• Basic Research •

Preliminary report on screening IGSF3 gene mutation in families with congenital absence of lacrimal puncta and canaliculi

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Abstract

• **AIM:** To investigate the variation of IGSF3 gene in three families with congenital absence of lacrimal puncta and canaliculi, and to lay a foundation for further research on the pathogenic gene of congenital lacrimal duct agenesis.

• **METHODS:** The members of the three families were recruited. The ophthalmologic examinations in details, including slit-lamp biomicroscope, intraocular pressure and fundus examination, *etc.* were carried out. All patients were checked with paracentesis of puncta membrane and lacrimal duct probing, as well as the computed tomography-dacryocystography (CT-DCG). Peripheral blood of 14 participants (3 normal) from three families were collected, 4 mL each, for genomic DNA extraction, and 11 exon fragments of IGSF3 gene were amplified and sequenced by polymerase chain reaction (PCR) to determine whether there were IGSF3 genetic variation.

• **RESULTS:** A total of 14 members from three families were screened for 4 synonymous variants: c.930C>T (p.Pro366=), c.1359T>C (p.Ser709=), c.1797G>A (p.Ser855=), c.1539G>A (p.Ser769=), and 6 missense variants: c.1507G>A (p.Gly759Ser), c.1783T>C (p.Trp851Arg), c.1952G>T (p.Ser 907Ile), c.3120C>G (p.Asp1040Glu), c.3123C>G (p.Asp1041Glu), c.3139_3140insGAC (p.Asp1046_Pro1047insAsp), and the latter three were only found in two patients with absence of lacrimal puncta and canaliculi combined with congenital osseous nasolacrimal canal obstruction from the first family.

• **CONCLUSION:** The same IGSF3 gene mutation c.3139_3140insGAC is found in the patients with congenital absence of lacrimal puncta and canaliculi combine with osseous nasolacrimal canal obstruction.

• **KEYWORDS:** congenital absence; lacrimal puncta; lacrimal canaliculi; IGSF3 mutation; DNA sequencing
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INTRODUCTION

Lacrimal duct disease is common and frequently-occurring in ophthalmology, among which the congenital lacrimal duct absence is a more serious one. The congenital lacrimal duct absence includes lacrimal duct partial absence or total lacrimal duct absence. The congenital absence of lacrimal puncta and canaliculi is one of the cause of epiphora in infants and adolescents, and the dysplasia of other anatomical parts of lacrimal passage is often complicated, making its clinical diagnosis and treatment difficult[1-3]. Clinical practice has shown that patients diagnosed as the absence of puncta have multiple onsets in one family, with obvious genetic tendency[4]. At present, achievements of research abroad show that some genovariation may cause the absence of lacrimal puncta and canaliculi, but most cases are combined with the abnormal development of other organs and tissues of the body, there is no accurate location of pathogenic genes for the congenital absence of lacrimal puncta so far[5-8]. Foster *et al*[9] had reported a family of congenital dacryocystocele with c.2995delc of IGSF3 gene (immunoglobulin superfamily 3), and suggested IGSF3 gene may be the pathogenic genes. The abnormal anatomy of congenital dacryocystocele is shown as the atresia of Hasner valve at the end of the nasolacrimal canal accompanied (or not) by Rosenmüller valve dysfunction[10]. According to the embryonic development characteristics of the lacrimal duct, the canaliculus and valve are both the extension of same type of epithelial tissue. Therefore, it is speculated that the congenital absence of lacrimal puncta and congenital dacryocystocele may have the same pathogenic genes. In the study, IGSF3 gene was sequenced directly in three families with congenital absence of lacrimal puncta and canaliculi to preliminarily screen the IGSF3 gene variation in patients.

SUBJECTS AND METHODS

Ethical Approval The study was approved by the Institutional Research Ethics Committee of Third Medical Center of PLA General Hospital, and all the 14 participants voluntarily joined this study with informed consents.

Clinical Research Methods Detailed ophthalmic examination, including slit-lamp microscopy, intraocular pressure and fundus examination, computed tomography-dacryocystography (CT-DCG), and CT images of the lacrimal duct by 3D reconstruction if necessary, was conducted for participants.

Molecular Genetics Research Methods Peripheral venous blood was collected from 14 members of three families, including 11 patients and 3 normal participants. Genomic DNA of the subjects was extracted (Qiagen, Hilden, Germany), and the concentration and purity of the DNA samples were determined by UV spectrophotometer.

All the 11 exons of IGSF3 gene (GeneBank accession number: NM001542.3) were designed with primer3.0 software (Table 1) and amplified *in vitro*. The polymerase chain reaction (PCR) reaction system includes MIX 25 mL (10×buffer, dNTP, DNA Tag), ddH_2O 21 mL, forward and reverse primer 1 mL each, and DNA sample 2 mL. The specific steps were set as a denaturation at 95℃ for 30s, annealing at 60℃ for 30s, the extension at 72℃ for 45s, totaling 35 cycles, and the last cycle was extended at 72℃ for 7min.

Table 1 IGSF3 exon primer sequence

Exon	Primer sequence (5'-3')	Tm
E1	Forward, CCCAACTCCCTCCTTCTGA Reverse, GCCTCTTCGTACTCTACTCCAA	60℃
E2	Forward, TTGGAGATTGCTGGCTACTT Reverse, GTTGGGAGTTTTGGGATTTT	57℃
E3	Forward, TACACCCCATGTTTTATCCA Reverse, AGTCTCCACATTCTCCCAGTA	57℃
E4	Forward, CCTCCCCGCAGTTAGTATT Reverse, TCACCTTCACCACGCTATTC	59℃
E5	Forward, TAGAGTAGGTGCTCGCTGAA Reverse, GCGCCATTAAAATCCAAG	56℃
E6	Forward, GAGCAAAGCCCAGAACCA Reverse, TATCCCTCAGGAGCCCCATC	60℃
E7	Forward, GCAGGATACTAAAAGCAACACT Reverse, AAATGCCGTCAGACAACAA	55℃
E8	Forward, CTGAAGCCAGTCGGTGTTA Reverse, TAGGGTGATGGTCCGTGTA	58℃
E9	Forward, GTGCTGCCGTCCTTCTGTA Reverse, GACGTGCAATTTTCCATTCA	59℃
E10	Forward, TAGCACTGCCTATGCCTTTT Reverse, CCAATCCCACCATATCTCAA	58℃
E11	Forward, ACTGGGTCAGGTGAGGTCTT Reverse, TGCTGTCAACTGTCCAATCA	60℃

Table 2 Lacrimal puncta manifestation in 11 patients

Patients		Left eye		Right eye	
		Superior	Inferior	Superior	Inferior
Family 1	II:12	-	+	+	+
	III:9	-	-	-	+
Family 2	I:1	-	-	+	-
	II:1	+	+	-	+
	III:1	-	+	-	+
	III:2	-	-	-	-
Family 3	III:4	-	-	-	-
	III:8	-	-	-	-
	III:12	+	-	+	-
	IV:1	-	-	-	-
	IV:2	-	-	+	-

+: Normal lacrimal puncta; -: Absence of lacrimal puncta.

RESULTS

The 14 participants from three pedigrees in the study, including 11 patients, 3 normal family members (Table 2). The members and their spouses are all Chinses Han nationality without consanguineous marriage. There were two participants of the first pedigree in the study (II:12, III:9; Figure 1A). The proband suffered from left congenital dacryocystocele and congenital obstruction of the osseous nasolacrimal canal. Besides the absence of lacrimal puncta, her mother was also diagnosed as bilateral congenital obstruction of osseous nasolacrimal canal (Figure 2). The three generations of the second pedigree (Figure 1B) included four participants in the study (I:1, II:1, III:1, III:2). The five generations of the third pedigree (Figure 1C) included eight participants in the study (III:1, III:3, III:4, III:8, III:11, III:12, IV:1, IV:2).

A total of 10 gene variants from 4 exons were detected in IGSF3 genetic screening, of which 4 synonymous mutations: c.930C>T(p.Pro366=), c.1359T>C(p.Ser709=), c.1797G>A (p.Ser855=), c.1539G>A (p.Ser769=), three missense mutations: c.1507G>A (p.Gly759Ser), c.1783T>C (p.Trp851Arg), c.1952G>T (p.Ser907Ile), were also present in a normal family member, and there was no co-separation of genotypes and disease. The variants c.3120C>G (p.Asp1040Glu), c.3123C>G (p.Asp1041Glu), and c.3139_3140insGAC (p.Asp1046_Pro1047insAsp) were detected in both of the patient from the first pedigree, but not in other subjects (Figure 3, Table 3).

DISCUSSION

The lacrimal duct arises embryologically from a core of surface 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 the nasolacrimal canal[11]. Failure of horizontal direction cell column budding or arrest of development caused by any factors in this embryological process can lead to the congenital absence of lacrimal puncta or even canaliculi[12]. According to literature, 86% patients of puncta absence may be combined with canalicular atresia, making its clinical treatment more

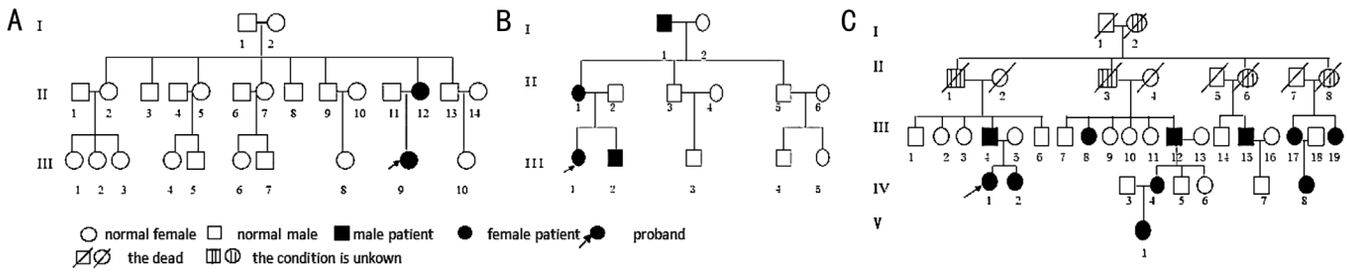


Figure 1 Three pedigrees A: Family 1. In addition to the absence of lacrimal puncta, they also suffered from pyorrhea because of the binocular obstruction of osseous nasolacrimal canal. B: Family 2; C: Family 3.

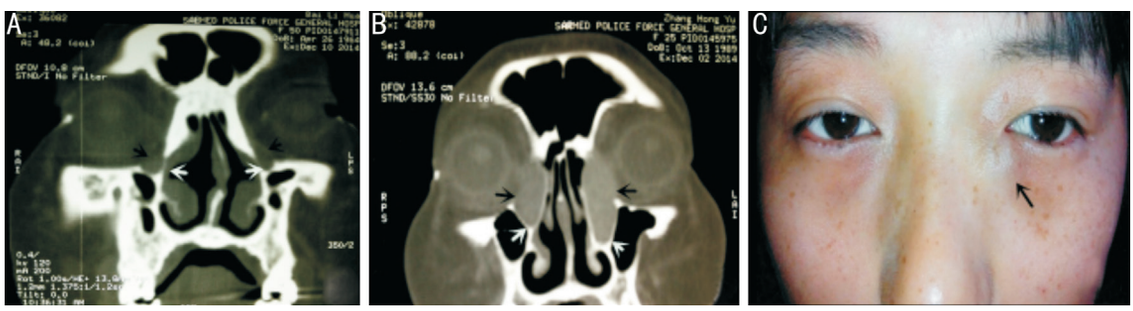
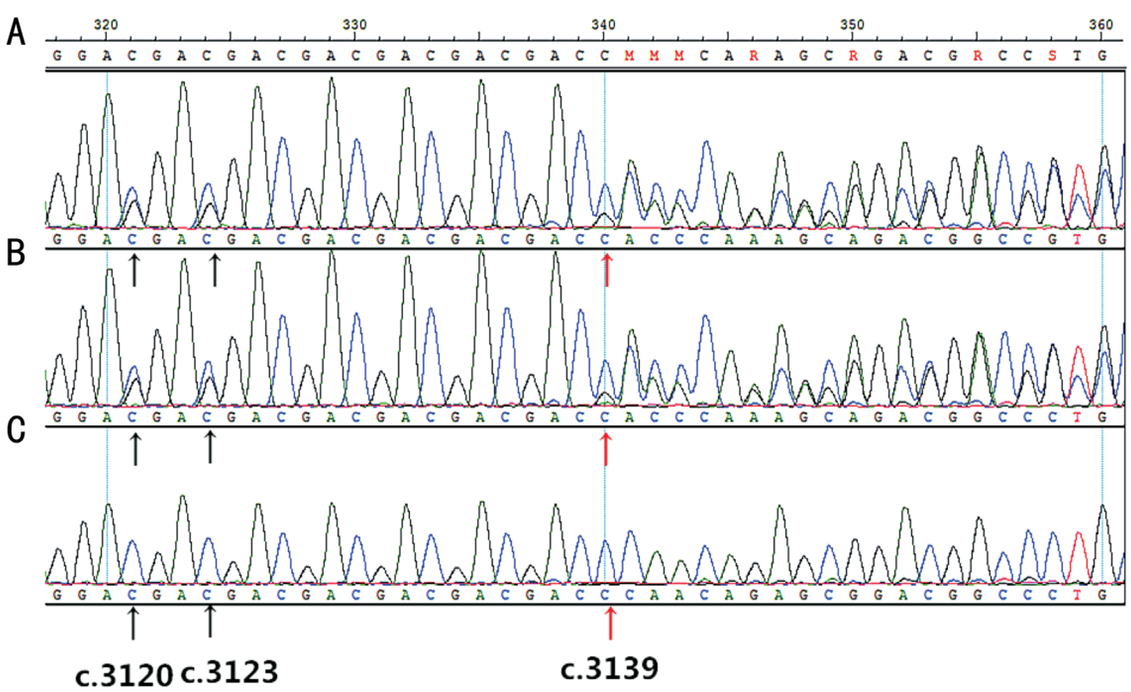


Figure 2 The proband and her mother in the first pedigree A, B: CT images of the lacrimal duct by 3D reconstruction of the mother and proband, respectively. Both the image manifested as a bony obstruction at the end of the nasolacrimal canal (white arrow) and dilation of the nasolacrimal duct (black arrow). C: A livid swelling was seen over the lacrimal sac area, inferior to the medial canthal ligament (arrow).



Firgure 3 IGSF3 variants in genetic screening A, B: The base sequence of exon 10 in the first family; C: The normal sequence accordingly. The black arrow pointed to c.3120C>G (rs647711), c.3123C>G (rs114915440), respectively. The red arrow pointed to c.3139_3140insGAC.

difficult[13-14]. All the patients in this study combined with the absence of canaliculi.

As absence of lacrimal puncta and canaliculi may be part of several congenital syndromes, combing with other organs dysplasia, such as the absence of salivary gland, parotid gland, submandibular gland, cleft lip and palate, finger (toe) deformities, deafness, *etc*[15-17]. Simple familiar congenital lacrimal duct dysplasia[18-19], such as the study on 340 patients of congenital obstruction of the nasolacrimal canal which founding its inheritance patterns can be autosomal dominant, autosomal recessive and sporadic, had been reported in succession[20]. In the clinical practice, it was found that although the absence of lacrimal puncta was mostly sporadic, there was also familial aggregation[4]. The three families

Table 3 IGSF3 variants screening results

Participants		E4	E6	E7	E10
Family 1	II:7	-	-	c.1783T>C (p.Trp851Arg), c.1952G>T (p.Ser907Ile), c.1797G>A (p.Ser855=)	c.3139_3140insGAC (p.Asp1046_Pro1047insAsp), c.3120C>G (p.Asp1040Glu), c.3123C>G (p.Asp1041Glu)
	III:9	-	-	c.1783T>C (p.Trp851Arg), c.1952G>T (p.Ser907Ile), c.1797G>A (p.Ser855=)	c.3139_3140insGAC (p.Asp1046_Pro1047insAsp), c.3120C>G (p.Asp1040Glu), c.3123C>G (p.Asp1041Glu)
Family 2	I:1	-	c.1507G>A (p.Gly759Ser), C.1359T>C (p.Ser709=)	-	-
Family 3	III:3 (normal)	c.930C>T (p.Pro366=)	c.1539G>A (p.Ser769=), c.1507G>A (p.Gly759Ser), c.1359T>C (p.Ser709=)	c.1783T>C (p.Trp851Arg), c.1952G>T (p.Ser907Ile), c.1797G>A (p.Ser855=)	-
	III:12	-	c.1507G>A (p.Gly759Ser)	c.1783T>C (p.Trp851Arg), c.1952G>T (p.Ser907Ile), c.1797G>A (p.Ser855=)	-
	IV:1	c.930C>T (p.Pro366=)	c.1507G>A (p.Gly759Ser)	c.1783T>C (p.Trp851Arg), c.1952G>T (p.Ser907Ile), c.1797G>A (p.Ser855=)	-
	IV:2	c.930C>T (p.Pro366=)	c.1539G>A (p.Ser769=), c.1359T>C (p.Ser709=)	c.1783T>C (p.Trp851Arg), c.1952G>T (p.Ser907Ile), c.1797G>A (p.Ser855=)	-

included in the study showed the characteristics of autosomal dominant inheritance.

Studies on the pathogenic gene of congenital lacrimal duct dysplasia are rarely reported. As mentioned above, Foster *et al*[9] speculated that c.2995delC of IGSF3 gene might be the cause of congenital dacryocystocele. IGSF3 is a kind of immunoglobulin-like protein. According to the literature, its mRNA is highly expressed in placenta, kidney, and lung, but not in peripheral lymph node cells, liver, spleen, *etc*., considering it is not associated with the immune condition of the body[21-22]. The congenital absence of puncta and dacryocystocele may share the same pathogenic gene based on the knowledge of the characteristics of normal lacrimal embryo development. Transcription of IGSF3 was found in the lacrimal duct and the gland in the mice at 19 and 30d after birth[9], which provided a supporting evidence for this study's hypothesis.

c.1507G>A (p.Gly759Ser), c.1783T>C (p.Trp851Arg), and c.1952G>T (p.Ser907Ile) detected in the study are existing in the patients and normal individuals, and it suggested that the amino acid changes do not affect the protein structure. c.3120C>G, c.3123C>G, and c.3139_3140insGAC are also not the pathogenesis of congenital absence of puncta and canaliculi because they are only existed in the patients with absence of lacrimal puncta and canaliculi combined with congenital osseous nasolacrimal canal obstruction from the first family. c.3120C>G and c.3123C>G are both the SNPs in PubMed database.

We also didn't detect the c.2995delC in the proband of the first pedigree who diagnosed as congenital dacryocystocele. It was considered that the cause of dacryocystocele in the proband was congenital osseous nasolacrimal canal obstruction combined with absence of puncta and canaliculi, and there were also membranous and bony lacrimal duct structural abnormalities. But the patients were all diagnosed as abnormal lacrimal valve development in Foster *et al*'s[9] study. It is suggested that different phenotypes of congenital lacrimal duct dysplasia may be caused by different genes or multiple locus variations.

c.3120C>G, c.3123C>G, and c.3139_3140insGAC were only detected in the patients from the first family, and they might be the cause of congenital osseous nasolacrimal canal obstruction. The difference between the diagnosis of the patients from the first family with others was congenital osseous nasolacrimal canal obstruction. However, due to the complex phenotype, congenital osseous nasolacrimal canal obstruction and absence of puncta and canaliculi, the mutation is related to the coexistence of the two phenotypes needs further research and confirmation.

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