Targeted next-generation sequencing identifies two novel *COL2A1* gene mutations in Stickler syndrome with bilateral retinal detachment

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Abstract. Stickler syndrome is a group of inherited connective tissue disorders characterized by distinctive facial and ocular abnormalities, hearing loss and early-onset arthritis. The aim of the present study was to investigate the genetic changes in two Chinese patients with Stickler syndrome, manifested as bilateral retinal detachment and peripheral retinal degeneration. Complete ophthalmic examinations, including best-corrected visual acuity, slit-lamp examination and fundus examination, were performed. Genomic DNA was extracted from leukocytes of the peripheral blood collected from the patients, their unaffected family members and 200 unrelated control subjects from the same population. Next-generation sequencing of established genes associated with ocular disease was performed. A heterozygous collagen type II α1 chain (*COL2A1*) mutation c.1310G>C (p.R437P) in exon 21 was identified in Family 1 and a heterozygous *COL2A1* mutation c.2302-1G>A in intron 34 was identified in Family 2. The functional effects of the mutations were assessed by polymorphism phenotyping (PolyPhen) and sorting intolerant from tolerant (SIFT) analysis. The c.1310G>C mutation was predicted to damage protein structure and function, and the c.2302-1G>A mutation was predicted to result in a splicing defect. The findings of the current study expand the established mutation spectrum of *COL2A1*, and may facilitate genetic counseling and development of therapeutic strategies for patients with Stickler syndrome.

Introduction

Stickler syndrome [Online Mendelian Inheritance in Man (OMIM) nos. 108300, 609508, 604841,184840, 614134 and 614284], first reported in 1965 by Stickler *et al* (1), is a group of inherited connective tissue disorders, with an incidence of 1 in 10,000 (2,3). Stickler syndrome is frequently misdiagnosed due to its widely varied clinical manifestations, which may resemble other diseases (4,5). It commonly involves distinctive ocular and facial abnormalities, hearing loss and joint problems (3,6-8). Patients with Stickler syndrome typically present with shallow supraorbital ridges, hypoplastic short nose with anteverted nares, buphthalmic eyes, a flat hypoplastic midface with a depressed nasal bridge, long philtrum and micrognathia (9).

Stickler syndrome is caused by mutations in collagen genes during fetal development, and can be divided into various subtypes based on the clinical manifestations and underlying genetic mutations (10). The most common form, Type 1 Stickler syndrome, is caused by a collagen type II α1 chain (*COL2A1*) mutation (OMIM no. 120140), and is characterized by membranous vitreous anomaly and megalophthalmos (11,12). Type 2 Stickler syndrome with

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an underlying collagen type XI α1 chain (*COL11A1*) mutation (OMIM no. 120280) accounts for a minority of patients and presents with a typical beaded vitreous phenotype (13). Type 3 or non-ocular Stickler syndrome, caused by collagen type XI α2 chain (*COL11A2*) mutation (OMIM no. 120290), often manifests as systemic malformations, including midface hypoplasia and osteoarthritis (14,15). Type 4 Stickler syndrome, caused by collagen type IX α1 chain (*COL9A1*) or collagen type IX α2 chain (*COL9A2*) mutation (OMIM no. 120210), is associated with sensorineural deafness, myopia, vitreoretinopathy and epiphyseal dysplasia (16).

Stickler syndrome can lead to a variety of ocular abnormalities, including vitreoretinal degeneration, retinal detachment, cataract, ocular hypertension and high myopia (17). The development of Stickler syndrome is progressive and can ultimately lead to blindness (3). The molecular mechanism of Stickler syndrome is not fully characterized. However, type 1 Stickler syndrome arises from aberrant type II collagen, which is the major collagen type synthesized in the adult human vitreous (18). Under physiological conditions, the strongly adherent collagen fibrils (typically types II, XI and IX) are interspersed in the extracellular matrix, which is predominantly composed of water and glycosaminoglycans. The interaction between collagen and hyaluronan, the most prevalent glycosaminoglycan in the vitreous, provides swelling pressure required to maintain the ocular structure (19). Mutation in the *COL2A1* gene can result in an abnormal fibrillar lamellar structure of the vitreous gel (20), disrupt collagen helices, alter fibrillogenesis and reduce collagen secretion (20,21).

Characterizing the Stickler syndrome phenotypes and identifying the underlying genetic mutations are initial steps to understand the disease pathogenesis and will be useful for future genetic counseling. The current study aimed to characterize the clinical presentation of two young patients with Stickler syndrome and bilateral retinal detachment, and to identify the genetic changes in these patients using targeted next-generation sequencing (NGS).

Materials and methods

Study subjects and clinical examinations. Two patients from two different families presenting with bilateral retinal detachment and peripheral retinal degeneration were recruited in the present study. All experimental protocols were performed according to the guidelines approved by the Ethics Committee of Zhongshan Ophthalmic Center (Guangzhou, China), and in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects.

Complete ophthalmic examinations were performed at the Zhongshan Ophthalmic Center. The best-corrected visual acuity (BCVA) was measured using the ETDRS chart (Precision Vision, Woodstock, IL, USA). Anterior segment images were obtained using a BX 900 Slit Lamp (Haag-Streit, Bern, Switzerland). Anterior segment measurements were performed using Pentacam HR version 70700 (Oculus, Wetzlar, Germany). Fundus photography was performed using Heidelberg Retina Angiograph (Heidelberg Engineering, Heidelberg, Germany) or ultra-wide-field 200Tx Optos system (Optos plc, Dunfermline, UK). Optical coherence tomography (OCT) was performed by Cirrus HD OCT (Zeiss GmbH, Jena,

Germany). Physical examinations were performed to exclude systemic diseases. Venous blood samples from the patients, their unaffected family members and 200 unrelated control subjects from the same population were collected.

Target capture, NGS and mutation validation. NGS was used to identify the potential variants. The parameters used for whole exome sequencing have been described in our previous studies (22,23). Identified mutations were validated using conventional polymerase chain reaction (PCR)-based sequencing methods (24-29,23). Briefly, exons 21-22 and 33-34 of the *COL2A1* gene were amplified by PCR with respective primers (Table I). PCRs were conducted in 50 μl total reaction volume using an ABI2720 system (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The cycling conditions included one cycle at 94˚C for 5 min, followed by 40 cycles at 94˚C for 45 sec, 59-60˚C for 45 sec, 72˚C for 45 sec, and one cycle at 72˚C for 10 min. The PCR products were sequenced in both directions using an ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA, USA). The sequencing results were analyzed using Seqman (version 2.3; Technelysium Pty Ltd., Brisbane, Australia), and compared with the reference sequences in National Center for Biotechnology Information (NCBI) databases (26-28,30).

Interpretation of the genetic variants. To predict the effect of missense variants, polymorphism phenotyping (PolyPhen) and sorting intolerant from tolerant (SIFT) were used to predict the potential impact of an amino acid substitution on the protein structure and function, using physical and comparative considerations (23,31,32). Variants were predicted to be pathogenic when at least one of the two programs predicted deleterious effect of the amino acid substitution on the protein structure and function. The Human Gene Mutation Database (hgmd.cf.ac.uk/ac/index.php) was used to screen mutations reported in previously published studies. HomoloGene (ncbi. nlm.nih.gov/homologene) was used to assess the conservation of the altered amino acid residues across different species (22,33).

Results

Clinical presentations of the patients. The patients reported in the present study were from the southern area of China (the family pedigrees are illustrated in Fig. 1). The clinical manifestations of Patient 1 in Family 1 (II:2 in Fig. 1A) are summarized in Table II. The patient was a 24-year-old female without a known familial history of ocular disease. The BCVA was 0.0 in the right eye and 0.2 in the left eye. Anterior segment photography demonstrated transparent lenses in both eyes. When Patient 1 was 21 years old, she exhibited a decreased vision in the left eye and retinal detachment of the left eye was diagnosed. Fundus photography revealed inferior retinal detachment and peripheral retinal degeneration (Fig. 2A). B-scan indicated localized retinal detachment (white arrow; Fig. 2B). OCT revealed a partially damaged macular area (Fig. 2C). Retinal detachment surgery was performed, and her vision improved. After one year, vision was decreased in the right eye and retinal detachment of the right eye without macular involvement was diagnosed. Fundus

Table I. Primers used for the amplification of *COL2A1* in the current study.

Gene	Exon	Forward (5'-3')	Reverse (5'-3')	Product size (bp)	Annealing temperature (°C)
COL2A1	*21-22*	GCCAAAGGATCTGCTGTGAG	GCCCTGTTAAGTCTCCTCCA	599	60
COL2A1	*33-34*	CCTGGGTCCTATGCTCCTG	AGCTTTGGTGAGAGGCTGTA	581	59

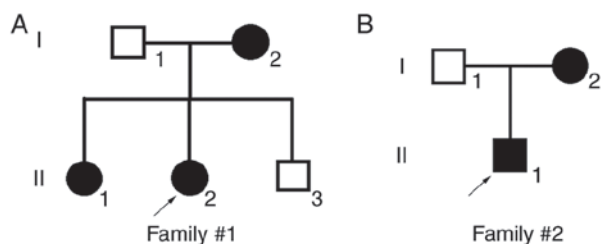*COL2A1*, collagen type II α1 chain.

Figure 1. (A and B) Pedigrees of two Chinese families with Stickler syndrome. Square symbols denote males, and circle symbols denote females. The solid symbols indicate ophthalmologist-confirmed Stickler syndrome, and the open symbols indicate unaffected individuals. The arrows point to the proband.
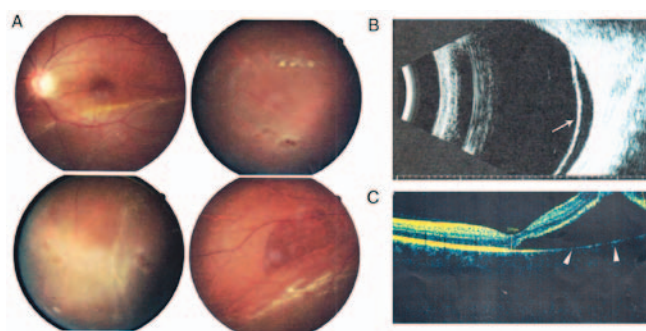

Figure 2. Clinical manifestation of the left eye of Patient 1 (II:2 in Family 1). (A) Fundus imaging shows inferior retinal detachment and peripheral retinal degeneration. (B) B-scan shows localized retinal detachment. (C) OCT shows a partially damaged macular area. OCT, optical coherence tomography.
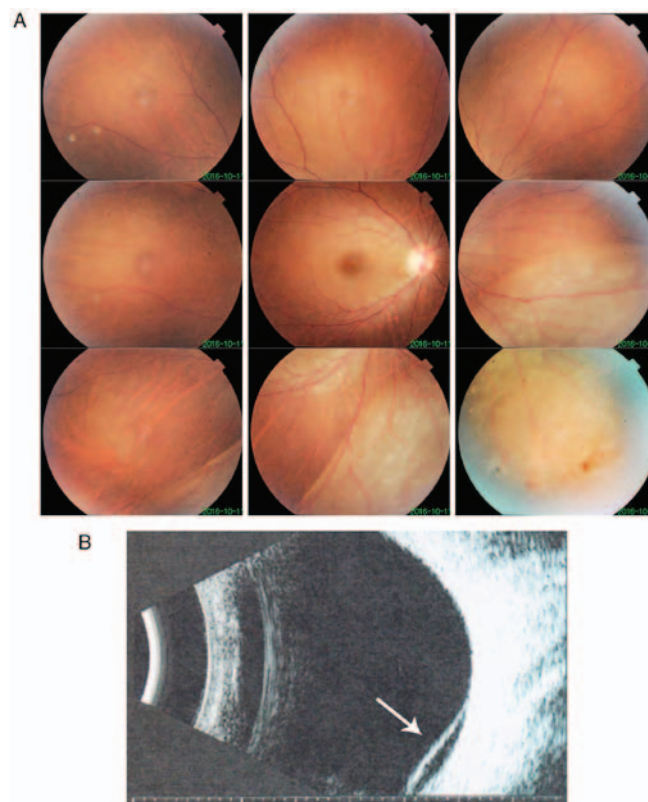

Figure 3. Clinical manifestation of the right eye of Patient 1 (II:2 in Family 1). (A) Fundus photography shows inferior retinal detachment and peripheral retinal degeneration. (B) B-scan displays localized retinal detachment.

imaging revealed inferior retinal detachment and peripheral retinal degeneration (Fig. 3A). B-scan indicated localized retinal detachment (white arrow; Fig. 3B). The elder sister of this patient (II:1) also exhibited myopia and peripheral retinal degeneration (Fig. 4).

Patient 2 in Family 2 (II:1 in Fig. 1B) was a 17-year-old male. Retinal detachment of the left eye and the right eye of Patient 2 was diagnosed at 14 and 17 years old, respectively. Following surgery, the BCVA was 0.7 in the right eye and 0.3 in the left eye. Fundus imaging revealed peripheral retinal scars (Fig. 5). The mother of patient 2 also had bilateral retinal detachment. The left eye of the mother was blind at birth and exhibited severe atrophy. Right retinal detachment was diagnosed at 30 years old. Patient 2 and the mother had cleft palate.

Mutation screening and bioinformatics analysis of the mutations. A novel heterozygous *COL2A1* mutation c.1310G>C

(p.R437P) in exon 21 was identified in Family 1 (I:2, II:1, II:2; Table II, Figs. 6 and 7A). Multiple sequence alignment indicated that the arginine residue at position 437 of collagen type II α1 chain is highly conserved (Fig. 7B). PolyPhen and SIFT predicted that this mutation is damaging (Fig. 7C). A novel heterozygous *COL2A1* mutation c.2302-1G>A in intron 34 was identified in Family 2 (I:2, II:1; Figs. 6 and 7A). This mutation is likely to result in a splicing defect as it occurs at the exon-intron border. These two mutations were not present in the unaffected family members and the other unrelated control subjects from the same population.

Discussion

The clinical manifestation of Stickler syndrome is heterogeneous (3-5). Retinal detachment is the most severe consequence of Stickler syndrome (34), and there is a high incidence of blindness. Approximately 55-73% of Caucasian patients with a

Table II. Summary of clinical manifestations and mutations in Family 1.

Patient	Sex	Age	Clinical manifestation							Mutation
			BCVA	Optometry	IOP	Lens/Cornea	Fundus	B-scan	OCT	
I:1	M	48	OD:0.0(0.0); OS:0.0(0.0)	N/A	Normal	Normal	Normal	N/A	N/A	-
I:2	F	45	OD:0.0(0.0); OS:0.0(0.0)	N/A	Normal	Normal	Normal	N/A	N/A	c.1310G>C (p.R437P)
II:1	F	24	OD:0.7(0.0); OS:0.7(0.0)	OD:-4.25DS&-0.50DC; OS:-3.75DS&-1.00DC	Normal	Normal	Peripheral retinal degeneration	N/A	N/A	c.1310G>C (p.R437P)
II:2	F	20	OD:2.0(0.0); OS:2.0(0.2)	OD:-3.00DS&-1.50DC; OS:-1.00DS&-1.00DC	Normal	Normal	Retinal detachment of the right eye	Retinal detachment	Retinal detachment	c.1310G>C (p.R437P)
II:3	M	19	OD:0.0(0.0);OS:0.0(0.0)	N/A	Normal	Normal	Normal	N/A	N/A	-

M, male; F, female; N/A, not available; BCVA, best-corrected visual acuity; IOP, intraocular pressure; OCT, optical coherence tomography.

clinical diagnosis of Stickler syndrome exhibit retinal detachment (35,36). Thus, Stickler syndrome should be considered and excluded if a patient presents with multiple peripheral degeneration spots in both eyes (37-40). In the current report, both patients exhibited sequential bilateral retinal detachment and multiple peripheral retinal degeneration at a young age. In Patient 1, the localized retinal detachment of the right eye did not extend to the macular area; thus, the visual impairment was less severe.

The diagnostic criteria for Stickler syndrome have not been well-established (5,10). Adult patients diagnosed with Stickler syndrome typically do not present with typical facial anomalies as children (41). In Family 2, the patient and his mother had cleft palate, which is an important clinical indicator of Stickler syndrome (6,37,42,43). Other typical extraocular collagenopathies include achondrogenesis, hypochondrogenesis and early onset osteoarthritis (44). However, diagnosing Stickler syndrome only based on clinical manifestations is often challenging. Genetic analysis, therefore, is an important tool for the diagnosis of Stickler syndrome, particularly in patients with myopia and peripheral retinal degeneration (12,39,45-47). Early diagnosis and close-follow up will help to decrease the incidence of the retinal detachment (3,38). Currently, the Cambridge prophylactic cryotherapy protocol has been demonstrated to be a safe intervention and can markedly reduce the risk of retinal detachment in patients with Stickler syndrome (48).

Although the affected patients in the present study had different genetic mutations, they exhibited similar clinical presentations of retinal detachment and degeneration. Previous studies have also reported that different mutations in *COL2A1* can lead to similar phenotypes, with various degrees of expressivity (42,49). The majority of *COL2A1* mutations identified in Stickler syndrome are loss-of-function mutations, as they are predicted to result in nonsense-mediated decay of transcripts (42). Splice site mutations, as identified in Family 2 in the current study, are commonly identified in Stickler syndrome, and are likely to cause unusual RNA isoforms with premature stop codons (42). Additionally, silent mutations in *COL2A1* can also result in splicing defects and reading frame shifts (50).

In summary, the present study characterized the clinical presentation of two Chinese families with Stickler syndrome, and identified two novel mutations in the *COL2A1* gene in the affected family members. These findings expand the known mutation spectrums of *COL2A1*, and may facilitate genetic counseling and development of therapeutic strategies for patients with Stickler syndrome.
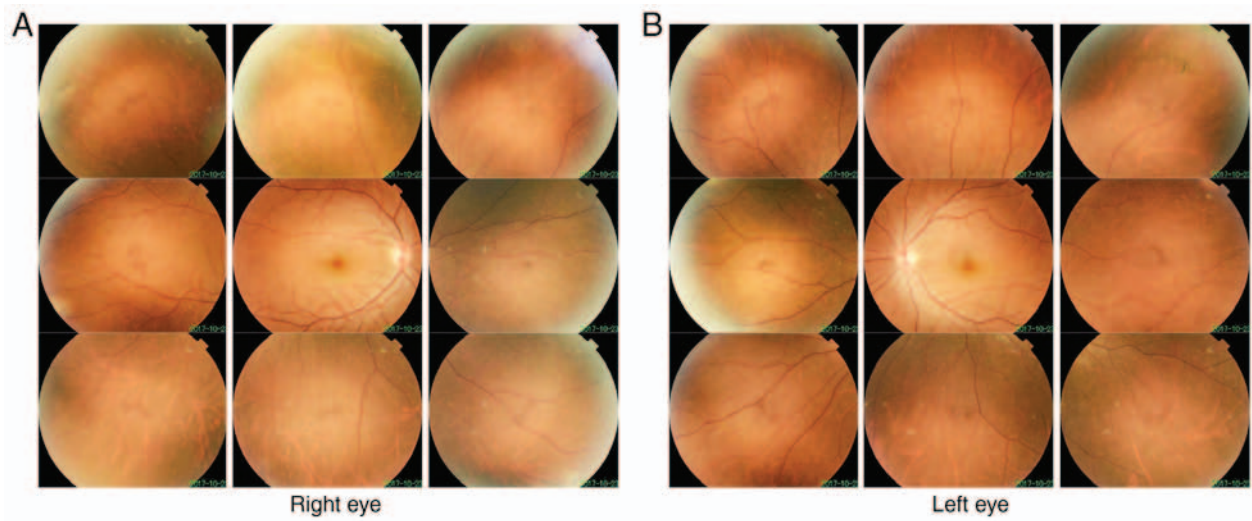
Figure 4. Clinical manifestation of the elder sister of Patient 1 (II:1 in Family 1). Fundus photography shows peripheral retinal degeneration in the upper temporal areas of (A) the right and (B) the left eye.
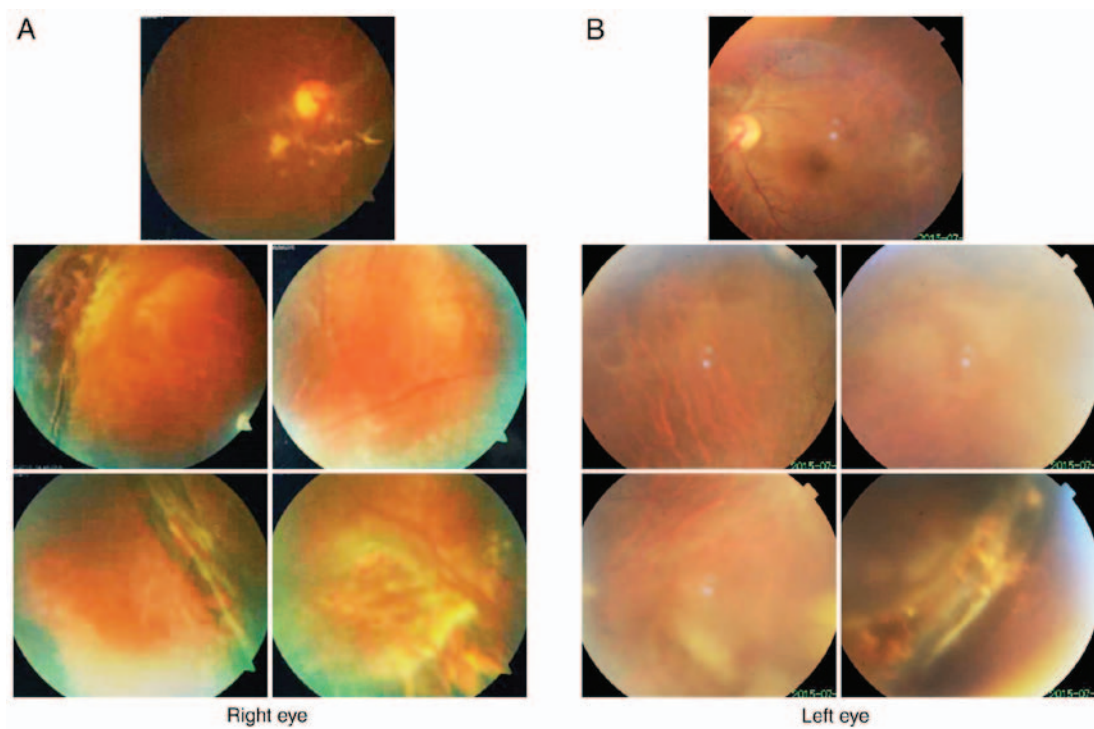

Figure 5. Clinical manifestation of Patient 2 (II:1 in Family 2). Fundus photography shows peripheral retinal scar in (A) the right and (B) the left eye.
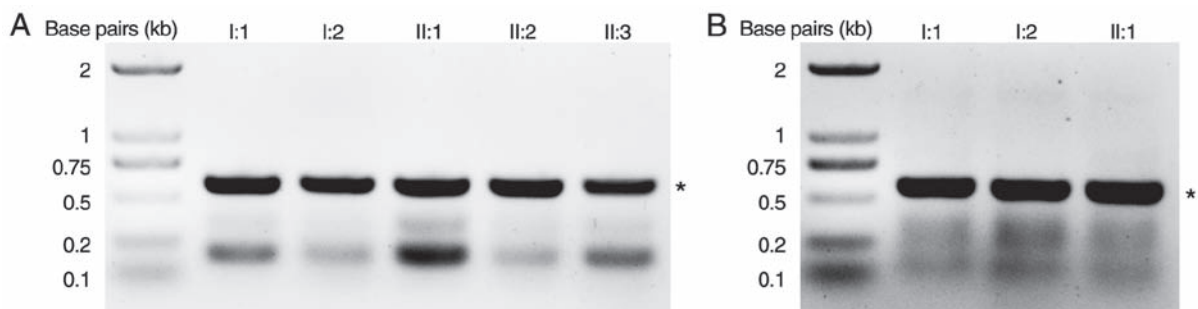

Figure 6. Amplification of exons 21-22 and 33-34 of the *COL2A1* gene by PCR. (A) Exons 21-22 in Family 1 were amplified by PCR, yielding a product of 599 bp (denoted next to the gel image by an asterisk). (B) Exons 33-34 in Family 2 were amplified by PCR, yielding a product of 581 bp (denoted next to the gel image by an asterisk). *COL2A1*, collagen type II α1 chain; PCR, polymerase chain reaction.
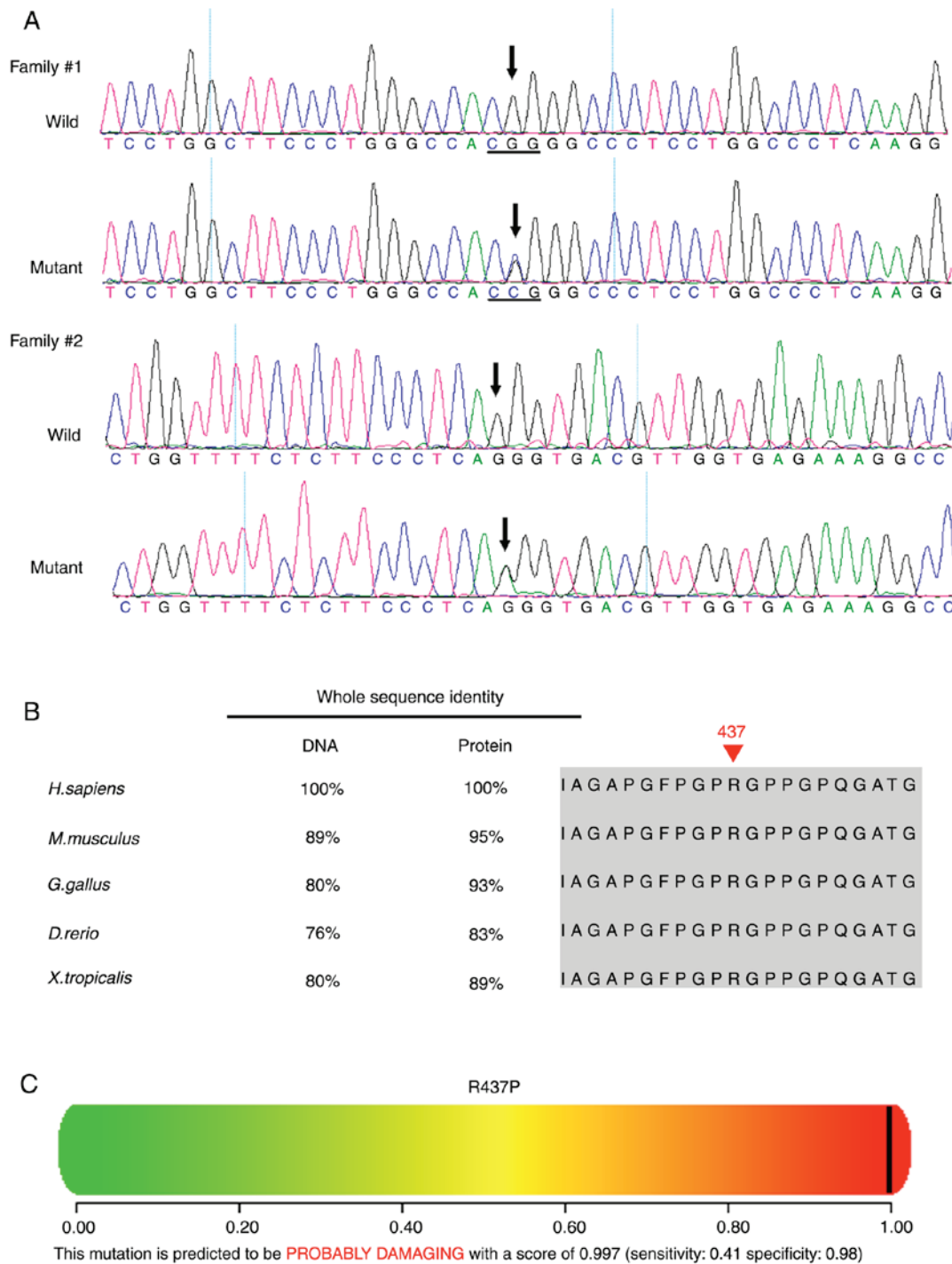
Figure 7. Genetic mutations identified in the two families. (A) A heterozygous *COL2A1* mutation c.1310G>C (p.R437P) in exon 21 was identified in Family 1 (I:2, II:1, II:2). A heterozygous COL2A1 mutation c.2302-1G>A in intron 34 was identified in Family 2 (I:2, II:1). (B) Multiple sequence alignment of the *COL2A1* gene and collagen type II α1 protein from different species. The red arrowhead indicates the location of the p.R437P mutation. The Arg 437 residue is highly conserved across species. (C) Polymorphism phenotyping predicts that the amino acid substitution p.R437P is damaging. *COL2A1*, collagen type II α1 chain.

Availability of data and materials

The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

XH, YL, TL, CJ, XL and LL analyzed and interpreted the patient data. HG, BL, CL, YH, QW and HL examined the patients and performed PCR and gene sequence analysis. YL, CC and YZ interpreted the sequencing data, drafted the manuscript and revised it critically. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experimental protocols were approved by the ethics committee of Zhongshan Ophthalmic Center (Guangzhou, China). Informed consent was obtained from all subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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