• Basic Research •

Identification of a novel mutation in the *FGF10* gene in a Chinese family with obvious congenital lacrimal duct dysplasia in lacrimo-auriculo-dento-digital syndrome

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Abstract

● **AIM:** To identify the pathogenic gene variant in a family with lacrimo-auriculo-dento-digital syndrome [LADD (MIM 149730)] showing congenital lacrimal duct dysplasia as the main clinical manifestation and lay the foundation for future research on the pathogenic gene.

● **METHODS:** Ophthalmological examinations, including slit-lamp biomicroscopy and lacrimal duct probing, and computed tomography dacryocystography (CT-DCG) were performed for all participants. The family pedigree was drawn, genetic features were analyzed, and the genomic DNA of the subjects was extracted. Pathogenic genes were screened *via* whole exome sequencing (WES) and confirmed using Sanger sequencing.

● **RESULTS:** Six patients belonged to this three-generation family, and their clinical manifestations included congenital nasolacrimal duct obstruction, congenital absence of lacrimal puncta and canaliculi, lacrimal fistulae, and limb deformities. This pattern indicates autosomal dominant inheritance. Diagnosis was based on the clinical characteristics of LADD syndrome, which presented in all the patients in this family. A novel frameshift mutation in the *FGF10* gene (NM_004465.1), c.234dupC (p.Trp79Leus*15), was identified in all patients *via* WES. The variant was confirmed by Sanger sequencing and classified as a "pathogenic mutation" according to the American College of Medical Genetics and Genomics (ACMG) variant interpretation guidelines.

● **CONCLUSION:** A novel frameshift mutation in the *FGF10* gene is found in all patients. This finding helps this family with LADD syndrome receiving a more accurate clinical diagnosis and genetic counseling by extending the mutation range of the *FGF10* gene.

● **KEYWORDS:** *FGF10* gene; frameshift mutation; congenital lacrimal duct dysplasia; LADD syndrome; pedigree

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INTRODUCTION

Congenital lacrimal duct dysplasia is a common ophthalmological condition. Atresia of the Hasner valve at the end of the nasolacrimal canal is the primary cause of congenital nasolacrimal duct obstruction (CNLDO), which affects 5%–20% of newborns[1-2]. As the eyes develop, the majority of children aged between a few months after birth and 12mo are able to open the membrane obstruction at the distal end of the nasolacrimal duct (NLD), and by the age of one year, 96% of cases of symptomatic epiphora spontaneously resolve[3]. However, congenital lacrimal duct dysplasia is not only a simple membranous obstruction at the distal end of the NLD, but also accompanied with partial or total congenital absence of the lacrimal duct, aplasia of the lacrimal and salivary glands, among others, and dysplasia of other organs, making its clinical diagnosis and treatment difficult[4]. The main clinical manifestations include epiphora and discharge in childhood, accompanied with redness, swelling, and pain in the lacrimal sac region, occasionally complicated by dacryocystitis fistula, orbital cellulitis, and intracranial infection, among others[5-6]. Congenital lacrimal duct dysplasia may be present at

birth as a component of several congenital syndromes, such as the acro-dermato-ungual-lacrimal-tooth (ADULT) syndrome, Rubinstein-Taybi (RTS) syndrome, and lacrimo-auriculo-dento-digital (LADD) syndrome[7].

Fibroblast growth factor 10 (FGF10) is a member of the FGFs family, which comprises 22 ligands grouped into 7 subfamilies that can bind to 4 receptors (FGFR1–4)[8]. FGFRs are members of the tyrosine kinase receptor family, which is predicted to encode proteins with an extracellular domain comprising either two or three immunoglobulin-like domains, a cytoplasmic tyrosine kinase domain, and a transmembrane domain[9]. In 1997, Emoto *et al*[10] examined the chromosomal localization of human FGF10 using *in situ* hybridization. The gene was located in the 5p12-p13 region on chromosome 5 and contained three exons. The literature on genetic mutations of *FGF10* reported broad involvement of ectodermal dysplasia, mainly congenital lacrimal duct dysplasia, which is usually associated with congenital and inherited disorders such as LADD syndrome and aplasia of lacrimal and salivary glands (ALSG) syndrome[11-12]. To lay the foundation for further research on the pathogenesis of congenital lacrimal duct dysplasia, facilitate clinical diagnosis, and provide more efficient genetic counseling for affected families, it is necessary to study the genetic and clinical characteristics of congenital lacrimal duct dysplasia and identify the pathogenic genes.

In this study, we investigated a multi-generational family with LADD syndrome showing congenital lacrimal duct dysplasia as the major clinical manifestation. A novel frameshift mutation in *FGF10* in this family was detected by whole exome sequencing (WES) and confirmed by Sanger sequencing.

SUBJECTS AND METHODS

Ethical Approval The study was approved by the Ethical Review Board of the Third Medical Center, Chinese PLA General Hospital (KY2023~012), and followed the principles of the Declaration of Helsinki. All 14 subjects voluntarily participated in this study and provided informed consent.

Clinical Data Investigation of the Family Members We identified 14 members of a pedigree from Lian Yungang City, Jiangsu Province, China. The members and their spouses were all of Chinese Han nationality without consanguineous marriages (Figure 1A).

Clinical Research Methods A field survey was conducted on this family. Ophthalmological examinations, including slit-lamp biomicroscopy, visual acuity, intraocular pressure, and fundus examination, were performed. All patients were examined using puncta membrane paracentesis, lacrimal duct probing, and computed tomography dacryocystography. Family history, marital history, and personal medical history were collected from all members, and inheritance patterns were investigated.

Molecular Genetics Research Methods Six milliliters of peripheral blood was collected from each of the 14 participants (6 patients and 8 healthy participants). Blood samples were stored at -80°C for prolonged storage. Genomic DNA was extracted from the subjects using the QIAamp® DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany). WES was performed on all the participants using Illumina® DNA Prep with Enrichment, and (S)Tagmentation (Illumina, San Diego, CA, USA) for exon capture. Paired-end sequencing was performed using the Illumina NovaSeq 6000 instruments (Illumina). The quality and concentration of the captured library were assessed by fragment analysis and quantitative polymerase chain reaction (PCR). First-generation sequencing techniques were used for the pedigree co-segregation analysis.

Multiple Sequence Alignments and Bioinformatic Prediction of Mutation The ISoGenentic analysis platform was used to analyze the genotypes (human genome build GRCh37/hg19 for analysis) for the WES data. We screened pathogenic variants in public databases (dbSNP, UCSC, and HGMD) by alignment, interpretation, and annotation, evaluated the experimental data quality, and eliminated unqualified variants. The sequencing variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines for pathogenicity assessment of variants. The pathogenicity of the variants was predicted using Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://provean. jcvi.org/index.php). The databases dbSNP, gnomAD, the 1000 Genomes Project (https://www.genome.gov/27528684/1000-genomes-project), and the Exome Aggregation Consortium (http://exac.broadinstitute.org), in addition to a native database, were used to predict the variant frequency in the population. To determine carrier rates in the population, the screened variant sites were aligned in databases (dbSNP, gnomAD, the 1000 Genomes Project, and local laboratory databases). Additionally, the target gene was queried in the HGMD, OMIM, Clinvar, UCSC, Ensembl, RefGene, and NCBI databases to further assess the pathogenicity of the gene variants.

Mutation Sequencing Sanger sequencing was used to confirm the variants. Specific primers were designed using the NCBI and Primer Premier 5 online software. Target fragments of target genes were amplified using PCR under optimal conditions. The concentrated, purified, and amplified fragments were sequenced using the QIAGEN MinElute® Gel Extraction Kit (QIAGEN GmbH). Sanger sequencing data were analyzed using ABI 3500 Dx (Applied Biosystems, Waltham, MA, USA) and Chromas. The following primers were used for PCR amplification: the *FGF10* sense primer 5' to 3', TTGCCTTGCATCGGGTCT; and the *FGF10* anti-sense primer 5' to 3', TGGGTTTGCTGGGTTGAT.

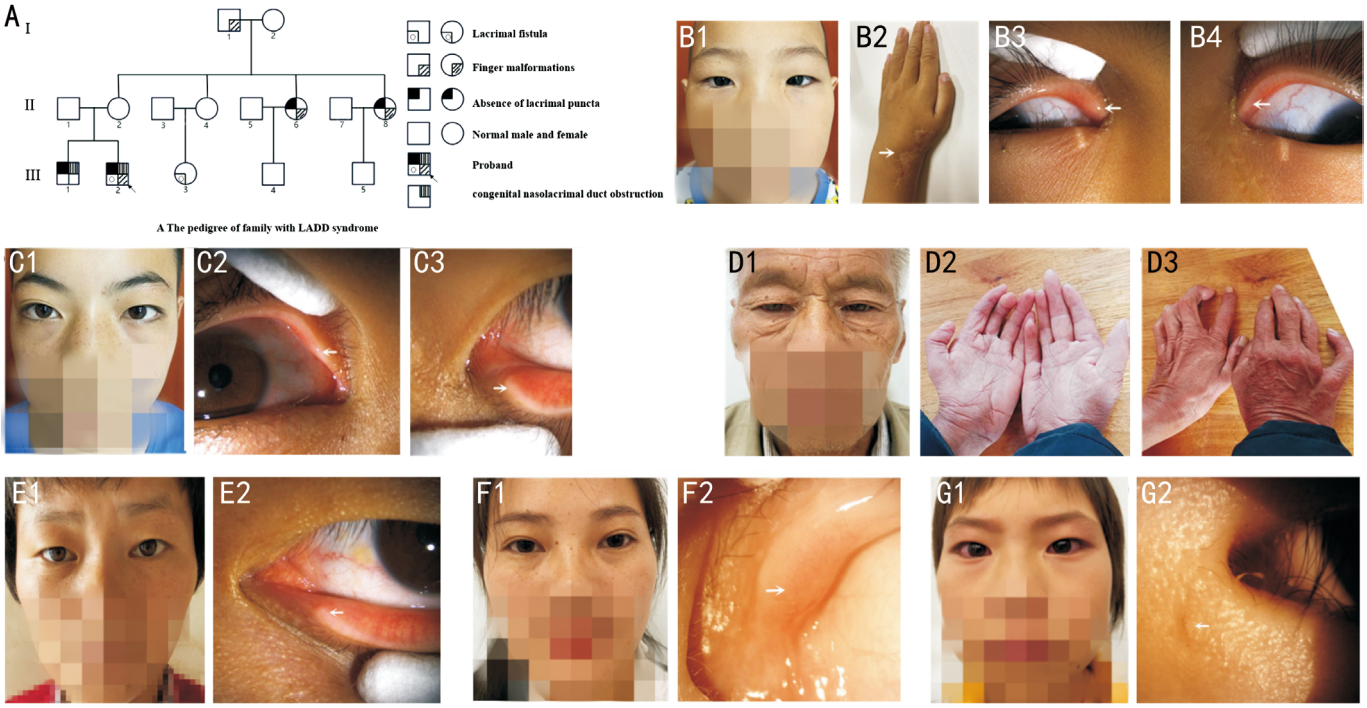

Figure 1 Family pedigree of the proband and clinical photographs of all patients A: Pedigree of family with lacrimo-auriculo-dento-digital syndrome shows six affected individuals in consanguineous family. Square symbol represents male, round symbol represents woman, filled symbol represents the affected, unfilled symbol represents the unaffected, arrow represents proband. II:2 and II:4 were carriers of pathogenic mutation. B1: Facial photograph of proband (III:2). B2: Postoperative scar of polydactyly and syndactyly at left thumb of proband. B3, B4: Arrows indicate lacking lacrimal papilla and superior lacrimal puncta in the proband's lacrimal apparatus, which also lacks superior canaliculi in both eyes. C1: Facial photograph of the patient (III:1). C2, C3: Absence of superior lacrimal puncta and canaliculi in the right eye and absence of inferior lacrimal puncta and canaliculi in the left eye of the patient (III:1). D1: Facial photograph of the patient (I:1). D2, D3: Long, tapering fingers with ulnar deviation at the proximal interphalangeal joints of these digits and partial syndactyly of the third and fourth fingers of the bilateral hands without skin line at the distal interphalangeal joint of the third and fourth fingers of the right hand of the patient (I:1). E1: Facial photograph of the patient (II:6). E2: Absence of inferior lacrimal puncta and canaliculi in the left eye of the patient (II:6). F1: Facial photograph of the patient (II:8). F2: Absence of inferior lacrimal puncta and canaliculi in the left eye of the patient (II:8). G1: Facial photograph of the patient (III:3). G2: Lacrimal fistula in the left eye of the patient (III:3).

RESULTS

Clinical Manifestations We identified a family with LADD syndrome that had congenital lacrimal duct dysplasia as the main clinical manifestation, as determined by detailed clinical and CT examinations. Fourteen members (I:1, I:2, II:1, II:2, II:4, II:5, II:6, II:7, II:8, III:1, III:2, III:3, III:4, and III:5) across three generations from this family participated in this study.

The proband (III:2; Figure 1, B1), a 5-year-old boy, was born with limb malformations, epiphora, and pus in both eyes. He had long, tapering fingers, left-hand partial polydactyly, and left-hand syndactyly of the thumb/multi-finger (Figure 1, B2). At the age of 7mo, he underwent a deformity correction surgery for limb malformation due to obvious left-hand extra thumb. In 2017, he was admitted to the Third Medical Center, Chinese PLA General Hospital, and diagnosed with congenital absence of upper lacrimal puncta and canaliculi in both eyes, lacrimal fistula in his left eye (Figure 1, B3 and B4), and congenital nasolacrimal duct obstruction in both eyes (Figure 2, A1) when he was one and a half years old. He underwent reconstruction of the lacrimal canaliculus by transposition of the lacrimal duct fistula, a dacryocystorhinostomy, and a bicanalicular intubation surgery in his left eye, with epiphora and pus largely alleviated after removing the lacrimal stent. In 2021, at the age of five, he underwent reconstruction of the superior lacrimal canaliculus with a pedicled conjunctival flap, a dacryocystorhinostomy, and a bicanalicular intubation surgery in his right eye at our hospital. Upon removing the lacrimal stent after six months of the procedure, he showed mild epiphora and discharge. This occurred when a chilly breeze was blown on his face. We observed that the proband had eye and limb malformations, and did not show any abnormalities in the lacrimal and salivary glands or other organs.

The elder brother (III:1; Figure 1, C1), a 16-year-old boy, had the same ocular symptoms in his left eye as the proband, but without other systemic abnormalities. He was diagnosed with congenital lacrimal duct dysplasia in both eyes at the age of seven and performed a bicanalicular intubation surgery in his left eye; however, the symptom persisted following

the removal of the lacrimal stent after three months of the operation. Later, he was hospitalized for diagnosis and treatment at our hospital when he was 12 years old. We observed that the patient had congenital absence of the inferior lacrimal puncta and canaliculi in his left eye, congenital absence of the superior lacrimal puncta and canaliculi in his right eye (Figure 1, C2 and C3), and congenital nasolacrimal duct obstruction in his left eye (Figure 2, A2). No other evident systemic abnormalities were observed. The patient underwent reconstruction of the inferior lacrimal canaliculus, a dacryocystorhinostomy, and a bicanalicular incubation surgery of the left eye. Two months later, pus-related symptoms disappeared. However, he briefly displayed epiphora when a chilly breeze was blown on his face after removing the lacrimal stent six-month after the operation.

The grandfather (I:1; Figure 1, D1) had severe defects in both hands, long tapering fingers with ulnar deviation at the proximal interphalangeal joints of these digits, and partial syndactyly of the third and fourth fingers of both hands. In addition, he did not have a skin line at the distal interphalangeal joint of the third and fourth fingers of the right hand (Figure 1, D2 and D3). We observed that the grandfather had a limb malformation without lacrimal duct system abnormalities. The two aunts (II:6, II:8; Figure 1, E1 and F1) had additional thumb deformities, similar to that of the proband, in their left hands, and showed congenital absence of the lacrimal puncta and canaliculi (Figure 1, E2 and F2). They also underwent multi-finger surgical resection. The cousin (III:3; Figure 1, G1), a 12-year-old girl, had lacrimal stenosis in both eyes and lacrimal duct fistula in the left eye (Figure 1, G2). Other family members were normal.

Mutation Analysis From the pedigree, we predicted that the disease was inherited in an autosomal dominant manner. We examined the possible causative genes and performed multiple sequence alignments and bioinformatic prediction of the mutations in all subjects, confirming a novel frameshift mutation (c.234dupC; p.Trp79Leus*15; Figure 3) in the *FGF10* gene in the proband (III:2), his grandfather (I:1), mother (II:2), aunts (II:4, II:6, and II:8), brother (III:1), and cousin (III:3). The mutation was not identified in his father (II:1) or in other members (I:1, II:5, II:7, III:4, and III:5). This newly identified mutation indicated a change in insert C at nucleotide position 234, leading to a substitution of the amino acid tryptophan (Trp) with leucine (Leus) at position 79. Two carrier (II:2 and II:4) members showed a frameshift variant, indicating incomplete penetrance of the autosomal dominant inheritance gene. Sanger sequencing verified the variant and showed that the mutation co-segregated in the affected family members. The variant was not found in the HGMD, OMIM, Clinvar, UCSC, Ensembl, RefGene, and NCBI databases. The

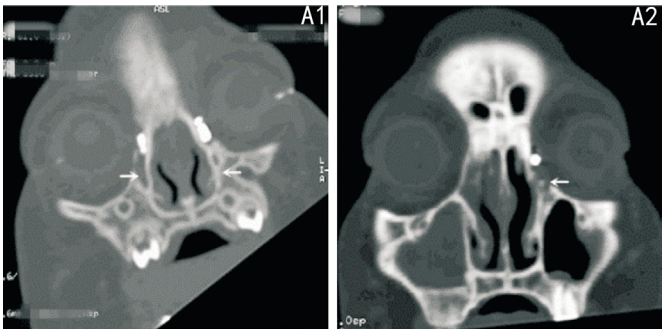

Figure 2 Computed tomography dacryocystography of proband and the patient (III:1) in the family in our study A1: Note congenital nasolacrimal duct obstruction in the both eyes of proband (III:2); A2: Note congenital nasolacrimal duct obstruction in the left eye of the patient (III:1).

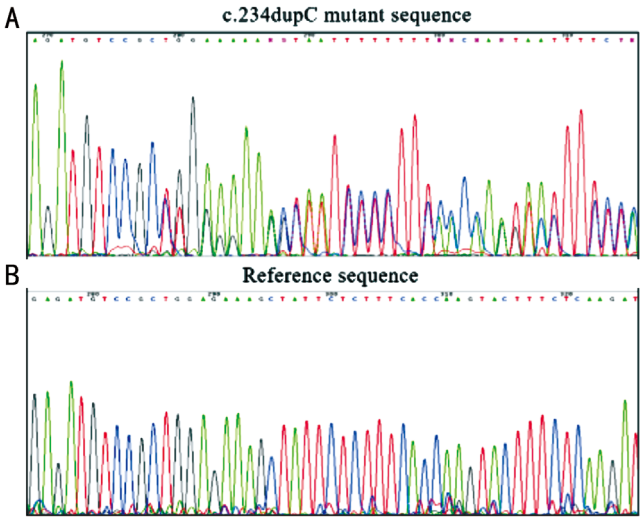

Figure 3 Sequencing results for the FGF10 mutation Sequence chromatograms indicate a heterozygous missense mutation (c.234dupC; p.Trp79Leus*15) in the affected individual. A: The mutant sequence found in the six patients and two carriers; B: The reference sequence.

mutation was classified as a "pathogenic mutation" according to the ACMG variant interpretation guidelines.

DISCUSSION

The lacrimal duct arises embryologically from canalization of the surface ectoderm, which begins as a solid cord of epithelium that invaginates between the maxillary and frontonasal processes at the 6th week of the embryo, giving rise proximally to the canaliculi and distally to the lacrimal sac and nasolacrimal canal[13]. Regardless of the cause, failure of horizontal cell column budding or arrest of development in this embryological process can lead to congenital lacrimal duct dysplasia[2,7]. The canalization process may be segmental, giving rise proximally to a continuous central lumen from multiple isolated canalization cavities or simultaneously throughout the length of the nasolacrimal apparatus, with the exception of the puncta and distal end of the nasolacrimal duct, which remain occluded by a combination of conjunctiva and

canalicular epithelium until the seventh month of gestation[14]. According to the literature, congenital lacrimal duct dysplasia includes partial or complete absence of the lacrimal duct, abnormal canalization of the lacrimal duct, or hypoplasia at the distal end of the NLD[1].

Congenital lacrimal duct dysplasia may occur as part of several congenital syndromes, combined with other organ dysplasia; Zhao and Zhang[15] described 340 patients with congenital lacrimal duct dysplasia and reported that its inheritance patterns may be autosomal dominant, autosomal recessive, or sporadic and that congenital lacrimal duct dysplasia is associated with other congenital syndromes in the majority of patients.

LADD syndrome is inherited as an autosomal dominant disorder. It was first described in 1967[16] and is also known as the Levy-Hollitser syndrome[17]. At present, diverse phenotypes of LADD syndrome have been reported in the literature, including congenital lacrimal duct dysplasia (partial or total congenital absence of the lacrimal duct, atresia of the lacrimal duct or CNLDO, or aplasia of the lacrimal and salivary glands, among others), ear malformations (small, low-set cup-shaped ears and mixed dysaudia), dental issues (dental caries, hypodontia, micrognathia, peg-shaped lateral incisors and developmental enamel defects), and limb malformations (thumb duplication deformity, triphalangeal thumb and congenital syndactyly)[18-19]. In our study, the proband (III:2) and his grandfather (I:1) and two aunts (II:6, II:8) showed several hand deformities. the proband and his two aunts all had fingers malformation. The other patients did not exhibit any hand anomalies. In this investigation, the proband and his older brother and two aunts did not have lacrimal puncta and canaliculi. At the distal end of the NLD, both the proband and his older brother developed blockage. The proband and his cousin also developed lacrimal fistulae. None of the patients showed symptoms of xerostomia or dry eyes, and the lacrimal and salivary glands appeared normal in the CT scan. According to these clinical manifestations and previous literature, the inheritance pattern in our study inferred autosomal dominant inheritance. In our study, none of the patients had dysplasia of the lacrimal or salivary glands. All patients in this family met the diagnostic criteria of LADD syndrome.

LADD syndrome is caused by mutations in *FGF10*, *FGFR*2, and *FGFR*3[20-21]. FGF10 is a member of the FGFs family, a highly evolutionarily conserved group of proteins that trigger signaling via receptor tyrosine kinases. FGF10 is a paracrine factor that regulates tissue and organ formation during embryonic development[22-23]. Studies of the mouse homolog have suggested that this gene is required for embryonic epidermal morphogenesis, including brain development, lung morphogenesis, and initiation of limb bud formation[24]. Current evidence suggests that FGF10 plays an important role in the morphogenesis of the branching organs in the craniofacial complex, and that FGF10 mRNA can be expressed in the stroma near the developing epithelial bud of the mouse lacrimal and salivary glands[25].

In this study, a novel frameshift mutation (c.234dupC; p.Trp79Leus*15) in the *FGF10* gene in exon 1 was detected in the proband and seven other members of the family, including two carriers of recessive pathogenic mutations. This mutation is expected to cause protein truncation or mRNA degradation *via* nonsense-mediated mRNA decay. There is no description of this mutation site in domestic or foreign literature. The proband and other seven members, including the two carrier members, showed a frameshift variant c.234dupC (p.Trp79Leus*15) of the *FGF10* gene on chromosome 5, indicating a change in insert C at nucleotide position 234, leading to a substitution of the amino acid tryptophan with leucine at position 79, which may cause protein truncation or mRNA degradation by nonsense-mediated mRNA decay, leading to premature termination of the translation process. There are two carriers of recessive pathogenic mutations in this family, which may be related to the genetic background, environmental conditions, and gene expression differences, a phenomenon known as incomplete penetrance of the autosomal dominant gene[26]. There may be some association of modifiers that lead to incomplete dominance. Since this variant has not been previously reported and was classified as “pathogenic” according to the ACMG variant interpretation guidelines, further research is necessary to confirm its pathogenicity. This may be achieved by studying more families and running genetic tests to determine other potential mutations.

In summary, according to the literature, the main phenotypes of LADD syndrome were lacrimal glands, ears or teeth dysplasia. However, in this study, we found this family with LADD syndrome characterized by congenital lacrimal duct dysplasia as the main manifestation. Meanwhile, a novel frameshift mutation in the *FGF10* gene (c.234dupC; p.Trp79Leus*15) was found in this family, our data expand the mutation spectrum of *FGF10*. This article proposed a novel frameshift mutation in the FGF10 in a family with obvious congenital lacrimal duct dysplasia in LADD syndrome which is suggestive of the possibility of genetic diagnosis of LADD syndrome. Further clinical and basic research might focus on collecting more evidence of the impact of this mutation on LADD syndrome.

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