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Case Report

Exploring the Genetic Landscape of Knobloch Syndrome: Novel Variant Identification and Literature Review

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Abstract

Knobloch Syndrome (KS) is a rare genetic disorder characterized by ocular abnormalities and central nervous system (CNS) defects, which are attributed to collagenopathy. The primary gene implicated in KS is *COL18A1*, which encodes the alpha chain of type XVIII collagen. This type of collagen functions as a proteoglycan, predominantly located in the basement membrane of human tissues. This study examined an Iranian female presenting with symptoms of horizontal nystagmus, strabismus, and suspected vision loss, with a potential diagnosis of KS. Genomic DNA was subjected to whole-exome sequencing (WES). The identified pathogenic variant was subsequently confirmed using Sanger sequencing. The impact of the identified pathogenic variant on the structure of *COL18A1* was assessed using I-TASSER. Analysis of WES data revealed two pathogenic compound heterozygous variants, c.2416C>T (p.Arg806Ter) and c.1698delC (p.Gly567AspfsTer45), in exons 7 and 16, respectively, of the *COL18A1* (NM_030582) gene. Furthermore, a standard literature review of clinical data highlighted the heterogeneity of phenotypic manifestations, ranging from mild ocular abnormalities to severe neurodevelopmental impairments. This study and literature search offer valuable insights into the genetic landscape of KS, thereby expanding our understanding of the disease and its clinical implications. The identification of novel variants in key genes sheds light on the underlying molecular mechanisms and potential therapeutic targets.

Keywords

Knobloch syndrome; *COL18A1*; frameshift; exome sequencing; novel variant; case report

1. Introduction

Defects in collagen genes can cause various collagenopathies. Collagen is essential for establishing connective tissues and acts as a signaling molecule. So far, 28 different types of collagen have been identified in the human body [1]. Therefore, the pathogenic variants of these genes can affect multiple organs.

Knobloch Syndrome (KS) (OMIM: 267750) is a rare collagenopathy that was first reported by Knobloch and Layer. It is mainly caused by a biallelic pathogenic variant of *COL18A1*, which is associated with retinal detachment and encephalocele [2]. Ocular manifestations in the posterior and anterior segments can be irregular, including lens subluxation, high myopia, smooth irides (iridodonesis), early-onset cataracts, retinal detachment, vitreoretinal degeneration, and retinal degeneration with severe cone-rod dystrophy [3, 4]. Patients also commonly experience non-ophthalmic symptoms such as brain defects (polymicrogyria and Dandy-Walker Malformation), mid-face hypoplasia, occipital changes (encephalocele, cutis aplasia, bone defects), micrognathia, flat

nasal bridge, hyperextensible joints, early onset renal failure, associated with increased creatinine levels and ultrastructural changes in the basement membrane of the kidney, as observed in *COL18A1*-deficient mice [5, 6], lung hypoplasia, epilepsy, high arched palate, dental abnormalities [7], and delayed skin healing due to dysfunction of the dermal-epidermal junction during wound healing [8].

The *COL18A1* gene, found on chromosome 21q22.3, plays a key role in connecting the eye's inner limiting membrane to the vitreous fibrils. It encodes the alpha chain of collagen type XVIII, a proteoglycan with multiple functions commonly found in the basement membrane of human tissues [9]. Furthermore, it has been identified that the *COL18A1* protein, upon proteolytic cleavage, generates endostatin, which serves as an inhibitor of endothelial cell proliferation, migration, and angiogenesis [10]. *COL18A1* comprises three distinct isoforms, which vary in their N-terminal non-collagenous domain, length, and tissue distribution. These isoforms are produced through alternative promoter usage and splicing mechanisms [11]. The long isoform is involved in maintaining the structure of the human eye. In contrast, the short isoform appears to play a crucial role in neural migration, eye structure, and neural tube closure during embryogenesis [12]. A 'delay in ocular developmental regression' refers to the prolonged retention of transient embryonic ocular structures, such as the hyaloid vasculature, which typically dissolve during postnatal maturation. For instance, in *COL18A1*-deficient mice, this delay disrupts the timely remodeling of blood vessels, which is essential for normal visual development [13].

This study aimed to examine a patient experiencing vision problems through the application of whole-exome sequencing (WES) to identify underlying genetic pathogenic variants. This study aimed to establish a comprehensive understanding of the genotypic and phenotypic spectrum associated with KS, with a particular emphasis on ocular manifestations and non-ophthalmic symptoms, through a standardized literature review. The findings of this study will contribute to expanding our knowledge about KS and its underlying genetic mechanisms.

2. Materials and Methods

2.1 Clinical Report

The patient was a 10-year-old female who presented with symptoms of horizontal nystagmus, strabismus, and suspected vision loss. The patient's parents were non-consanguineous, and she was the only known individual with this condition in her family, with no similar clinical signs observed in other family members (Figure 1a).

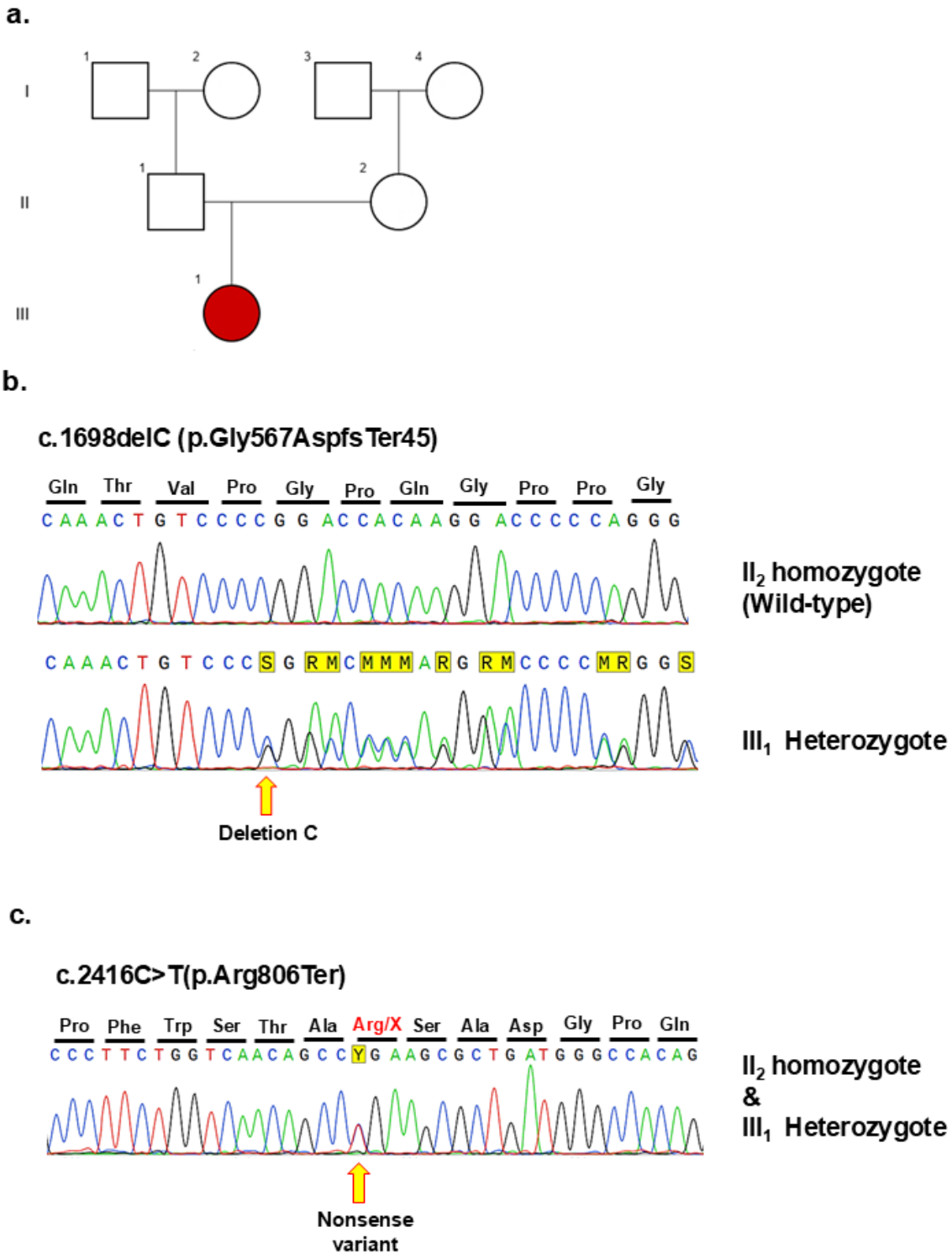

Figure 1 The schematic pedigree of the proband's family and Sanger Sequencing findings. **a**) The pedigree was depicted by using a user-friendly online Invitae family history tool (https://familyhistory.invitae.com/). The red color indicates KS. **b**) Frameshift pathogenic variant in the exon 7; and **c**) Premature stop codon in the exon 16.

2.1.1 Prenatal and Birth History

The patient was delivered via cesarean section at 38 weeks of gestation, with a birth weight of 2800 grams and a length of 50 cm, both within the normal range for the gestational age. Her head

circumference was normal. The patient's mother was hospitalized for one week due to a gynecological infection during pregnancy.

2.1.2 Early Infancy (1 Month to 7 Months)

The signs began at the age of one month, initially presented with horizontal nystagmus, which later became associated with strabismus. Initial neonatal examinations were normal, and the patient had a normal appearance. At seven months of age, she was observed to have persistent symptoms of horizontal nystagmus and strabismus, raising concerns about potential vision loss.

2.1.3 Childhood (Up to 5 Years)

Throughout her early childhood, the patient neither exhibited any motor or learning difficulties nor had any speech disorders. However, she experienced joint laxity until the age of 5. There was no history of renal or skin disorders, and the patient had no history of seizures. Ocular signs showed a brief improvement over time.

2.1.4 Age 6 Years

At the age of 6, the patient began taking L-carnitine and vitamin B1. The reason for administering these supplements is not explicitly linked to the diagnosis of mitochondrial disorders in the context provided.

2.1.5 Current Status (Age 10 Years)

At the age of 10, the patient presented with eye deviation and persistent horizontal nystagmus, accompanied by a high degree of nearsightedness (high myopia). The patient’s clinical features included horizontal nystagmus, high myopia, visual loss, hypermobile joints, and strabismus. There was no significant family history of similar symptoms.

2.2 Compliance with Ethical Standards

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Arak University of Medical Sciences and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Medical Ethics Committee of Arak University of Medical Sciences (Approval no: IR.ARAKMU.REC.1401.116). Written informed consent was obtained from the patient’s parents to publish this report in accordance with the journal's patient consent policy.

2.3 DNA Extraction

The salting-out procedure [14] was used to extract genomic DNA from 10 mL of peripheral blood samples obtained from the proband and her mother. A NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) was used to assess the concentration and quality of genomic DNA.

2.4 Whole Exome Sequencing (WES)

The Illumina HiSeq4000 platform produced 101-bp paired-end reads for WES on the genomic DNA of the proband. The SureSelectXT2 V6 kit was used to enrich the exonic and surrounding exon-intron border areas. Raw sequencing data (approximately 100 gigabases) were aligned to the human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner (BWA) [10]. The Picard tool (v. 1.118) eliminated the polymerase chain reaction (PCR) duplicates. Sequencing covered more than 95% of the targeted regions with a sensitivity of over 99% and a mean coverage of 100×. The Genome Analysis Toolkit (GATK v 3.7) called variants, and the ANNOVAR program annotated them. Variants were identified using a minor allele frequency of 0.01 in databases such as the 1000 Genomes Project, dbSNP 138, NHBL Exome Variant Server (EVS), ESP6500, and Iranome. Additional filtering based on pathogenic variant pathogenicity and impact, prevalence in the public population, clinically relevant variant databases, and known associations with the phenotype was performed according to the literature. The variants were prioritized based on their phenotypic plausibility.

2.5 Sanger Sequencing

Sanger sequencing verified the pathogenic variants in the proband. The c.2416C>T was also assessed in the mother of the proband for segregation analysis. The ABI Sequencer 3500XL PE and BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies; Thermo Fisher Scientific, Shanghai, China) were used for Sanger sequencing (Applied Biosystems, CA, USA). Standard procedures were followed for PCR amplification, product purification, and Sanger sequencing. Specific primers targeting exons 7 and 16 of the *COL18A1* locus and its flanking intronic regions were designed using the Primer3 Web-based server. The quality and specificity of the designed primers were checked using the OligoAnalyzer Tool and NCBI primer BLAST, respectively (Table 1).

Table 1 The sequence of designed primers that were used in this study.

Exon 7	*COL18A1*-EX7F	CCGAGCCCTGTGTTCTGTTTATTC
	COL18A1-EX7R	AGCAACGGTGGGCCTAAGGA
Exon 16	*COL18A1*-EX16F	GAATGAGCTGACCCGAGAC
	COL18A1-EX16R	TCCGAAGAACAAGGGTGGC

The acquired sequences were aligned with the reference sequence in GenBank using Chromas Lite software (Technelysium Pty Ltd., Australia) and CodonCode Aligner v.8.0.2. In silico tools, including BayesDel_addAF, MutationTaster2, FATHMM software, and DANN, were utilized to evaluate the pathogenicity of the identified variants.

2.6 Prediction of Protein Structure Using I-TASSER

The entire *COL18A1* protein sequence was introduced into Iterative Threading ASSEmbly Refinement (I-TASSER) to predict the protein structure and structure-based functions. Similarly, the sequence of the frameshifted protein was introduced into I-TASSER to compare the wild type and its mutated counterpart. Pymol 2.5.4 visualized the predicted structures.

2.7 Compiling Reported Cases of KS Molecularly and Clinically

An extensive literature search compiled all reported cases of KS, both molecular and clinical. Multiple electronic databases, including PubMed, ClinVar, and OMIM, were systematically searched for relevant keywords and MeSH terms. The search included studies published in English up to July 2024 and studies reporting on patients with a confirmed diagnosis of KS, focusing on pathogenic variants in the *COL18A1* gene and their corresponding phenotypes. The exclusion criteria were studies unrelated to KS or insufficient molecular or clinical information. Two independent reviewers screened titles, abstracts, and full texts to select relevant studies. Discrepancies were resolved through consensus or consultation with a third reviewer. Information obtained from the selected studies included demographic information, clinical manifestations, identified pathogenic variants in the *COL18A1* gene, and outcomes. These standard literature reviews create an overview of KS-reported cases in terms of molecular findings and clinical characteristics, as presented in Table 2.

Table 2 Overview of molecular findings and clinical characteristics of the reported cases of KS.

										Ocular manifestations								**Non- ophthalmic symptoms**			
Reference	**Study**	**Consanguinity**	**Gender**	**pathogenic variant at the cDNA level**	**pathogenic variant at the protein level**	**Zygosity**	**ACMG Classification**	**The same presentations in family**	**nationality**	Visual loss/Congenital cataract/Lens subluxation/High myopia/Nystagmus	Band keratopathy/Poor pupillary dilation/Absent iris crypts/Iris transillumination/Elevated intraocular pressure/Glaucomatous optic disc cupping	Pigment dispersion syndrome/Persistent pupillary membrane/Retinal detachment (childhood)/Syneresis/Vitreous attachment at the disc/Vitreoretinal degeneration	Persistent fetal hyaloid vasculature/Attenuated retinal vessels/Peripapillary atrophy/Chorioretinal atrophy, central/Generalized retinal atrophy	Generalized retinal pigment epithelium atrophy/Chorioretinal atrophy, central/	Phthisis bulbi/Macular hypoplasia/Pale optic disc/Irregular white dots at the vitreoretinal interface	Absent foveal pits seen on OCT /Extensive loss of outer retinal structures seen on OCT	Cone-rod dysfunction seen on ERG/Reduced or undetectable responses seen on ERG	Congenital hydronephrosis/Duplex kidneys/Bifid ureters/Ureteric anomaly	Hypermobile joints Skull/Midline occipital bone defect/Alopecia at the occipital defect	Occipital encephalocele/Occipital dermal sinus tract/Cognitive decline/Cerebellar ataxia, adult-onset	Seizures/Ventricular dilatation/Subependymal heterotopic nodules/Polymicrogyria/Cerebellar atrophy/Cerebral atrophy
	Present study	-	F	c.2416C>T / c.1698delC	p.Arg806Ter / p.Gly567AspfsTer45	Compound Het	P / P	-	Persian	+/-/-/+/+	-/-/-/-/-/-	-/-/-/-/-/-	-/-/-/-/-/-	NA	NA	NA	NA	NA	NA	NA	NA
1	[15]	-	M	c.2908C>T	p.Arg970Ter	Mutant Hom	P (nonsense)	+	Indian	-/-/+/+/+/-	-/-/-/-/-/-	-/-/-/-/-/+	-/-/-/+/+/-	-/+	-/-/-/-	-/-	-/-	-/-/-/-	-/+/-	-/-/-	-/-/--/-/-

2		-	M	c.2908 C>T	p.Arg970Ter	Mutant Hom	P (nonsense)	+	Indian	-/-/+/-/-	-/-/+/-/-/-	-/-/-/-/-/-	-/-/-/+/-	-/+	-/-/-/-	-/-	-/-	-/-/-/-	-/+/+	-/-/-	-/-/-/-/-/-
3	[16]	NA	M	c.2159 dupC / c.5014 G>A	p.Gly956ArgfsX20 / p.Asp1672Asn	Compound Het	LP / LB (missense)	+	British	-/+/-/+/+	-/-/-/-/-/-	+/-/+/-/-/-	+/-/-/-/-/-	-/+	-/+/-/-/	-/-	+/-	-/-/-/-	NA	NA	NA
4		NA	M	c.2159 dupC / c.5014 G>A	p.Gly956ArgfsX20 / p.(Asp1672Asn)	Compound Het	LP / LB	+	British	-/+/+/+/+	-/-/-/+/-/-	+/-/-/-/-/-	-/-/-/+/-	-/+	-/+/-/-/-	-/-	-/-	-/-/-/-	NA	NA	NA
5	[17]	-	M	c.1883_1891 dup / c.1787 C>T	p.Pro628_Pro630dup / p.Pro596Leu	Compound Het	V (in frame) / V (missense)	-	Spanish	-/-/-/-	-/-/-/-/-/-	-/-/-/-/-/-	-/-/-/-/-/-	-/-	-/-/-/-/-	-/-	-/-	-/-/-/-	-/-/-	-/-/+/-	-/-/-/-/-/-
6	[18]	-	F	c.1762-1G>A c.4462_4463delinsT	NA / p.Gly1311Serfs*2	Compound Het	LP (noncoding) / LP	+	Chinese	-/-/-/+/-	-/-/-/-/-/-	-/-/-/-/-/-	-/-/-/-/+/-	-/-	-/-/-/-/-	-/-	-/-	-/-/-/-	-/+/-	+/-/-/-/-	-/-/-/-/-/-
7		-	M	c.1762-1G>A c.4462_4463delinsT	NA / p.Gly1311Serfs	Compound Het	LP (noncoding) / LP	+	Chinese	-/-/-/+/-	-/-/-/-/+/-	-/-/+/-/-/-	-/-/-/-/+	-/-	-/-/-/-/-	-/-	-/-	-/-/-/-	-/+/-	+/-/-/-/-	-/-/-/-/+/-

8	[19]	-	F	c.4269_4287del c.4194_4221del	p.Pro1424LeufsTer13 p.Pro1399LeufsTer35	Compound Het	P/P	-	Jewish	-/-/+/+	-/-/-/+/-/-	+/-/+/+/-	-/-/-/-/+	+/-	-/+/-/-	-/-	+/-	-/-/-/-	-/+/-	-/-/-/-	-/-/-/-/-
9		+	F	c.4164dupC	p.Gly1389ArgfsTer1	Mutant Hom		-	Arabic	-/-/+/+	-/-/-/-/-/-	-/-/+/+/-	-/-/-/-/+	-/-	-/+/-/-	-/-	+/-	-/-/-/-	-/+/-	-/-/-/-	-/+/-/-/-/-
10		+	F	c.4759_4760delCT	Leu1587Valfs*72	Mutant Hom	P	+	Arabic	-/-/+/+	-/-/-/-/-/-	-/-/+/+/-	-/-/-/-/+	-/-	-/+/-/-	-/-	+/-	-/-/-/-	-/+/-	-/-/+/-	+/+/-/-/+/-
11		+	M	c.4759_4760delCT	Leu1587Valfs*72	Mutant Hom	P	+	Arabic	-/-/+/+	-/-/-/-/-/-	-/-/+/+/-	-/-/-/-/+	-/-	-/+/-/-	-/-	+/-	-/-/-/-	-/+/-	-/-/+/-	+/+/-/-/+/-
12	[20]	NA	F	c.4054_4055delCT / c.2992G>A	p.Leu1352Valfs*72 p.Gly998Arg	Compound Het	P/V (missense)	+	Chinese	-/-/+/-	-/-/-/-/-/-	-/-/-/+/+	-/+/+/-	-/-	-/-//-/-	-/-	-/-	-/-/-/-	-/+/-	NA	NA
13		NA	M	c.4054_4055delCT / c.2992G>A	p.Leu1352Valfs*72 / p.Gly998Arg	Compound Het	P/V (missense)	+	Chinese	+/+/-/+/-	-/-/-/-/-/-	-/-/-/+/+	-/+/+/-	-/-	-/-//-/-	-/-	-/-	-/-/-/-	-/+/-	NA	NA
14		NA	M	c.4054_4055delCT /	p.Leu1352Valfs*72 / p.Gly998Arg	Compound Het	P/V (missense)	+	Chinese	+/-/-/+/-	-/-/-/-/-/-	-/-/-/+/+	-/+/+/-	-/-	-/-//-/-	-/-	-/-	-/-/-/-	-/+/-	NA	NA

				c.2992G>A															
15		+	F	c.3810dup	p.Val1271Arg fs*27	Mutant Hom	P	-	Chinese	-/-/-/+/+	-/-/-/-/-/-	-/-/-/-/-/-	-/-/-/+/+	+/+	-/+/-/-	-/-	-/+	-/-/-/-/-	-/-/-	NA	NA
16		-	M	c.3364_3372delGGCCCCCCAinsC	p.Gly1122ArgfsTer142	Mutant Hom	B	-	Chinese	-/-/-/+/+	NA	-/-/-/-/-/-	-/-/-/+/+	+/+	NA	NA	NA	-/-/-/-/-	-/-/-	-/-/-	-/-/-/-/-/-
17		-	M	c.1999C>T / c.3213dup	p.Arg667Ter / p.Gly1072ArgfsTer9	Compound Het	P (nonsense) / P	-	Chinese	-/-/-/+/-	NA	-/-/-/-/-/-	-/-/-/+/+	-/+	-/+/-/-	-/+	+/+	-/-/-/-/-	-/-/-	-/-/-	NA
18	[21]	-	F	c.2673del	p.Gly892Aspfs*17	Mutant Hom	LP	+	Pakistani	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
19		-	M	c.2673del	p.Gly892Aspfs*17	Mutant Hom	LP	+	Pakistani	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
20	[22]	+	F	c.4224_4225delinsC	p.Pro1411Leufs*35	Mutant Hom	P	-	Chilean	-/-/-/+/-	-/-/-/-/+/-	-/-/-/-/-/-	-/-/+/+/-	-/+	-/-/-/-	-/-	-/-	-/-/-/-/-	-/+/+	-/-/-/-	-/-/-/-/-/-
21	[23]	NA	M	c.4290_4299del	p.Gly1431Glufs*9	Mutant Hom	P	NA	Chinese	-/-/-/+/+	NA	-/-/-/-/-/-	-/-/+/+/+	-/+	-/+/+/-	NA	NA	-/-/-/-/-	-/-/-	NA	NA
22		NA	M	c.4259-28_4265del	NA	Mutant Hom	LP (splice junction)	NA	Chinese	-/-/-/+/+	NA	-/-/-/-/-/-	-/-/+/+/+	+/+	-/+/+/-	NA	NA	-/-/-/-/	-/+/-	NA	NA

23		NA	M	c.4759_4760del	p.Leu1587Valfs*72	Mutant Hom	P	NA	Chinese	-/+/-/+/+	NA	-/-/-/-/-/-	-/-/+/+/+	+/+	-/+/+/-	NA	-/+	-/-/-/-	-/-/-	NA	NA
24	[7]	-	F	c.2960_2969dup / c.3514_3515del	p.Gly991Argfs*96 / p.Leu1172Valfs*72	Compound Het	LP / LP	NA	Belgian	-/-/-/+/+	-/-/-/-/-/-	-/-/-/-/-/-	-/-/-/-/-/-	-/-	-/-/-/-	-/-	-/-	-/-/-/-	-/+/-	+/-/-/-	-/-/-/-/-/-
25		+	M	c.1610del	P.537Glnfs*16	Mutant Hom	P	NA	Belgian	-/-/+/+/+	-/-/-/+/-/-	-/-/-/-/-/-	-/-/-/+/+	+/+	-/NA/-/-	-/-	-/-	+/+/-/-	-/+/+	-/-/-/-	-/-/-/-/-/-
26		+	F	c.4492del G	p.Glu1498Lys*fs	Mutant Hom	LP	NA	Belgian	-/-/-/+/+	-/-/+/-/-/-	-/-/+/-/-/-	-/-/-/-/-/-	+/-	-/+/-/-	NA	NA	-/-/-/-	-/-/-	-/-/-/-	-/-/-/-/-/-
27	[24]	NA	F	c.2978_2987del	p.Pro993Leufs*35	Mutant Hom	P	NA	Arabic	-/-/-/+/+	-/-/+/+/-	-/-/-/-/-/-	-/-/-/+/+	-/+	-/+/-/-/	NA	NA	-/-/-/-	-/+/+	-/-/-/-	-/-/-/-/-/-
28	[25]	NA	F	c.4759_4760delCT	p.Leu1587ValfsX72	Mutant Hom	P	+	Chinese	-/+/+/+/+/+	-/-/-/-/-/-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA/NA/NA/NA/+/NA
29		NA	M	c.4759_4760delCT	p.Leu1587ValfsX72	Mutant Hom	P	+	Chinese	-/+/+/+/+/+	-/-/-/-/-/-	-/-/+/-/-/-	NA	NA	NA	NA	NA	NA	NA	NA	NA/NA/NA/NA/+/-

30	[26]	NA	F	c.4166G>A	p.Arg1389His	Mutant Hom	V (missense)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-/-/-	+/+/-/-/-/-
31	[27]	-	M	c.2970_2971delAGinsC	p.Gly991AlafsTer40	Mutant Hom	P	+	NA	-/-/-/+/+	-/-/-/-/-/-	-/-/-/-/-/-	-/-/-/+/+	+/+	-/+/+/-	NA	+/+	-/-/-/-	-/-/-/-	-/-/-/+/-/-
32		-	F	c.2970_2971delAGinsC	p.Gly991AlafsTer40	Mutant Hom	P	+	NA	-/+/-/+/+	-/-/-/-/-/-	-/-/-/-/-/-	-/+/-/-/-/+	-/-	-/+/+/-	+/+	+/+	-/-/-/-	-/-/-/-	-/-/-/+/-/-
33	[28]	-	M	c.4759_4760delTC	p.L1587Vfs*72	Mutant Hom	P	+	Palestinian	-/-/-/+/+	NA	NA	NA	NA	NA	NA	NA	-/-/-/-	-/+/-	-/-/+/-	+/-/-/+/+/-/-
34		-	M	c.4759_4760delTC	p.L1587Vfs*72	Mutant Hom	P	+	Palestinian	-/-/-/+/+	NA	NA	NA	NA	NA	NA	NA	-/-/-/-	-/+/-	-/-/+/-	+/-/-/+/+/+
35	[12]	-	F	c.3690G>A / c.4063_4064delCT	p.Trp1230* / p.Leu1355Valfs*72	Compound Het	P (nonsense) / P	+	Northern European	-/+/+/NA/NA	-/+/+/-/-/-	-/-/+/-/-/-	-/-/+/+/-	-/-	-/+/+/-	NA	NA	-/-/-/-	NA	NA	+/-/-/+/+/+
36	[29]	+	F	c.3213dupC	p.Gly1072ArgfsStop9	Mutant Hom	P	-	NA	-/-/-/+/+	-/-/+/+/-/-	-/-/+/-/-/-	-/+/-/+/+	-/+	-/+/+/-	NA	-/-	-/-/-/-	-/-/-/-	-/-/-/+/-/-
38	[4]	-	M	c.4063_64delCT	p.Leu1355Valfs*72	Mutant Hom	P	+	Indian	-/-/-/+/+	-/+/+/-/-/-	-/-/+/-/-/+	-/-/+/+	+/+	-/+/-/-	NR/NR	NR/NR	-/-/-/-	-/-/-/-	-/-/-/+/-/-

39	-	F	c.4063_64delCT	p.Leu1355Valfs*72	Mutant Hom	P	+	Indian	-/-/-/+/+	-/+/+/-/-/-	-/+/+/-/+/-	-/-/+/+	+/+	-/+/-/-	NR/NR	NR/NR	-/-/-/-	-/+/-	+/-/-/-	-/-/-/+/-/-
40	-	M	c.2437-2A>G / c.3213delC	NA / p.Gly1072Aspfs*17	Compound Het	LP (noncoding) / P	+	British	-/-/-/+/+	-/+/-/+/-/-	-/+/+/-/-/-	-/-/-/+	+/+	-/+/-/-	NR/NR	NR/NR	-/-/-/-	+/-/-	-/-/-/-	-/-/-/-/-/-
41	-	M	c.2437-2A>G / c.3213delC	NA / p.Gly1072Aspfs*17	Compound Het	LP (noncoding) / P	+	British	-/-/-/+/+	-/+/-/+/-/-	-/+/-/-/-/-	-/-/-/+	-/-	-/+/-/-	NR/NR	NR/NR	-/-/-/-	+/-/-	+/-/-/-	-/-/-/+/-/-
42	+	M	c.3459dupC	p.Gly1154Argfs*110	Mutant Hom	P	+	Slovak	-/-/-/+/+	-/+/+/-/-/-	-/-/-/-/-/-	-/-/-/+	+/+	-/+/-/-	NR/NR	NR/NR	-/-/-/-	-/+/-	-/-/+/-	NR/NR/NR/NR/NR/NR
43	+	F	c.3459dupC	p.Gly1154Argfs*110	Mutant Hom	P	+	Slovak	-/-/-/+/+	-/+/+/-/-/-	-/-/-/-/-/-	-/-/-/+	+/+	-/+/-/-	NR/NR	+/-	-/-/-/-	-/-/-	-/-/-/-	NR/NR/NR/NR/NR/NR
44	-	F	c.3356_7insT	p.Gly1122Argfs*145	Mutant Hom	P	+	Arabic	-/-/-/+/+	-/-/-/-/-/-	-/-/-/-/-/-	-/-/-/+	-/+	-/+/-/-	NR/NR	NR/NR	+/-/-/-	+/+/-	-/-/-/-	NR/NR/NR/NR/NR/NR
45	-	M	c.3356_7insT	p.Gly1122Argfs*145	Mutant Hom	P	+	Arabic	-/-/-/+/+	-/-/+/-/-/-	-/+/-/-/-/-	-/-/-/+	-/+	-/+/-/-	NR/NR	NR/NR	-/-/-/-	+/+/-	-/-/-/-	NR/NR/NR/NR/

																			NR/NR
46		+	F	c.3213dupC	(p.Gly1072Argfs*9	Mutant Hom	P	-	British	-/-/-/+/+	-/+/-/+/-/-	+/-/-/-/-/-	-/-/-/+	-/+	-/+/-/-	NR/NR	NR/NR	-/+/+/-	-/-/-	-/-/-/-	-/-/-/-
47		+	F	c.2658dupC	p.Gly887Argfs*23	Mutant Hom	P	+	Northern European	-/-/-/+/+	-/-/-/+/-/-	-/-/-/-/-/-	-/-/-/+	-/+	-/+/-/-	NR/NR	+/-	-/-/-/-	-/-/-	-/-/-/-	NR/NR/NR/NR/NR/NR
48		-	M	c.2658dupC	p.Gly887Argfs*23	Mutant Hom	P	+	Northern European	-/-/-/+/+	-/+/-/+/-/-	+/+/-/-/-/-	-/-/-/+	-/+	-/+/-/-	NR/NR	+/-	-/-/-/-	-/-/+	NR/NR/NR/NR	NR/NR/NR/NR/NR/NR
49		+	F	c.4173G>A	p.Gln1211	Mutant Hom	LB (synonymous)	-	African	-/-/-/+/+	-/+/-/+/-/-	-/+/-/-/-/-	-/-/-/+	-/+	-/+/-/-	NR/NR	+/+	-/-/-/-	-/-/-	+/-/-/-	NR/NR/NR/NR/NR/NR
50	[30]	NA	F	c.3213dupC	p.Gly1072fs	Mutant Hom	P	-	Afghan	-/-/-/+/+	-/-/-/-/-/-	-/+/-/-/-/+	-/+/-/+	+/+	+/+/+/-	+/+	NR/NR	NR/NR/NR/NR	-/-/-	NR/NR/NR/NR	+/-/-/+/-/-
51	[31]	+	M	c.4768_4769delCT	p.Leu1590ValfsX 72	Mutant Hom	P	+	Turkish	+/-/+/-/-	-/-/-/-/-/-	-/-/-/-/-/+	-/-/-/-/-/-	-/-	+/-/-/-	NA	NA	-/-/-/-	-/-/-	-/-/+/-	+/NA/NA/+/-/-
52		+	M	c.2309_2310ins C	p.Gly773Argfs X55	Mutant Hom	P	+	Turkish	+/-/+/-/-	-/-/-/-/+/-	-/-/-/-/-/-	-/-/-/-/-/-	-/-	-/-/-/-	NA	NA	-/-/-/-	-/-/-	-/-/+/-	+/NA/NA/+/-/-
53		+	F	c.5195_5196insTGCC	p.Ala1734CysfsX 14	Mutant Hom	V	+	Turkish	+/-/-/+/+	-/-/-/-/+/-	-/-/-/-/+/+	-/-/-/-/-/-	-/-	-/-/+/-	NA	NA	-/-/-/-	-/-/-	-/-/+/+	+/NA/NA/+/-/-

54		+	F	c.4768_4769del CT	p.Leu1590Valfs X72	Mutant Hom	P	+	Turkish	+/-/NA/-/-	-/-/-/-/-/-	-/-/+/-/-/-	-/-/-/-/-/-	-/-	-/-/-/-	NA	NA	-/-/-/-	-/+/-	-/+/+/-	-/NA/NA/-/+/-
55	[32]		F	c.3825_3838del	p.Ser1276Alafs*9	Mutant Hom	P	-	Persian	+/-/-/+/+	-/-/-/-/-/-	-/-/-/-/-/-	-/+/-/+/-	-/+	-/+/-/-	NA	+/+	NA	NA	NA	NA
56	[33]	-	M	C.2416C>T	p.Arg806Ter	Mutant Hom	LP (nonsense)	-	NA	+/-/-/+/+	NR/NR/NR/NR/-/NR	-/-/+/-/+/+	NR/NR/NR/NR/NR	-/+	NR/NR/NR/NR/NR	NR/NR	NR/NR	+/+/-	-/-/+	-/-/-/-	NR/NR/NR/NR/NR/NR
57	[34]	+	M	c.355delG	p.V119Sfs X5	Mutant Hom	P	-	Arabic	-/-/+/-/-	-/-/+/-/-/+	-/-/-/-/+/+	-/-/-/+	+/+	-/-/-/-	NR/NR	-/-	NR/NR/NR/NR	-/-/-	+/-/+/-	NR/NR/NR/NR/NR/NR
58		+	M	c.2743C>T	p.R915X	Mutant Hom	P (nonsense)	+	Arabic	-/-/+/-/+	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/-	-/-/-/-	NR/NR	+/+	NR/NR/NR/NR	-/-/+	+/-/+/-	NR/NR/NR/NR/NR/NR
59		+	M	c.2743C>T	p.R915X	Mutant Hom	P (nonsense)	+	Arabic	-/-/+/+/+	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/+	-/+/-/-	NR/NR	-/-	NR/NR/NR/NR	-/-/+	-/-/-/-	NR/NR/NR/NR/NR/NR
60		+	M	c.2743C>T	p.R915X	Mutant Hom	P (nonsense)	+	Arabic	-/-/+/+/+	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/-	-/-/-/-	NR/NR	-/-	NR/NR/NR/NR	-/-/+	-/-/-/-	NR/NR/NR/NR/NR/NR

61		+	F	c.3514_3515delCT	p.L1172VfsX72	Mutant Hom	P	+	Arabic	-/-/+/-/+	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/-	-/-/-/-	NR/NR	+/+	NR/NR/NR/NR	-/-/-	-/-/-/-	NR/NR/NR/NR/NR/NR
62		+	M	c.3514_3515delCT	p.L1172VfsX72	Mutant Hom	P	+	Arabic	-/-/-/-/+	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/+	-/+/-/-	NR/NR	+/+	NR/NR/NR/NR	-/-/-	-/-/-/-	NR/NR/NR/NR/NR/NR
63		+	M	c.1785_1786delinsA	p.P597LfsX127	Mutant Hom	LP	-	Arabic	-/-/-/-/-	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/+	-/+/-/-	NR/NR	-/-	NR/NR/NR/NR	-/-/+	-/-/-/-	NR/NR/NR/NR/NR/NR
64	[35]	NA	M	c.2743C>T	p.R915X	Mutant Hom	P (nonsense)	+	Arabic	-/-/+/+/+	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/+	-/+/-/-	NR/NR	-/-	NR/NR/NR/NR	-/-/+	-/-/-/-	NR/NR/NR/NR/NR/NR
65		NA	M	c.2743C>T	p.R915X	Mutant Hom	P (nonsense)	+	Arabic	-/+/+/+/+	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/+	-/+/-/-	NR/NR	-/-	NR/NR/NR/NR	-/-/+	-/-/-/-	NR/NR/NR/NR/NR/NR
66		NA	M	c.2743C>T	p.R915X	Mutant Hom	P (nonsense)	+	Arabic	-/+/+/+/+	-/-/+/-/-/-	-/-/-/-/+/+	-/-/-/+	+/+	-/+/-/-	NR/NR	-/-	NR/NR/NR/NR	-/-/+	-/-/-/-	NR/NR/NR/NR/NR/NR
67		NA	M	c.355delG	p.V119SfsX5	Mutant Hom	LP	+	Arabic	-/-/+/-/-	NA	NA	NA	NA	+-/-/-	NA	NA	NA	+/-/-	NA	NA

68		NA	M	c.355delG	p.V119SfsX5	Mutant Hom	LP	+	Arabic	-/-/+/-/-	NA	NA	NA	NA	+/-/-	NA	NA	NA	-/-/+	NA	NA
69	[36]	-	F	c.3514-3515delCT	p.1173ArgfsTer93	Mutant Hom	LP	+	El Salvador	-/-/-/+/+	-/-/-/-/+/-	-/-/+/+/+/-	+/-/+/+	NR/+	-/+/+/+	NR/NR	NR/NR	NR/NR/NR/NR	-/-/+	-/+/-/-	-/-/-/-/-/-
70		-	F	c3514-3515delCT	p.1173ArgfsTer93	Mutant Hom	LP	+	El Salvador	-/-/-/+/+	-/-/-/-/+/-	-/-/+/+/+/-	+/-/+/+	NR/+	-/+/+/+	NR/NR	NR/NR	NR/NR/NR/NR	-/-/+	-/+/-/-	-/-/-/-/-/-
71	[37]	+	F	c.3544+3A>C		Mutant Hom	P	+	Algerian	-/-/-/+/+	-/-/-/-/-/-	-/-/-/-/-/+	-/+/-/-	-/-	-/-/+	NR/NR	-/-	NR/NR/NR/NR	-/-/-	+/-/-/-	-/-/-/-/-/-

3. Results

The latest literature review, which introduces a new case of a unique variant in the *COL18A1* gene associated with KS, was included in this study. Seventy-one patients from previous studies, including our research, were identified (Table 2), with 47 reported variants in *COL18A1*. Among these variants, the truncating pathogenic variant c.4759_4760delCT was more prevalent in seven families. The data indicated that there were 31 female individuals, including our case, and 40 male individuals.

The analysis revealed a diverse distribution of *COL18A1* variants across different nationalities, with the highest representation from Arabic (18), Chinese (13), Turkish (5), and British (5) populations. Other nationalities represented include Indian (4), Belgian (3), Northern European (3) Pakistani (2), Palestinian (2), Slovak (2), El Salvadorean (2), African (1), Afghan (1), Spanish (1), Jewish (1), Chilean (1), Persian (1), and Algerian (1) Additionally, there are 5 cases with unspecified or missing nationality information (NA). Zygosity analysis indicated that the majority of patients exhibited a homozygous state for the identified gene variants, with 57 cases being mutant homozygous. Compound Heterozygous variants were detected in 14 patients.

The variants were classified into different pathogenic variant types. Nonsense pathogenic variants were the most prevalent, followed by missense and non-coding pathogenic variants. The classification of variants into different pathogenicity categories highlighted a significant number of pathogenic and likely pathogenic variants (28 pathogenic and 11 likely pathogenic), emphasizing their deleterious effects on KS.

KS is caused by homozygous or compound heterozygous pathogenic variants in the *COL18A1* and is characterized by both ophthalmic and non-ophthalmic clinical presentations. The presence of family history and consanguinity in affected families is consistent with this pattern of inheritance.

Genetic counseling is vital in light of these findings. Furthermore, the coexistence of comorbidities such as infection, proteinuria, hypoalbuminemia, and various other manifestations underscores the intricate multisystem involvement and complexity associated with KS.

In the present study, a total read base of 6.8 million bp was gathered for one case using WES. The proband had approximately 86000 variations. Variants with a lower frequency of 1% regarding gnomAD, 1K genome projects, ExAC, and ESP were selected for further study; consequently, 700 variants remained for further consideration. The causative variants, identified by their presence in the HGMD and ClinVar databases, were further assessed for conservation and pathogenicity using tools such as PhastCons100way, PhyloP100way, SiPhy29way, fitCons-gm, statistics, and various BayesDel and MutationTaster models (Table 3).

Table 3 The pathogenicity and conservation score according to online databases.

Variant	Location	Zygosity			Allele Frequency	Pathogenicity	Conservation
		Proband	Father	Mother			
NM_030582.4: c.1698del (p.Gly567 AspfsTer45)	*COL18A1*; chr:21; g45477899	Het	NR	Hom (WT)	gnomAD: NR 1K genome: NR ExAC: NR Iranome: NR ESP 6500: NR TOPMed Bravo: NR GME Variome: NR 4.7KJPN: NR GenomeAsia: NR Mexican DB: NR	BayesDel noAF: NR BayesDel addAF: NR FATHMM-MKL: NR MutationTaster: NR EIGEN: NR	PhyloP100way: -0.315 PhastCons100way: 0.000 fitCons-gm: NR SiPhy29way: NR Bstatistic: NR
NM_030582.4): c.2416C>T (p.Arg806Ter)	*COL18A1*; chr:21; g45487489	Het	NR	Het	gnomAD: 0.0000131 1K genome: NR ExAC: NR Iranome: NR ESP 6500: NR TOPMed Bravo: 0.000011 GME Variome: NR 4.7KJPN: NR GenomeAsia: NR Mexican DB: NR	BayesDel noAF: Damaging BayesDel addAF: Damaging FATHMM-MKL: Neutral MutationTaster: Disease causing automatic EIGEN: Benign	PhyloP100way: -0.511 PhastCons100way: 0.000 fitCons-gm: 0.5881 SiPhy29way: 11.3059 Bstatistic: 641

Het: Heterozygous; Hom: Homozygous; NR: not reported.

The pathogenicity ranking of homozygous/compound heterozygote variants, considering the non-consanguinity of the parents, prioritized splicing region, stop gain, and frameshift pathogenic variants, followed by the assessment of amino acid changes for nonsynonymous pathogenic variants**.** WES analysis identified two compound heterozygous variants in the *COL18A1* gene: the c.2416C>T (p.Arg806Ter) variant, which was inherited from the mother, and the c.1698delC (p.Gly567AspfsTer45) variant, for which the inheritance status is unknown due to the unavailability of paternal testing.

Both variants were classified as pathogenic according to ACMG and ClinVar guidelines [38]. The c.2416C>T (p.Arg806Ter) variation has previously been reported as a disease-causing variant [39]. The novel frameshift pathogenic variants c.1698delC (p.Gly567AspfsTer45) has not been reported in the 1K genome project, gnomAD, ExAC, HGMD, ESP 6500, TOPMed Bravo, 4.7 KJPN, Iranome, GME Variome, Mexican DB, and GenomeAsia (Table 3). Sanger sequencing revealed that c.2416C>T (p.Arg806Ter) was heterozygous in both the proband and her mother. The proband carried the second variant, c.1698delC (p.Gly567AspfsTer45), while her mother had a normal, wild-type genotype. This suggests that the first variant was inherited from the mother, while the second variant may have originated from the father or occurred spontaneously. Because the father's information was unavailable, we could not determine whether the second variant, c.1698delC (p.Gly567AspfsTer45), was inherited from the father or occurred *de novo*. However, we confirmed that the first variant, c.2416C>T (p.Arg806Ter), was in a heterozygous state in both the proband and her mother, indicating segregation through the maternal lineage. This observation revealed a compound heterozygote state in the proband, causing KS (Figure 1b & c).

Furthermore, we used *the I-TASSER* online software to predict the potential effect of candidate pathogenic variants on the protein domain structure [40]. LOMETS identified PDB hints 2pffB (Z-score of 10.05), 1vt41 (Z-score of 4.74), and 2uur (Z-score of 3.54) as the most reliable PDB structure templates for threading alignment with the *COL18A1* wild-type, c.2416C>T (p.Arg806Ter), and c.1698delC (p.Gly567AspfsTer45) variants, respectively. Based on pair-wise structure similarity, the SPICKER program predicted final protein models with a C-score = -0.19 for wild-type form, C-score = -2.88 for the stop codon, and-1.79, respectively (Figure 2).

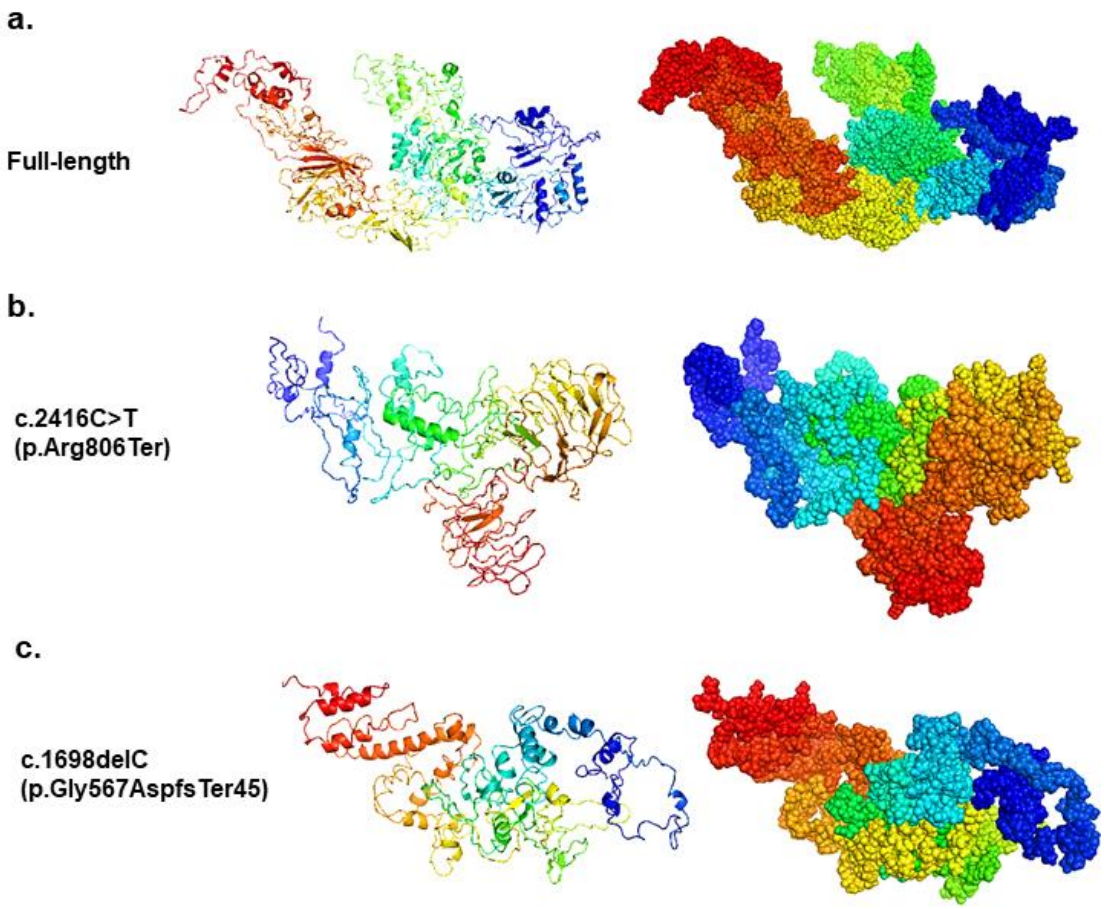

Figure 2 The schematic view of the stimulated domain proteins using *I-TASSER*. a) Wild-type; b) The premature stop codon; and c) Frameshift pathogenic variant.

4. Discussion

In this study, we investigated a 10-year-old Iranian female with clinical symptoms that were suggestive of KS. WES analysis of gDNA identified two pathogenic compound heterozygous variants in the *COL18A1* gene, which encodes the alpha chain of collagen type XVIII. The variants identified include a previously documented premature stop codon, c.2416C>T (p.Arg806Ter) [41], and a novel frameshift pathogenic variant, c.1698delC (p.Gly567AspfsTer45), located in exons 7 and 16 of *COL18A1*, respectively. The identified variants were validated through Sanger sequencing. Furthermore, the impact of the identified pathogenic variant on the *COL18A1* structure was assessed using I-TASSER.

The findings of this study align with existing literature on KS, which has documented the heterogeneity of the disease's phenotypic manifestations [42]. These manifestations range from mild ocular abnormalities to severe neurodevelopmental impairment. The presence of diverse clinical presentations further underscores the complex nature of the disease and the necessity for genetic counseling [43]. Given the rarity of KS and the fact that *COL18A1* is the sole causative gene, elucidating the spectrum of pathogenic variants is of paramount importance [20].

In the analysis of previously documented cases of KS, 47 distinct variants of the *COL18A1* gene were identified. The most frequently observed variant was the truncating pathogenic variant c.4759_4760delCT, which was present in seven families. This finding highlights the importance of

understanding the spectrum of gene variants associated with KS, as different variants may result in varied clinical outcomes. The distribution of *COL18A1* variants showed variation across different nationalities, with the highest prevalence observed in populations of Arabic, Chinese, Turkish, and British descent. These findings suggest that ethnicity-specific genetic factors may contribute to the development of KS. Further studies examining the genetic landscape of KS in different populations would provide valuable insights into its pathogenesis.

Our WES analysis identified a variety of pathogenic variant types in the *COL18A1* gene, with nonsense pathogenic variants being the most common, followed by missense and non-coding pathogenic variants. This classification of variants into different pathogenic variant types further emphasizes the deleterious effects of these pathogenic variants on KS. The pathogenicity of the identified variants was assessed using a range of computational tools, including PhastCons100way, PhyloP100way, SiPhy29way, fitCons-gm, and MutationTaster. These tools facilitated the prioritization of the variants based on their conservation and pathogenicity scores. Both the identified variants, c.2416C>T (p.Arg806Ter) and c.1698delC (p.Gly567AspfsTer45), were classified as pathogenic according to the ACMG and ClinVar guidelines. The c.2416C>T (p.Arg806Ter) variant has been previously identified as a pathogenic variant [41], while the c.1698delC (p.Gly567AspfsTer45) variant is characterized as a novel frameshift pathogenic variant.

To elucidate the potential structural impacts of the identified pathogenic variants on the *COL18A1* protein, I-TASSER online software was employed. This software predicted alterations in the protein domain structure for both variants. The resulting models indicated a possible disruption of the protein structure, suggesting that these pathogenic variants may have significant functional implications.

Overall, this study contributes to the growing body of knowledge regarding KS and its genetic basis. The identification of pathogenic variants of *COL18A1* expands our understanding of the disease and its clinical implications. The heterogeneity of the phenotypic manifestations highlights the need for personalized and comprehensive clinical management. The application of advanced genomic technologies, such as whole-exome sequencing (WES) and bioinformatics tools, provides significant insights into the genetic landscape of KS and may facilitate the development of targeted therapies. Future research should prioritize elucidating the molecular mechanisms underlying KS and identifying potential therapeutic targets. Collaborative efforts across diverse populations and countries are essential to enhance our understanding of the genetic basis of KS and improve clinical management strategies. Furthermore, long-term follow-up studies are necessary to assess the efficacy of targeted therapies and evaluate the impact of genetic counseling on individuals and families affected by KS.

In conclusion, this study advances the disciplines of genetics and ophthalmology by offering novel insights into the genetic framework of KS. The identification of novel variants of *COL18A1* expands our understanding of the disease and its clinical implications. Further research and collaboration are needed to fully understand the molecular mechanisms underlying KS and develop effective therapeutic interventions.

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ensure the English language quality of our writing. These tools were employed solely to refine the language and improve readability, not to generate or alter the scientific content. We reaffirm that we have reviewed and edited the section generated by the AI-assisted tool for accuracy, and acknowledge full responsibility for the content of the manuscript.

Author Contributions

Mohammad-Reza Ghasemi: Conceptualization, Investigation, Methodology, Formal analysis, Writing—original draft; Maryam Mirahmadi: Investigation, Methodology, Formal analysis, Writing—original draft; Hadi Bayat: Investigation, Formal analysis, Writing—review and editing; Morteza Sheikhi Nooshabadi: Investigation, Methodology; Sanaz Jamshidi: Investigation, Methodology; Shadab Salehpour: Validation, Resources; Reza Mirfakhraie: Validation, Resources; Fatemeh Fazeli: Investigation, Methodology; Mohammad Miryounesi: Conceptualization, Validation, Supervision, Writing—review and editing; Milad Gholami: Conceptualization, Investigation, Validation, Resources, Supervision, Project administration, Funding acquisition, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Competing Interests

The authors declare that they have no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available upon request from the corresponding author. The data were not publicly available because of privacy or ethical restrictions.

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