal defects, such as abnormal nails and an increase in bone thickness and/or density, are variable in affected individuals. In this study, the penetrant features included kinky, shaggy hair, edentulous jaws at a very young age caused by pulpitis and fragile nails. According to these clinical manifestations, we came to the diagnosis of TDO. The hair defects observed by electron microscopy confirmed the diagnosis. To further confirm the diagnosis, we conducted DNA sequencing in this family, which revealed a novel heterozygous variant (c.534G>C) in *DLX3*.

The conservation analysis of the DLX3 protein in eight different species indicated that the homeodomain sequence of DLX3 is highly conserved. This conservation suggests that DLX3 plays an essential role in regulating the development of organisms, as *DLX3* variants are responsible for TDO in humans and a disruption in the *DLX3* gene in mice is embryonically lethal due to placental failure (Morasso et al., 1999). The novel variant identified in this study, c.534G>C, caused the substitution of glutamine to histidine at position 178, which was predicted to be damaging according to MutationTaster, SIFT, PROVEAN,

Table 2
Variants identified in tricho-dento-osseous syndrome.

References	Mutation type	Gene (NG_023063.1)	cDNA (NM_005220.3)	Protein (NP_005211.1)
Price (1998)	Frameshift	g.8405_8418delGGGG	c.561_574delGGGG	p.Gly191Argfs*66
Wright et al. (2008)	Frameshift	g.8405_8406delCT	c.561_562delCT	p.Tyr188Glnfs*13
Lee et al. (2008)	Frameshift	g.8405_8406delCT	c.561_562delCT	p.Tyr188Glnfs*13
Mayer et al. (2010)	Missense	g.8368T > C	c.524T > C	p.Ile175Thr
Nieminen et al. (2011)	Missense	g.8389C>T	c.545C>T	p.Ser182Phe
Nieminen et al. (2011)	Missense	g.8242G>C	c.398G>C	p.Arg133Pro
Li et al. (2015)	Missense	g.8377A>G	c.533A>G	p.Gln178Arg
Jain et al. (2017)		a 4-bp mutation		
Whitehouse et al. (2019)	Frameshift	g.8418delG	c.574delG	p.Glu192Argfs*66
Whitehouse et al. (2019)	Missense	g.8320G>T	c.476G>T	p.Arg159Leu
Whitehouse et al. (2019)		a heterozygous deletion of the entire coding region of *DLX3*		
This study	Missense	g.8378G>C	c.534G>C	p.Gln178His

PolyPhen-2 and fathmm software. The c.534G>C variant is located within the homeobox region, which encodes the homeodomain of the DLX3 protein. Three-dimensional structural analysis showed that a change from a Gln to a His side chain may result in a change to the protein's function. The homeodomain is the DNA-binding region characterized by a helix-turn-helix motif (Bobola & Merabet, 2017). Most vertebrates have approximately 250 homeobox genes that act as transcription factors to control gene expression by binding specifically to their targets (Holland, 2013). They are essential in embryonic stem cells, indicating that the organism's developmental process may be incorrect without the appropriate expression of homeodomain proteins (Young, 2011). As a homeodomain protein, DLX3 plays an important role in regulating the development of several organs, particularly the bone, teeth, and hair, which has been confirmed by numerous reports (Choi et al., 2008, 2009; Hassan et al., 2004; Hwang et al., 2008; Isaac et al., 2014; Morasso et al., 1996; Zhang et al., 2015; Zhao et al., 2016).

To date, ten variants responsible for TDO have been identified in the *DLX3* gene (Table 2)(Jain et al., 2017; Lee et al., 2008; Li et al., 2015; Mayer et al., 2010; Nieminen et al., 2011; Price, 1998; Whitehouse et al., 2019; Wright et al., 2008). The most common disorders in individuals with different *DLX3* variants are enamel defects and taurodontism, which were observed in almost every patient in the current study. However, the abnormal hair, nail, and bone features were not consistent. Increased thickness of the cranial bone was not observed in the radiographs of the proband in this study. These results suggest that variation in the phenotype among *DLX3* variants needed to be solved in subsequent studies.

In conclusion, we identified a novel missense variant (c.534G>C; p.Gln178His) in the *DLX3* gene in a Chinese family diagnosed with TDO. We previously reported a variant in the *DLX3* gene in a Chinese patient; however, the patient described in that report was a sporadic case. In this study, we identified a variant in the *DLX3* gene in an integrated family of Han nationality for the first time. We further confirmed that the *DLX3* variant is responsible for TDO syndrome in the Han Chinese population and expanded the variant spectrum of *DLX3*. However, the precise relationship between the structure and function of the DLX3 protein needs further investigation.

CRediT authorship contribution statement

Haochen Liu: Investigation, Data curation, Writing-Original draft **Yue Wang:** Methodology, Data curation, Writing-Original draft **Hangbo Liu:** Investigation, Software, Formal analysis, Writing-Original draft **Miao Yu:** Investigation, Validation, Writing-Original draft **Jinglei Zheng:** Investigation, Software, Writing-Original draft **Hailan Feng:** Conceptualization, Funding acquisition, Writing-Review& Editing **Yang Liu:** Validation, Writing-Review& Editing **Dong Han:** Project administration, Supervision, Writing-Review& Editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability Statement

The data presented in this study are openly available in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), Submission ID: SCV002074133.

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