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Original Article

Identification of a novel heterozygous mutation in the DLX3 gene of a Chinese family with tricho-dento-ossenous syndrome

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Abstract: Tricho-dento-ossenous (TDO) syndrome is a rare autosomal dominant disorder, characterized by abnormal hair, teeth and bone. Previous studies have shown that TDO syndrome is associated with the DLX3 homeobox gene mutation, a transcriptional activator regulating target gene expression by binding to cognate DNA sequences. In this study, we reported a five-generation Chinese family with typically features of TDO syndrome. The affected family members have presented with thin or fine hair, sparse or even absent eyebrows and eyelashes and markedly congenitally missing teeth. To investigate the potential involvement of disease-related gene mutation in the development of TDO syndrome in this family, we analyzed the genomic DNA sequences of family member blood samples by PCR amplification and Sanger sequencing, and mapped the disease locus of this identified family to human chromosome 17q21 and found a novel heterozygous mutation in exon 3 of DLX3. This guanine to cytosine transversion at nucleotide position 534 (c.534 G > C) in DLX3 gene leads to a glutamine to histidine substitution of amino acid 178 (p.Gln178His), a severe missense mutation in the primary sequence of DLX3 protein. Our results suggest that defective DLX3 expression caused by multiple genomic mutations functions as the principal pathogenic mechanism mediating the development of TDO syndrome.

Keywords: Tricho-dento-ossenous syndrome, DLX3, heterozygous mutation

Introduction

Tricho-dento-ossenous (TDO) syndrome, a rare genetic disorder inherited in autosomal dominant pattern, is characterized by significant morphologic abnormalities mainly involving the hair, teeth and bone [1]. As previously revealed by a number of clinical reports, patients with TDO syndrome usually exhibit significant clinical symptoms such as kinky/curly hair in infancy, dysplastic nails, a wide variety of tooth developmental defects such as taurodontism and enamel hypoplasia, and also abnormal bone development featured by increased thickness and density like bone sclerosis of calvarium and long bones [2-4]. Although this severe hereditary disorder has been investigated for several decades, the causative genetic mutation and the underlying mechanisms has not been fully understood.

Early in 1997, Hart et al. have first linked the TDO syndrome locus to markers on chromosome 17q21 through investigating a four multiplex American families by genome-wide search strategy [5]. Later on, Price et al. further showed that a 4 bp deletion mutation in the DLX3 gene, one of the two genes localized in the chromosome 17q21, caused a functionally altered DLX3 protein by frame shift and premature termination codon that was associated with TDO syndrome in multiple families [6, 7]. DLX3, a homeobox gene of the Distal-less family, is a transcriptional activator that regulates the transcription of its target genes through binding to cognate DNA sequences [8, 9]. Previous experimental and clinical studies have revealed that DLX3 plays a crucial role in the patterning of hair, teeth and bone [10, 11]. Moreover, the involvement of DLX3 in the development of TDO syndrome has also been demonstrated by large number of recent reports, strongly suggesting the key roles of DLX3 gene mutations in the development of multiple typical symptoms of TDO syndrome [12-15]. The genetic analysis of

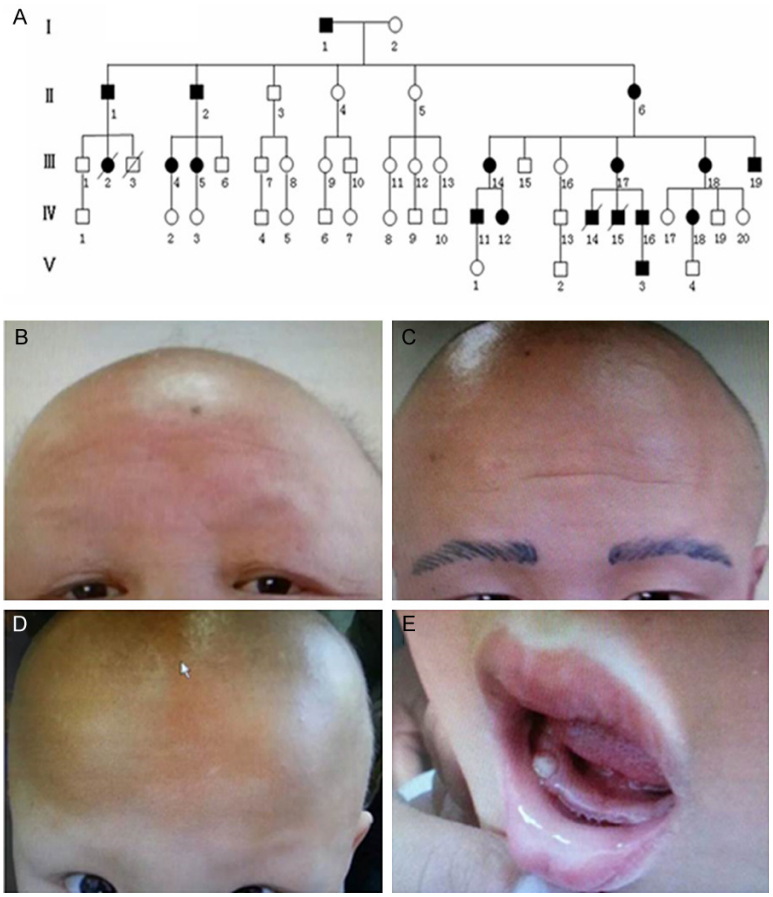

Figure 1. Clinical phenotypes of a Chinese family with TDO syndrome. (A) Family pedigree of the Chinese family registered in this family. (B-D) Similar typical hair features among affected members of three generations. The hair feature of three affected members III: 17 aged 50 years (B), IV: 16 aged 27 years (C) and V: 3 aged 3 years and 5 months (D) were shown here. (E) Missing deciduous teeth in the affected member V: 3 aged 3 years and 5 months.

a Chinese patient with the typical TDO traits discovered another causative mutation for TDO, a novel missense mutation in the conserved homeodomain of the DLX3 gene [16]. More recently, a recurrent 2-bp deletion mutation in DLX3 gene has been identified in a family with autosomal dominant amelogenesis imperfecta (AI) [17]. All these identified DLX3 mutations associated with TDO symptoms convincingly demonstrated the critical role of DLX3 gene in the pathogenesis of this severe hereditary disorder. However, the molecular mechanisms underlying the progression of TDO syndrome have not been fully addressed and the identification of more genetic mutations linked with this hereditary disorder might deepen our understanding of its pathogenesis, which could greatly facilitate the early detection and treatment.

In the present study, we investigated a five generation Chinese family with typical features of TDO syndrome, and the disease locus of this family was also mapped to human chromosome 17q21. Further genetic analysis identified a novel guanine to cytosine transversion at nucleotide position c.534 in exon 3 of DLX3 gene prevalent in the affected family members. Our results indicate that the novel mutation in exon 3 of DLX3 gene might contribute to the pathogenesis of TDO syndrome in this affected family, and further confirmed the role of DLX3 gene as the key causative factor for TDO syndrome.

Materials and methods

Subjects

A five-generation DLX3 family with 51 members was investigated in this study, including 4 decreased members. Clinical examinations and genetic analysis were performed on 14 family members, including 7 affected members and 7 unaffected members. The study protocol was approved by the Ethics Committee of Harbin Medical University in China and was carried out in accordance with the Declaration of Helsinki. Written informed consents were obtained from all the participants.

DNA sample collection

Peripheral blood samples of 14 members were collected from each participant for DNA analysis. Genomic DNA was isolated using a TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China), and stored at -20°C for further analysis by PCR amplification.

Genetic analysis

The following primers were designed to amplify exon 3 of DLX3: Forward, 5'-ATT GGG TTC TGG

Table 1. Clinical phenotypes of patients with Tricho-Dento-Osseous Syndrome

No.	Gender	Age	Presentation		
			Hair	Teeth	Bone
1	Female	47	Anaphalantiasis, alopecia	Teeth falling out	Digital clubbing, swelling of the ankle joint
2	Male	23	Anaphalantiasis, alopecia	Teeth falling out	Digital clubbing, swelling of the ankle joint
3	Male	33	Anaphalantiasis, alopecia	Shedding of deciduous teeth	Digital clubbing, swelling of the ankle joint
4	Female	45	Anaphalantiasis, alopecia	Teeth falling out	Digital clubbing, swelling of the ankle joint, and compressed fingernail and toenail
5	Female	20	Anaphalantiasis, alopecia	Teeth falling out	Digital clubbing, swelling of the ankle joint
6	Male	31	Anaphalantiasis, alopecia	Teeth falling out	Normal
7	Male	49	Anaphalantiasis, scant curly hair	Teeth falling out	Digital clubbing, swelling of the ankle joint
8	Female	32	Anaphalantiasis, alopecia	Teeth falling out	Digital clubbing, swelling of the ankle joint
9	Female	29	Anaphalantiasis, alopecia	Teeth falling out	Compressed fingernail with normal digital outline

CCT TTC TT-3'; Reverse, 5'-CCT CGA TGA TTC CTG AGT GG-3'. The PCR amplification of DLX3 sequence was performed for 35 cycles with an annealing temperature of 57°C. The 420 bp PCR products were further purified and analyzed by Sanger sequencing (MyGenositics, Beijing, China). Sequencing analysis was performed using the Genome analysis toolkit.

Results

Clinical findings

To explore the potential mechanisms mediating development of TDO syndrome, a Chinese family diagnosed with this hereditary disordered was registered in this study. Among all 51 members of the five generation family, 18 family members were affected by TDO syndrome with significant morphological abnormalities in both the hair and teeth (**Figure 1A**; **Table 1**). Affected family members exhibited typically thin or fine hair, combined with sparse or even absent eyebrows and eyelashes (**Figure 1B-D**). Meanwhile, affected members usually suffered from congenitally missing deciduous teeth (**Figure 1E**). The detailed symptoms of affected family members were listed in **Table 1**. The combination of above symptoms is characteristic of TDO syndrome associated with TLX3 gene mutation according to previous reports.

Mutational analysis of DLX3

To further analyze the relationship between these typical symptoms of TDO syndrome and DLX3 gene mutation in the affected members of this family, we performed PCR amplification of three exons in DLX3 gene and the PCR products were further sequenced for gene mutation validation. Blood samples of all 7 affected members (III: 4, III: 5, III: 17, III: 18, IV: 16, IV: 18 and V: 3) and 7 unaffected members (III: 6, III: 15, III: 16, IV: 13, IV: 17, IV: 19 and V: 4) were analyzed. As clearly shown in **Figure 2**, a novel heterozygous guanine to cytosine transversion at nucleotide position 534 in exon 3 of DLX3 gene was discovered in affected members by Sanger sequencing (**Figure 2A** and **2B**). This guanine to cytosine transversion at nucleotide position 534 (c.534 G > C) in DLX3 gene would result into a glutamine to histidine substitution of amino acid 178 (p.Gln178His), finally causing a severe missense mutation in the primary sequence of DLX3 protein (**Figure 2A**). The sequencing results of all members including the affected and unaffected members were presented in **Figure 2B**. The exon 3 sequences of DLX3 gene in all 7 affected members harbor such a guanine to cytosine transversion, strongly suggesting that this missense mutation in exon 3 of DLX3 gene might be responsible for the development of TDO syndrome in this five generation family.

Discussion

The mechanisms underlying severe human genetic defects have long been attached great importance by researchers in genetics and medical sciences. In the current study, we reported the clinical features and potential pathogenesis of a five-generation Chinese family affected with Tricho-dento-ossenous (TDO) syndrome, a congenital multisystem disease due to ectodermal dysplasias and characterized by specific clinical phenotypes include curly or kinky hair, taurodontism, enamel hypoplasia and increased bone mineral thickness and density [2-4]. However, previous reports revealed great variability of clinical phenotypes

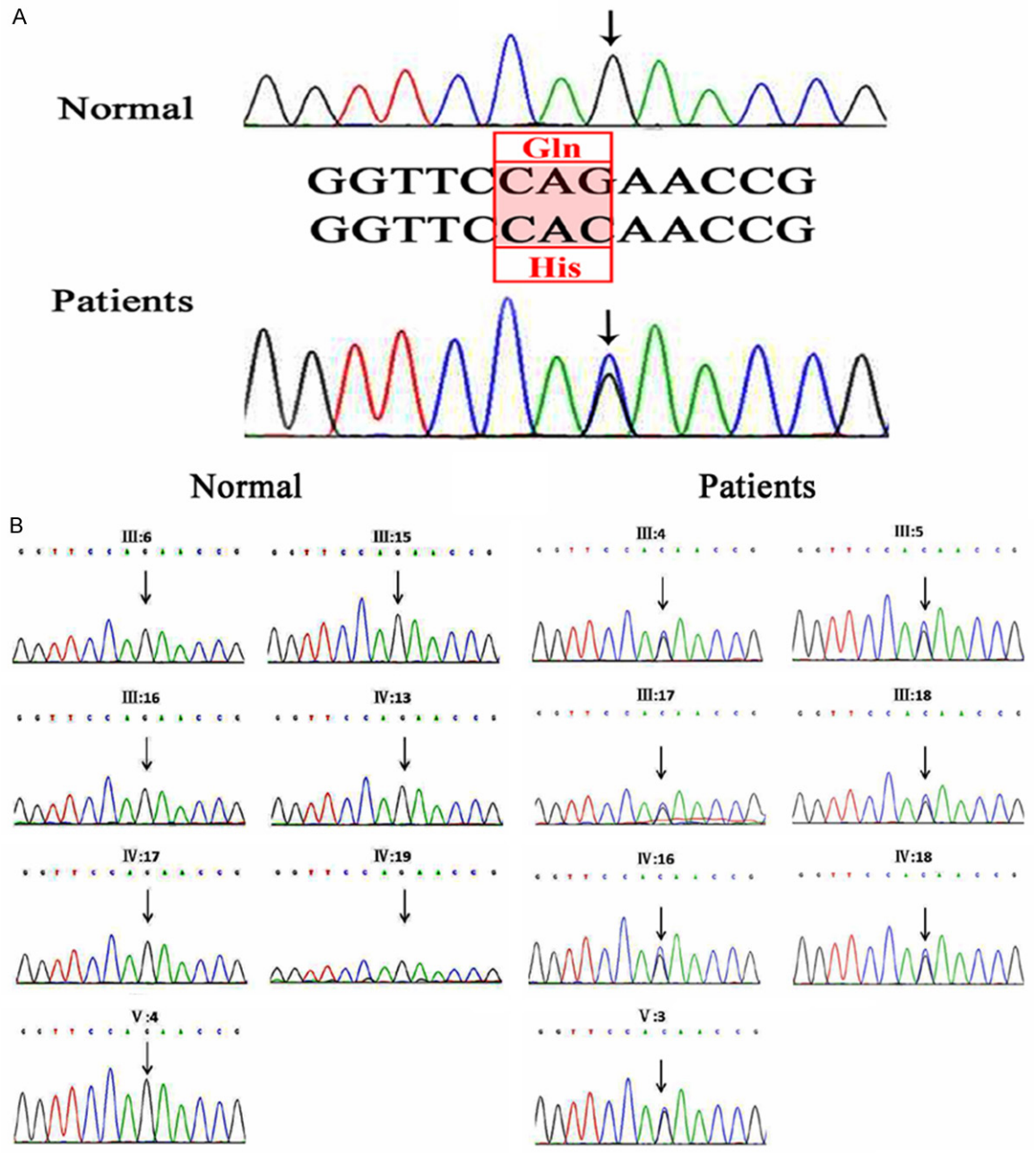

Figure 2. Missense mutation in the exon 3 of DLX3 gene in patients with TDO. A: Representative sequences of missense mutation in family members. The genotype sequences of the nucleotide position 534 and its flanking sequences of one affected member (Patient) and one unaffected member (Normal) were representatively shown here. The amino residues encoded by the wild-type and missense mutation sequences were also shown in the middle. B: Sequencing results of all affected and unaffected family members. The normal group with wild-type genotyping includes unaffected members III: 6, III: 15, III: 16, IV: 13, IV: 17, IV: 19 and V: 4, while the patient group with DLX3: c.534 G > C missense mutation includes affected members III: 4, III: 5, III: 17, III: 18, IV: 16, IV: 18 and V: 3. The amino residues encoded by the wild-type and missense mutation sequences were also shown in the corner.

among different TDO families, and even individual patients within the same affected family usually exhibited distinct morphological abnormalities [18]. The affected members of this five-generation Chinese family described here showed prominent TDO syndrome features especially in hair and teeth, and sparse or colorless hair and early loss of teeth in childhood were the main reasons for their initial hospital visits to seek medical advice. The hair features

of patients with TDO syndrome in this Chinese family were similar with two other Finnish families with TDO syndrome reported by Nieminen et al. [19], although their main symptoms were slightly different from some other reported families especially in terms of the typical curly hair phenotype. Also, it is reasonable to speculate that the difference in major symptoms might be caused by distinct gene mutation styles.

Previous genetic studies have shown that the homeobox DLX3 gene localized in human chromosome 17q21 played important roles in embryonic development and was involved in the regulation of hair, teeth and bone development [10, 11]. There is growing evidence showing that the deletion and missense mutations in the DLX3 gene have been closely related with the pathogenesis of TDO syndrome. Early in 1998, Price et al. have mapped the disease locus for TDO to chromosome 17q21 and also identified a 4 bp deletion mutation in the human DLX3 gene in six families which closely correlated with the TDO phenotypes [6, 7]. Further research revealed that the DLX3, 4 bp deletion mutation could resulted into markedly increased bone density in TDO patients and suggested important role of the DLX3 gene in bone formation and appendicular skeleton homeostasis [20]. Similar deletion mutations have also been found in additional TDO families [13, 14]. Subsequently, multiple in vitro and in vivo studies further verified that the DLX3 deletion mutation could affect osteogenesis and amelogenesis, thus mediating TDO pathogenesis [21-24]. Besides deletion mutation, missense mutations in DLX3 homeobox gene have also been identified in individual patient or family members with TDO, showing the complexity of DLX3 gene involvement in the pathogenesis of this hereditary disorder [15, 19]. In this study, we identified a novel a heterozygous guanine to cytosine transversion in exon 3 of DLX3 gene, which would lead to a glutamine to histidine substitution of amino acid 178 (p. Gln178His), and might finally cause significant alteration of DLX3 protein structure and function. The abnormal development of hair and teeth in this TDO family might be attributed to this newly identified missense mutation, and the prevalence and underlying molecular mechanisms deserved further investigation.

Taken together, we described a novel missense mutation in the DLX3 homeobox gene associated with TDO syndrome in a five-generation Chinese family. Our results provided a new molecular pathogenic mechanism of TDO syndrome mediated by DLX3 gene and deepened our understanding of the pathogenesis of TDO syndrome as well as the regulating role of the DLX3 gene in hair, teeth and bone development.

Disclosure of conflict of interest

None.

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