·Clinical Research·

Identification of a *COL2A1* mutation in a Chinese family with Stickler syndrome type 1 via whole exome sequencing

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[Abstract] Objective To identify genetic mutations associated with Stickler syndrome type 1 in a Chinese family.

Methods A pedigree investigation was conducted of the members of a Chinese family that included some individuals with Stickler syndrome type 1 recruited from the Shantou International Eye Center in June 2012. Medical histories were collected and clinical examinations were conducted, which included visual acuity, intraocular pressure, slit lamp microscopy, and fundus autofluorescence. The diagnoses were made by experienced clinicians. Total genomic DNA was extracted from peripheral blood samples (5 mL) collected from five affected and four healthy family members. The potential variant of the father of the proband (subject III-5) was screened by whole exome sequencing and stepwise bioinformatics analyses. Segregation and mutation conformation of the variant were verified by Sanger sequencing. The pathogenicity of the variant was predicted by SIFT, Polyphen2, and MutationTaster. Conservation and three-dimensional structure of the amino acid mutation were determined by multiple sequence alignment and tools available from the UniProt website.

Results Autosomal dominant inherence was identified in 39 members of the family, spanning four generations, which included 15 affected and 24 phenotypically normal individuals. The proband (subject IV-4) had retinal detachment of the right eye, binocular strabismus, and high myopia. Subject III-5 had high myopia of the right eye and cataract and atrophy of the left eye. Subject Ⅳ-9 had high myopia of both eyes. A heterozygous variation, c.1693C>T (p.Arg565Cys), within exon 26 of *COL2A1* was found in the affected individuals, but not phenotypically normal individuals, demonstrating co-separation. The variant was predicted as deleterious by SIFT, Polyphen2, and MutationTaster. The amino acid residue at position 565, which was highly conserved among the human, mouse, rat, bovine, and *Xenopus laevis* sequences, exhibited an arginine to cysteine substitution in triple helix repeat region Gly-X-Y, thereby altering protein function.

Conclusions The *COL2A1* variant c.1693C>T (p.Arg565Cys) was identified as the cause of Stickler syndrome type 1 in this family. This is the first report of this variant in China.

[Key words] Stickler syndrome, type 1; Pedigree; Genetic testing; Whole exome sequencing; Retinal detachment; High myopia; *COL2A1* gene

Fund program: This study was financially supported by the Top-notch Talent Project of Guangdong Sailing Plan (grant no. 0142) and the Medical College Clinical Medicine High-level Key Discipline Construction Project of China

DOI: 10.3760/cma.j.cn115989-20200612-00425

Stickler syndrome is a genetic disorder of the connective tissues caused by mutations to collagen-encoding genes via autosomal dominant inheritance (*COL2A1*, *COL11A1*, or *COL11A2*) or autosomal recessive inheritance (*COL9A1*, *COL9A2*, or *COL9A3*)[1-2]. Collagens are important constitutes of the extracellular matrix of the chondrocyte, eye, and inner ear, and necessary for cartilage formation and growth, and maintenance of healthy joints, vision, and hearing[3]. Dysregulation of collagen biogenesis can affect the structure of eyes, ears, joints, and facial structures, resulting in defects of the ocular tissues (high myopia, vitreous degeneration, cataract, peripheral retinal degeneration, rhegmatogenous retinal detachment), sensorineural hearing loss, and facial deformities (saddle nose, micrognathia, cleft palate)[4-5]. Most cases of Stickler syndrome are due to *COL2A1* mutations[6-8]. In light of the wide spectrum of hereditary disorders associated with collagen proteins, clinical manifestations are varied, complex, and difficult to diagnosis. Moreover, effective treatments are lacking for such diseases, especially rhegmatogenous retinal detachment. Therefore, the identification of pathogenic variants of Stickler syndrome will help to elucidate the molecular pathogenesis and contribute to disease diagnosis and personized medicine to enhance the quality of life of patients. Whole exome sequencing (WES) is widely used to identify genetic mutations associated with inheritable diseases. Here, WES was employed to identify a pathogenic variant of *COL2A1* in members of a Chinese family with Stickler syndrome.

1 Materials and methods

1.1 General data

The study cohort included 39 members of a Chinese family (15 with Stickler syndrome and 24 phenotypically normal) spanning four generations and 200 healthy controls, which included older individuals with cataracts. Subject recruitment began in June 2021. Detailed clinic data were collected, which included information about deceased family members provided by relatives. The study protocol was approved by the Ethics Committee of the Joint Shantou International Eye Center (Shantou, Guangdong province, China) (approval no. EC20110310(2)-P02) and conducted in accordance with the ethical principles for medical research involving human subjects described in the Declaration of Helsinki. Prior to inclusion in this study, written informed consent was obtained from all subjects.

1.2 Methods

1.2.1 Clinic examinations Clinical examinations of all study subjects included visual acuity testing, slit-lamp examinations, and confocal laser scanning of the posterior segment of the eye (Heidelberg retina tomograph II).

1.2.2 Blood sample collection and DNA extraction Peripheral blood samples (5 mL) were collected from five affected individuals and four heathy controls in this family and 200 healthy individuals as controls recruited from the Joint Shantou International Eye Center. Genomic DNA was extracted from whole blood samples using the TIANamp Blood DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.,

Beijing, China) in accordance with the manufacturer's instructions and quantified with a NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA samples were immediately used for WES or stored at −20°C.

1.2.3 WES and screening of candidate pathogenic variants WES of the genomic DNA of subject III-5 was conducted by a commercial sequencing service (Annoroad Gene Technology Co., Ltd., Beijing, China). In brief, WES of 2 μg DNA was performed using the SureSelect Human All Exon Kit V5 (Agilent Technologies, Inc., Santa Clara, CA, USA). Paired-end sequencing of each sample was conducted with the HiSeq 2500 platform (PE100; Illumina, Inc., San Diego, CA, USA) with read lengths of 100 bp and average coverage depth of at least 100×. The raw WES data were used to screen for mutations. Single-nucleotide polymorphisms and insertion/deletions were detected with SAMtools (http://www.htslib.org/) by mapping the divergent reads against the University of California, Santa Cruz human reference genome hg19 with the Burrows–Wheeler Aligner (https://sourceforge.net/projects/bio-bwa/files/). To screen for candidate variants, high-frequency variants (minor allele frequency > 0.01) in the 1000 Genomes project (https://www.internationalgenome.org/) and Genome Aggregation Database (https://gnomad.broadinstitute.org/) were initially excluded, followed by all intergenic variants, intronic variants, and synonymous mutations. Then, the data were checked for mutations to all genes associated with Stickler syndrome. Remaining mutations were analyzed using the protein structure prediction programs Polymorphism Phenotyping v2 (Polyphen-2; http://genetics.bwh.harvard.edu/pph2/) and Sorting Intolerant From Tolerant (SIFT; https://sift.bii.a-star.edu.sg/). Polyphen-2 uses sequence- and structure-based predictive algorithms and generates a unique scale of reported scores of the effects of identified mutations on protein function. The SIFT tool is mainly used to identify the positions of conserved amino acids among different species to predict the effects of missense changes on protein structure.

1.2.4 Sanger sequencing and co-segregation analysis Amplification by polymerase chain reaction (PCR) and Sanger sequencing were conducted to confirm the identified candidate variants. PCR was performed using a real-time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with a primer pair specific for COL2A1 (forward: 5'-CCC TTG GCT TCA GAC CCT-3'; reverse: 5'-CCC CTG TCA CAA TTC TCA AAA TT-3') designed with Primer3 software (https://primer3.org/). The PCR products were purified with an E.Z.N.A.® Cycle Pure Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) and sequenced by Guangzhou IGE Biotechnology Ltd. (Guangzhou, China). The mutations detected among the five affected and four healthy family members were subjected to co-segregation analysis and excluded from the sequences of the 200 healthy controls by Sanger sequencing

1.2.5 Conserved amino acids and three-dimensional protein structures Alignments of multiple protein sequences and prediction of three-dimensional protein structures were performed with tools available from the UniProt website (https://www.uniprot.org/).

2 Results

2.1 Clinic manifestation

The cohort of 39 individuals from the same Chinese family spanning four generations included 15 individuals with Stickler syndrome and 24 otherwise healthy individuals (Figure 1). The proband (subject IV-4) was a 7-year-old boy with high myopia, retinal detachment, heterotropia of the right eye, and blindness of the left eye. Intraocular pressure of the right and left eye was 10 and 6 mmHg, respectively. Following retinal detachment surgery combined with silicone oil tamponade, unaided vision of the right eye recovered to 0.1 (1 year later) with intraocular pressure of 33.1 mmHg (considered secondary glaucoma) (Figure 2). Subject III-5 had high myopia and cataract of the right eye and atrophy of the left eye. Subject III-14 had staphyloma and sensory esotropia of the right eye and atrophy of the left eye. Subject IV-9 had high myopia of both eyes (right eye: −17.00 OD; left eye: −18.00 OD). Other affected individuals within this family showed similar clinic symptoms of Stickler syndrome.

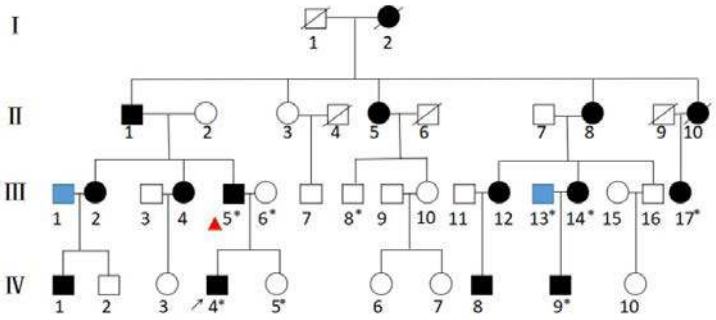

Figure 1 Pedigree of a family with Stickler syndrome type I □: normal male; ○: normal female; ■: affected male; ●: affected female; /: deceased; ▲: WES sequencing sample; *: peripheral blood sample; ↗: proband; ■: blind

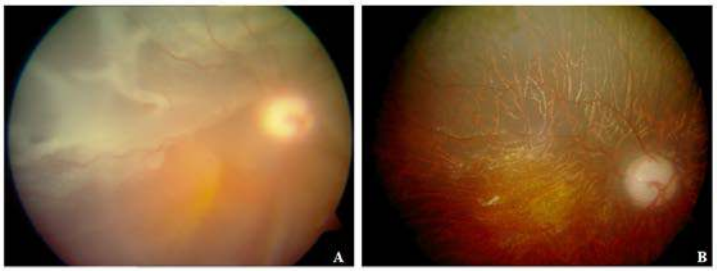

Figure 2 Fundus images of the proband (subject IV-4) A: Preoperative image showing a grayish white retinal bulge with a suspected oval hiatus at 9:00 in the right eye. B: An image after retinal detachment repair combined silicone oil tamponade showing a tigroid fundus with a cup to disc ratio of ~0.9

2.2 WES and identification of candidate variants

Step-wise screening of the WES data identified a heterozygous mutation to the *COL2A1* gene (c.1693C>T: p.565Arg>Cys), which was predicted as deleterious with SIFT, Polyphen2, and MutationTaster (https://www.mutationtaster.org/).

2.3 Sanger sequencing and co-segregation assay

Analysis of the variant revealed that all of the tested affected individuals carried the mutation, but not the healthy family members or 200 controls. As shown in Figure 3, the Sanger sequencing results confirmed that the variant co-segregated with the disease phenotype, suggesting the variant was a pathogenic mutation.

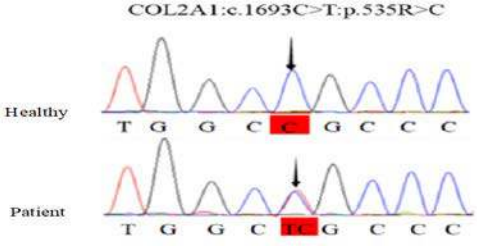

Figure 3 Variant confirmation by Sanger sequencing The mutation site is indicated by an arrow

2.4 Location of the mutated amino acid of the COL2A1 protein

Alignment of the partial COL2A1 protein sequences containing the variant of the human, mouse, rat, bovine, and African clawed frog showed that the mutated arginine residue at position 565 (Arg565) is highly conserved (Figure 4A). The Arg565 residue located within the triple-helical domain of COL2A1 mutated to cysteine (Cys) and might have compromised the stability of the Gly-X-Y sequence (Figure 4B), resulting in possible loss some important biological functions linked to the pathogenesis of Stickler syndrome.

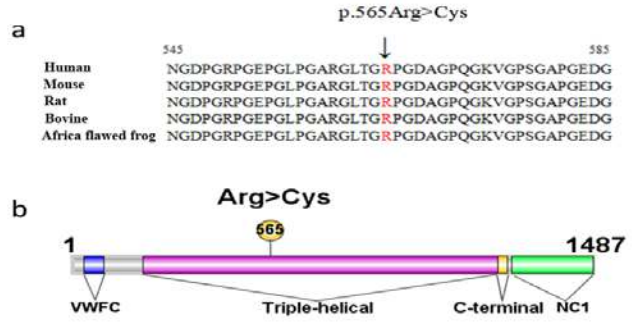

Figure 4 Multiple alignment of the amino acid sequences and proposed protein structure of COL2A1 **A**: Interspecies conservation of the Arg565 residue of COL2A1 B: Proposed secondary structure of COL2A1 composed of 1478 amino acids and four functional domains. The VWFC domain is shown in blue, triple helix domain in purple (Arg565Cys), C-terminal region in yellow, and non-collagenous region in green

3 Discussion

The common clinic manifestations of Stickler syndrome include high myopia, vitreous abnormalities, cataract, peripheral retinal degeneration, and rhegmatogenous retinal detachment. Other clinic symptoms include micrognathia (alone or as part of the Pierre Robin sequence), sensorineural hearing loss, early onset osteoarthritis, spondyloepiphyseal dysplasia, and cleft palate. These clinic manifestations tend to be more obvious in early childhood. Notably, high myopia is not present in all patients with Stickler syndrome and tends to be stable rather than progressive [5]. In this study, the diagnosis of Stickler syndrome was based on clinic examinations conducted at the Joint Shantou International Eye Center and other hospitals. The age of onset of high myopia was 7 years for subject IV-4 and 5 years for subject IV-9. Moreover, two of the affected family members had retinal detachment. However, some of the clinic data obtained from the other hospitals lacked information about hearing loss, skeletal/joint malformations, and craniofacial abnormalities. Nonetheless, the lack of clinic data did not affect the diagnoses or conclusions of this study that a deleterious variant of the *COL2A1* gene was associated with the pathogenesis of type 1 Stickler syndrome.

Mutations to *COL2A1*, which encodes type II collagen, *COL11A1* and *COL11A2*, which encode type XI collagen, and *COL9A1* and *COL9A2*, which encode type IX collagen, have been associated with type I, II, III, IV, and V Stickler syndrome, respectively. COL2A1 and COL11A1 are mainly expressed in the vitreous. Mutations to *COL2A1* usually result in a congenital membranous vitreous anomaly. In contrast, mutations to *COL11A1* result in a different vitreous phenotype where the gel that fills the posterior chamber forms beaded bundles with irregular diameters, probably reflecting the role of type XI collagen in the regulation of collagen fibrillogenesis [5-6]. COL11A2 is not expressed in the eye, thus a mutation leads to Stickler syndrome without eye involvement. Mutations in *COL9A1* result in rapid aging of the vitreous due to progressive liquefaction and degeneration, leading to formation of an optical vitreous cavity. Mutation to *COL9A2* results in similar phenotypes as types 1 and 2. *COL2A1* contains 54 exons, but pathogenic variants have not been identified in exons 3, 5, 22, 24, 31, 37, or 54 [9-31]. Some pathogenic variants are located within introns [10, 14-15, 29, 32]. Exon 2, which encodes the VWFC domain [9, 15, 17, 28], exon 23 [9, 10, 15, 28, 30-31], exon 42 [9, 10, 15], and exon 51 [9, 10, 14-15, 23] carry most variants, suggesting that these exons are mutation hotspots. Other exons contain fewer variants, but are more evenly distributed. Of the 166 reported variants of COL2A1, 155 (93.4%) are located within the triple-helical domain of COL2A1. Among the amino acid residues, the frequency of mutation is greater for the glycine and Arg residues. Most of the frame shift mutations cause vitreous-related symptoms. The COL2A1 variant found in the patient cohort of this study was previously reported in two sporadic cases. Sporadic case MS12 exhibited symptoms similar to those of subject IV-4, which included retinal detachment, high myopia, and heterotopia, while the symptoms of sporadic case MS16 were similar to those of subject III-5, which included high myopia, retinal detachment, and cataract. The Arg565Cys mutation, which was first reported in a Chinese patient, has been linked to the clinical manifestations of Stickler syndrome [10].

In conclusion, the results of this study found that the *COL2A1* variant c.1693C>T (p.Arg565Cys) was linked to the clinical manifestations of type 1 Stickler syndrome.

Conflict of interest None declared.

Author contributions DF perform the experiments and analyzed the data. CYJ and CSW analyzed the data. XLJ collected the clinical and diagnostic data. XXQ conceived and designed the study, arranged funding, performed the experiments, analyzed and interpreted the data, and revised the article.

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