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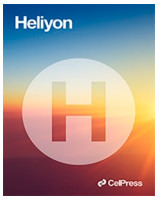

Research article

Genetic investigation in a four-generation Chinese family with congenital fibrosis of extraocular muscles and keratoconus

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ABSTRACT

Here, we have reported the genetic and clinical characteristics of four generations of a family patient from China with congenital fibrosis of extraocular muscles 1 (CFEOM1) and keratoconus (KC). The history of diseases, clinical observations, and blood samples of all family members were collected. A total of 100 healthy participants were recruited as normal controls. The whole exome sequencing of the genomic DNA and polymerase chain reaction were performed on samples obtained from the controls and their family members to verify the gene variants. The functional analyses of the variants were performed by using different software. Two single nucleotide polymorphisms were detected in the proband and other patients in his families, including a heterozygous missense variation, g.39726207C > T (c.2860C > T, p.R954W, rs121912585), in the third highly conserved coiled-coil domain of *KIF21A*, and a heterozygous missense variant, g.30664732A > C (c.136A > C, p.S46R, rs200111443) in *TGFBR2*. The variant p.R954W in *KIF21A* was predicted to be pathogenic using software, whereas p.S46R in *TGFBR2* was predicted to be of uncertain significance (VUS). Thus, KC might have occurred in the proband and his daughter because of a combination of genetic mutations and involuntary eye rubbing induced by CFEOM1. This is the first case of concomitant KC in a family having CFEOM1. Thus, the study provides new information about patients with KC having CFEOM1. Furthermore, the study suggests that attention should be paid to the early detection and diagnosis of KC in patients with CFEOM1.

1. Introduction

Congenital cranial dysinnervation disorders (CCDDs) are congenital, non-progressive, and genetically heterogeneous syndromes characterized by the sporadicalness or family abnormalities in the innervation of extraocular and cranial musculature, and they are

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caused by hypoplasia or the loss of one or more cranial moto-neurons with the primary or secondary neuromuscular disorder [1]. CCDDs belong to a spectrum that includes congenital fibrosis of extraocular muscles (CFEOM), Duane retraction syndrome, Moebius syndrome, horizontal gaze palsy with progressive scoliosis, and hereditary congenital facial paresis [2].

CFEOM1 is commonly regarded as "classic CFEOM" and its typical clinical features are non-progressive and congenital bilateral external ophthalmoplegia. The main manifestations are restricted vertical and horizontal eye movements and ptosis, which results in eyelid drooping [3,4]. In the past few years, the identification of several heterozygous missense variants of *KIF21A* has been shown to be associated with the classic CFEOM1 phenotype. However, for patients with CFEOM1, corneal morphology is rarely examined, which often results in the missed diagnosis of certain corneal abnormalities, such as keratoconus (KC). KC, a type of corneal ectasia (CE), is one of the most common clinical ophthalmological diseases. According to the optical coherence tomograph (OCT) sign, CE can be classified into KC, keratoglobus, and pellucid marginal degeneration (PMD). KC exhibits the thinning of the cornea at the center, whereas PMD exhibits the thinning of the cornea in the periphery. Keratoglobus exhibits a wide thinning in the whole cornea. The etiology of KC includes genetic, behavioral, and environmental factors [5,6]. The pathological characteristic of KC is the decreased corneal collagen or abnormalities in collagen fiber distribution, resulting in the reduced mechanical resistance of the cornea [7,8].

Previous studies on patients with CFEOM1 have focused on binocular alignment, ocular motility, the palpebral fissure height, and levator palpebrae muscle functions, without paying sufficient attention to corneal examinations. In the present study, the corneal topography of the family members was conducted in addition to the abovementioned examinations. The cumulative results suggested the importance of complete corneal topography for early KC diagnosis in patients with CCDDs before its clinical onset.

2. Materials and methods

2.1. Participants and examinations

A total of 109 participants— 9 family members from four generations of a Chinese family having CFEOM1 with or without KC and 100 unrelated healthy Chinese individuals, who were not diagnosed with hereditary corneal disorders including CFEOM1 and KC—were recruited in the study. All family members had no allergic history, trauma, or laser corneal surgery history.

All participants signed an informed consent form. Further, they underwent detailed ophthalmologic and physical examinations, which included the assessment of cycloplegic refraction, best-corrected visual acuity, binocular alignment, eye movements, palpebral fissure height measurement, levator palpebrae muscle functions, biomicroscopy, and fundus examination. In addition, the Scheimpflug camera system (Pentacam; Oculus Optikgeräte GmbH, Wetzlar, Germany) was used for cornea examination. This study was approved by the institutional review board of Fudan University (Shanghai, China) (approval no. 2022128) and was performed in accordance with the Declaration of Helsinki.

2.2. Whole exome sequencing (ES) technology

ES was performed on samples collected from four participants (showing the mutations IV:2, III:3, and II:3) using a method described in a previous study [9]. Briefly, genomic DNA extracted from leukocytes was used for ES and ES enrichment, followed by data processing and analysis. The 1000 Genomes Project was used to examine variants observed in CCDDs or corneal abnormalities that exhibited frequencies of $\leq$1%. Only mutations shared by the family members, namely IV:2, III:3, and II:3 were considered CCDD candidate mutations, whereas the mutations IV:2 and III:3 were considered KC candidate mutations.

2.3. Variant validation and cross-species conservation analysis

Variant validation and cross-species conservation analysis were performed as previously described. All missense variations observed were analyzed using online software including PolyPhen-2 (genetics.bwh.harvard.edu), SIFT (sift.jcvi.org), FATHMM-MKL (fathmm.biocompute.org.uk), CADD v1.4 (cadd.gs.washington.edu), Mutation Taster (mutationtaster.org), FAVOR (https://favor.genohub.org), and ACMG guidelines (American College of Medical Genetics and Genomics). Briefly, polymerase chain reaction (PCR) and Sanger sequencing were performed on samples collected from all family members and normal controls to confirm the candidate mutations. PCR primers were designed by using Primer3. The validation and analyses were performed using NCBI VARIANT, NCBI HomoloGene, and 1000 Genomes Project databases. Three-dimensional (3D) protein structures of the variants were generated using the online server I-TASSER (zhanggroup.org/I-TASSER/).

2.4. Network of interactions between the proteins

A protein–protein interaction network (PPI) was established using STRING (cn.string-db.org), which was further analyzed using Cytoscape (v3.9.0), and a degree of $\geq$5 was set to filter the key proteins in the middle of the network.

2.5. RNA isolation and qPCR

Reverse transcription-polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR) were performed to identify *KIF21A* and *TGFBR2* mutations in the peripheral blood cells of patients and healthy volunteers. An RNA extraction kit (Invitrogen) was used to extract the total RNA. The first-stranded cDNA was synthesized by using the reverse transcription system (Thermo). The primer

sequences for analysis were as follows: RT-KIF21A-F: ACTTCCTGTCAATGGGCATCAAT and RT-KIF21A-R: TATTAGCCA-CATTTTTATCTCCCTC; RT-TGFBR2-F: AAACAGTTTCACTTTCCTGTCATC and RT-TGFBR2-R: TACACTGCCATCGCCTCTTT. GAPDH was used as an internal control.

2.6. Statistical analysis

Data are presented as the mean + standard error. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test using GraphPad Prism 9.0.0 (GraphPad Software, Inc.). The results were considered statistically significant as $*p < 0.05$, $**p < 0.01$.

3. Results

3.1. Clinical manifestations

Fig. 1 presents the genograms, while Table 1 provides a comprehensive overview of the clinical data. Six individuals within the family exhibited congenital cranial dysinnervation disorders (CCDDs), specifically Congenital Fibrosis of the Extraocular Muscles type 1 (CFEOM1). Notably, those harboring mutations denoted as III:3 and IV:2 were diagnosed with Keratoconus (KC), whereas individuals carrying mutations I:1 and II:1 were diagnosed with strabismus and ptosis during their lifetime (binoculus). The proband, a 37-year-old male (III:3), manifested symptoms of left ptosis, hypotropia, and nystagmus during childhood, eventually receiving a CFEOM1 diagnosis at 20 years of age. Subsequent to undergoing frontal muscle suspension and strabismus surgery at 22 years of age, he has been utilizing rigid gas permeable (RGP) lenses due to a KC diagnosis 4 years ago. Corneal topography assessments were conducted for family members, revealing thinner central corneas in the proband and his KC-affected daughter. This analysis led to the identification of one known variant in *KIF21A* and a novel variant in *TGFBR2* gene. Nonetheless, the patient's ocular motility restrictions, strabismus, and fixation disorder significantly influenced the corneal topography results. Despite challenges, the topographic map displayed a distinct image indicating thin central cornea and abnormal posterior surface height, thereby reinforcing the KC diagnosis (Supplementary Fig. 1).

Subject IV:2, a 7-year-old daughter of the proband, received a CFEOM1 diagnosis at the age of 2, displaying clinical features such as bilateral ptosis, hypotropia, esotropia, and nystagmus. At 4 years of age, she underwent binocular frontalis suspension and strabismus surgery, followed by a KC diagnosis a year later, warranting continuous monitoring. Ocular motility constraints, strabismus, and fixation disorder posed challenges during pentacam examination; however, the resultant topographic image highlighted an abnormal increase in the posterior corneal surface, supporting the KC diagnosis (Supplementary Fig. 2).

3.2. Identification and analyses of the two variants

Two single nucleotide polymorphisms (SNPs) were found in the proband and other patients in the family, consisting of a heterozygous missense variation, g.39726207C > T (c.2860C > T, p.R954W, rs121912585), in *KIF21A*, and a heterozygous missense

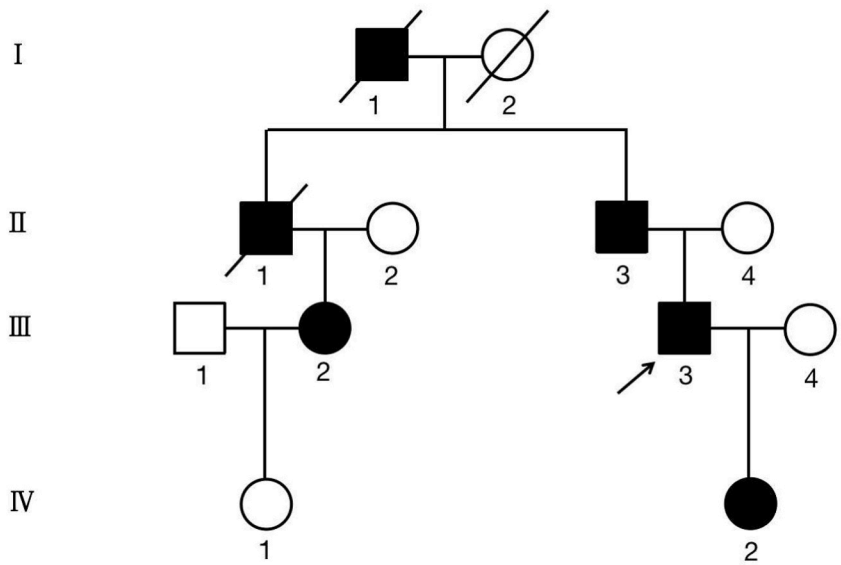

Fig. 1. The genogram of four generations of a Chinese family presented with CFEOM1 and KC. The squares indicate the males and the circles indicate the females in the family. The black symbols indicate individuals with CFEOM1. The white symbols indicate the unaffected members of the family. Subject III:3 in the family was the proband (arrow).

Table 1
The data of family members.

Family members	Gender/Age	Ptosis	Ocular alignment	Restriction of ocular movement		Other Eye Diseases	Genetic finding
				Horizontal	Vertical		
IV:1	F/11	–	–	–	–	–	–
IV:2	F/6	Bilateral	Esotropia, Hypotropia	Bilateral	Bilateral	Nystagmus, KC	KIF21A (p.R954W) TGFBR2 (p.S46R)
III:1	M/40	–	–	–	–	–	–
III:2	F/38	Bilateral	Bilateral Hypotropia	Bilateral	Bilateral	Nystagmus	KIF21A (p.R954W)
III:3	M/36	Left	Hypotropia	Bilateral	–	Nystagmus, KC	KIF21A (p.R954W) TGFBR2 (p.S46R)
III:4	F/35	–	–	–	–	–	–
II:1	M/pass away at 70 y	Bilateral	Hypotropia	Bilateral	–	–	NA
II:2	F/69	–	–	–	–	–	–
II:3	M/66	Bilateral	Esotropia, Hypotropia	Bilateral	Bilateral	Nystagmus	KIF21A (p.R954W)
II:4	F/65	–	–	–	–	–	–
I:1	M/pass away at 81y	Bilateral	Hypotropia	NA	NA	NA	NA
I:2	F/pass away at 79y	NA	NA	NA	NA	NA	NA

Notes: F, Female; M, Male.

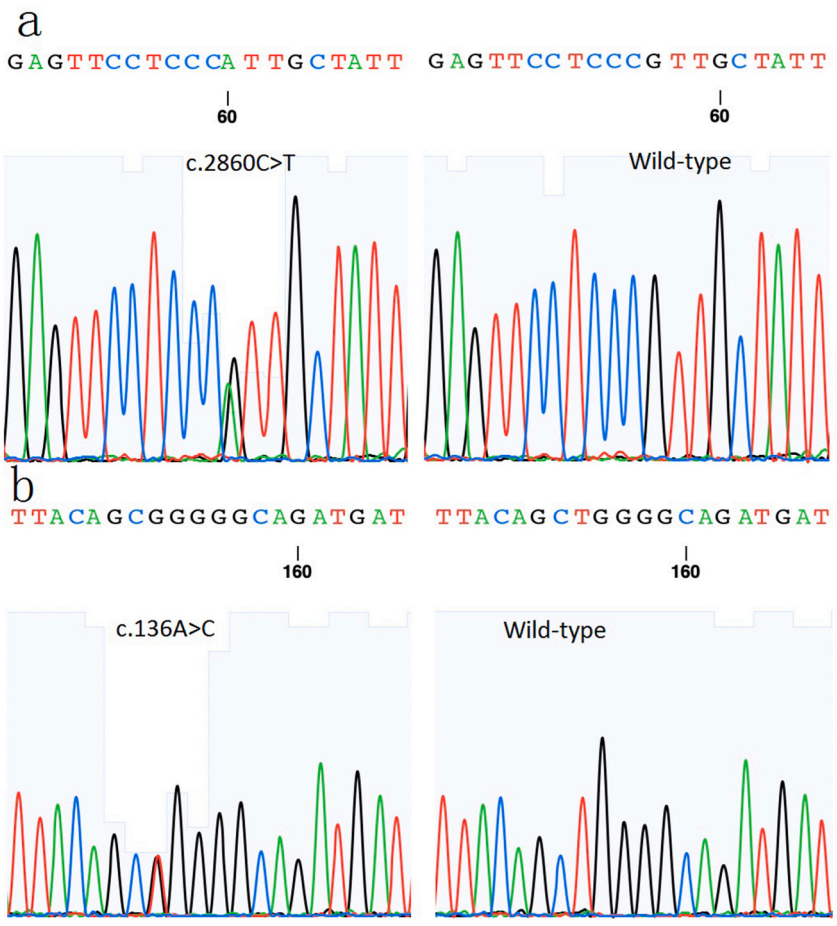

Fig. 2. a) A heterozygous missense variation g.39726207C > T (c.2860C > T, p.R954W, rs121912585) in *KIF21A*. **b)** A heterozygous missense variant (g.30664732A > C, c.136A > C, p.S46R, rs200111443) in *TGFBR2*.

variant, g.30664732A > C (c.136A > C, p.S46R, rs200111443), in *TGFBR2* (Fig. 2a and b). The mutation p.R954W in *KIF21A* was detected in all patients with CFEOM1 with or without KC in the family. The variant p.S46R in *TGFBR2* was only detected in two patients with KC (IV:2 and III:3) in the family. These variants were absent in healthy family members and the 100 controls. The variant p.R954W in *KIF21A* was likely pathogenic according to the analysis performed using Polyphen2, SIFT, MutationTaster, FAVOR, and Fathmm-MKL. The combined annotation-dependent depletion (CADD) score was 33, which indicated "deleterious" mutations. According to the ACMG guidelines [10], the variant was predicted to be "likely pathogenic" (PS4+PM2_Supporting + PP3). The prevalence in the affected populations increased significantly compared with that in the controls according to the online database (PS4), whereas the frequency of the gnomAD population database was very low (PM2). Computational prediction tools such as the Panther classification system used for predicting the effects of the variant on the protein structure and function suggested that this variant was conserved over time (GERP++ score = 3.66) [11]. Therefore, the p.R954W mutation may exert a deleterious effect (PP3). The variant p.S46R in *TGFBR2* was predicted to be pathogenic using SIFT and FATHMM. Uncertain significance (VUS) was predicted according to the ACMG guidelines (PM2_Supporting PP3), which had been reported in the Human Gene Mutation Database (HGMD), and the rate in the gnomAD population databases was extremely low (PM2) (Table 2). Moreover, the 3D modeling of a wild-type protein, p.R954W in *KIF21A*, and p.S46R in *TGFBR2* clearly showed the conformational changes caused by the mutations (Fig. 3a and b).

3.3. Topological analysis of the PPI network

Following a thorough exploration within the STRING database, we identified 11 genes, including TGFBR2, each possessing association scores surpassing 0.99 (https://cn.string-db.org/cgi/network?taskId=biteemii8gQ4&sessionId=bWEtAUcE4Xam, Supplementary Fig. 3). Subsequently, these 11 genes underwent meticulous functional and signaling pathway enrichment analyses by utilizing the GO database. The outcomes, featured in Barplot Supplementary Fig. 4, delineated the top 20 pathways, which notably encompassed the BMP signaling pathway and positively regulated the epithelial-to-mesenchymal transition, transforming growth factor beta receptor binding, and SMAD protein signal transduction. Supplementary Fig. 5 provides an exhaustive breakdown of Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) analyses. Noteworthy BPs included protein targeting to activin receptor signaling pathway, SMAD protein signal transduction, and transforming growth factor beta receptor signaling pathway. CCs comprised SMAD protein complex, receptor complex, transcription regulator complex, and collagen-containing extracellular matrix. Primary MFs included transforming growth factor beta receptor binding, I-SMAD binding, and transforming growth factor beta binding.

To further elucidate the interplay among these significantly enriched genes, we uploaded them to STRING, thereby facilitating the establishment of the Protein-Protein Interaction (PPI) network for TGFBR2. This resultant PPI network was subsequently imported into Cytoscape to construct sub-networks. As shown in Fig. 4a and b, within the PPI network, TGFBR2, distinguished by a maximum score of 20, is conspicuously highlighted in red, occupying a central position in the network.

3.4. qRT-PCR

The qRT-PCR results of these mutation carriers (III-3 and IV-2) and controls (II-4 and III-4) are presented as the mean +standard error (**$P < 0.01$). The qRT-PCR results showed that mRNA levels of *TGFBR2* in the patients were lower than those in the controls (Supplementary Fig. 6a). However, *KIF21A* mRNA levels decreased significantly in the patient IV2 (Supplementary Fig. 6b). These results suggested that *TGFBR2* variants could reduce its mRNA level in the patients. Moreover, the qRT-PCR results showed that the mutation carriers (III-3 and IV-2) in the family exhibited distinct *KIF21A* mRNA levels.

4. Discussion

Heterozygous missense variants in *KIF21A* have been identified as the primary cause of CFEOM1 [12]. CFEOM1 is characterized by bilateral external ophthalmoplegia and droopy eyelids and is unrelated to any additional neurologic abnormalities [2].

KIF21A comprises an amino-terminal motor domain, a middle stalk, and a carboxy-terminal tail domain (Fig. 5a). The motor domain, which can bind to microtubules, is highly conservative. KIFs can be differentiated based on their tail domains, which normally contain a site where cargo proteins can be loaded via an adaptor/scaffold protein or a protein complex [13]. The stalk region contains α-helical coiled-coil repeats, which can flexibly connect the motor domain with the tail domain. Normally, the stalk-region repeats near the motor domain of kinesins are the locus for the homo- or heterodimerization of KIF, which can allow two KIF motor domains to

Table 2
Identification and analysis of the novel variants.

GENE	Mutation	Phenotype	Conservation	Polyphen2 prediction	SIFT Prediction	Mutation Taster Prediction	Fathmm-MKL	ACMG guidelines
KIF21A	c.2860C > T (rs121912585)	Nonsynonymous (p.R954W)	HC	Possibly damaging	Deleterious	Disease causing	Deleterious	Likely Pathogenic
TGFBR2	c.136A > C (rs200111443)	Nonsynonymous (p.S46R)	HC	Benign	Deleterious	–	Deleterious	Uncertain significance (VUS)

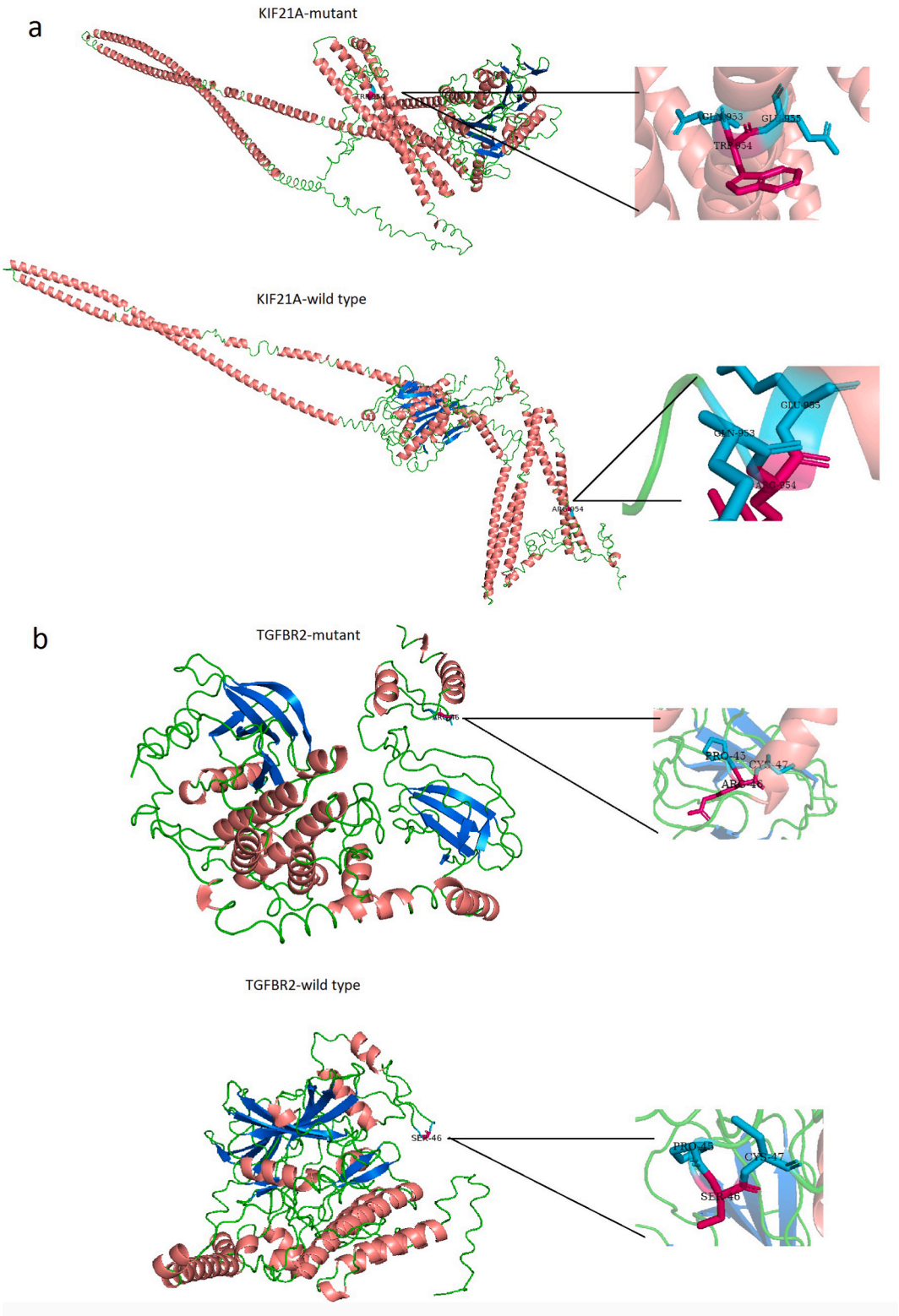

Fig. 3. Three-dimensional structures of the proteins indicating the sites of variants. The inset pictures are regional enlargement of the variants. **a)** Three-dimensional modeling of wild-type *KIF21A* and its p.R954W mutant. **b)** Three-dimensional modeling of wild-type *TGFBR2* and its p.S46R mutant.

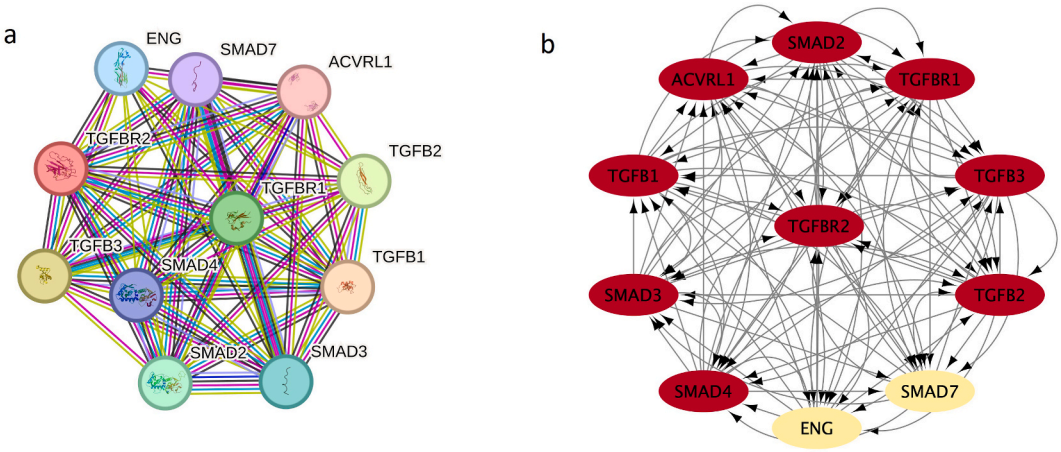

Fig. 4. a) The network of proteins interaction built by STRING; **b)** The network of PPI created with Cytoscape.

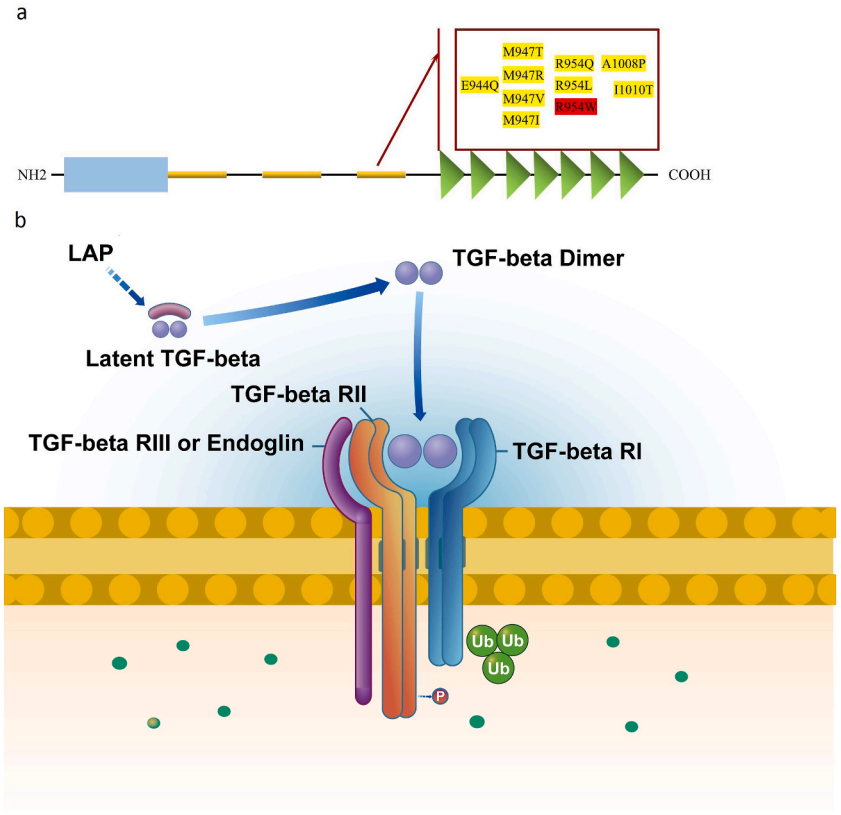

Fig. 5. a) The structure of the KIF21A protein. A total of five residues of amino acid in the third coiled-coil domain of KIF21A was previously reported in association with CFEOM1, including E944, M947, R954, A1008, and I1010. p.R954W in KIF21A located in the third coiled-coil domain. **b)** The receptor complex includes two TGFBR1 and two TGFBR2 molecules.

move to microtubules. Additionally, the coiled-coil domains toward the C terminal of some KIFs interact with cargo proteins [14]. Amino acids in this domain are typically internalized and can stabilize the dimeric structure. A study has shown that *KIF21A* variants can lead to CFEOM1 by decreasing the intramolecular interaction between the motor and the third coiled-coil regions of KIF21A both *in vivo* and *in vitro*, which is critical to auto-inhibition, thereby increasing interactions with microtubules [9].

Five amino acid residues, namely E944, M947, R954, A1008, and I1010, in the third coiled-coil domain of the KIF21A stalk are associated with CFEOM1, which may normally modulate the protein structure and interaction [15]. Previous studies have suggested that R954, the highly conserved and positively charged arginine residue, is an important site for KIF21A function. Herein, we

re-confirmed one of the reported "hotspot" CFEOM1 mutations in *KIF21A* using samples from four alive members in the four generations of the family, including the proband. The results showed a heterozygous missense variation, g.39726207C > T (c.2860C > T, p.R954W, rs121912585) located in the coiled-coil region of *KIF21A*, which can result in C-domain disfunction. We found that the mutation was predicted as pathogenic and deleterious based on Polyphen2, SIFT, mutation Taster, and Fathmm MKL databases. Thus, *KIF21A*-R954W mutation probably causes CFEOM1 by failing in effectively transferring the cargo proteins to the development of the neuromuscular junction and extraocular muscles. Past research has demonstrated that this mutation is located in the CpG islands and is associated with methylation, which causes genetic diseases in humans [13]. Combining this result with the published data, c.2860C > T in *KIF21A* was likely to induce CFEOM1 in the family members. The qRT-PCR results showed that the mutation carriers (III-3 and IV-2) in the family exhibited distinctive *KIF21A* mRNA levels. The observed discrepancy in *KIF21A* expressions between individuals III-3 and IV-2 may be attributed to variations in the severity of Congenital Fibrosis of the Extraocular Muscles type 1 (CFEOM1). Notably, III-3, exhibiting comparable *KIF21A* expression to the controls, manifests only left ptosis, whereas IV-2, with lower *KIF21A* expression, presents with bilateral ptosis. This difference in clinical severity suggests a plausible correlation between *KIF21A* expressions and the severity of CFEOM1 phenotypes. Furthermore, environmental exposures unique to each individual could contribute to epigenetic changes that may, in turn, influence the *KIF21A* expression. This proposition is particularly relevant as the observed mutation is situated within the CpG islands, and its association with methylation has been documented [13]. Considering that DNA methylation is a reversible process [16], it is conceivable that distinct environmental factors could induce epigenetic modifications, leading to compensatory changes in the *KIF21A* expression in individual III-3. However, due to the limited sample size in the patient group, the availability of an insufficient number of biological replicates hinders the execution of a robust statistical analysis for the assessment of *KIF21A* expression. Consequently, the preliminary outcomes derived from the comparison between each patient and the two controls do not provide a sufficient basis for drawing definitive conclusions. Therefore, any interpretation should be approached with caution.

The network of protein-protein interactions generated by STRING and visualized in Cytoscape provides a holistic view of the relationships among TGFBR2-related proteins. TGF-beta receptor type-2 (*TGFBR2*) encodes a transmembrane protein that has a protein kinase domain, which can form a hetero-dimeric complex with the other receptor protein (*TGFBR1*) to bind to TGF-beta. The receptor complex, which comprises two *TGFBR1* and two *TGFBR2* molecules, symmetrically binds to cytokine dimers to phosphorylate and activate *TGFBR1* and *TGFBR2* (Fig. 5b). Subsequently, activated *TGFBR1* phosphorylates *SMAD2*, and *SMAD2* dissociates from the receptor to interact with *SMAD4*. The *SMAD2–SMAD4* complex then translocates to the cell nucleus where it controls the transcription of TGF-β-regulated genes, thereby arresting the cell cycle in the corneal epithelium, corneal cell differentiation, wound healing, extracellular matrix production, and immunosuppression [17]. This constitutes the canonical SMAD-dependent TGF-β signaling cascade, which plays a crucial role in the cornea. This is also involved in a non-canonical process called the SMAD-independent TGF-β signaling pathway.

TGFBR2 belongs to the TGF-β receptor family that can secrete signaling molecules. These receptors contain a signal peptide (1-22aa), an extracellular region that binds ligands (23-166aa), a single transmembrane region (167-187aa), and a cytoplasmic catalytic kinase region (188-567aa). Type II receptors, such as *TGFBR2*, are high-affinity receptors that can bind to ligands to control autophosphorylation and *trans*-phosphorylation to activate low-affinity type I receptors. *TGFBR2* acts as a receptor for TGF-β, which is crucial to control growth and homeostasis in diverse tissues, including the corneal epithelium and limbal epithelial stem cells. It plays a role in regulating cell apoptosis and in maintaining the balance between self-renewal and cell loss.

Recently, genomic and RNA-seq studies showed that the dysfunction of TGF-β signaling might be associated with the etiology and pathogenesis of CE [18,19]. In 2023, Wang et al. [17] indicated that *TGFBR2* deficiency in keratocytes resulted in CE. Herein, a heterozygous missense variant p.S46R in *TGFBR2* was only detected in two members with KC (IV:2 and III:3) and were not detected in the healthy family members and the 100 controls. Based on the results obtained with the prediction tools described earlier, variant p.S46R in *TGFBR2* was predicted to be pathogenic. Moreover, the 3D modeling of the wild-type protein and p.S46R in *TGFBR2* mutations clearly presented the conformational changes. Thus, the variant p.S46R in *TGFBR2* might have significantly increased the risk of corneal abnormality. The qRT-PCR results also indicated that *TGFBR2* mRNA levels in the patients, including those with IV2 and III3, were lower than those with KC and unaffected family members, suggesting that variant p.S46R in *TGFBR2* might exert an effect on *TGFBR2* expression, resulting in the loss of TGFBR2 in the patients. TGFBR2 deficiency can affect the TGFB signaling pathway and thereby potentially alter the extracellular matrix (ECM) and the development of fibrotic phenotype in KC.

Although there is currently a lack of population data supporting a direct link between CFEOM1 and an increased risk of corneal abnormalities, existing literature indicates a correlation between *KIF21A*-associated CFEOM1 and the presence of ptosis [9]. The literature further suggests that ptosis, especially when severe, can introduce asymmetry and irregularities in the cornea, thereby potentially posing a risk for the development of keratoconus [20,21]. It is widely recognized that the etiology of keratoconus involves a combination of genetic and environmental factors, with environmental influences such as eye rubbing due to ptosis potentially triggering keratoconus in genetically susceptible individuals [22,23]. Consequently, the combination of CFEOM1, genetic mutations, and associated involuntary eye rubbing may significantly elevate the risk of corneal damage, impede wound healing, and consequently enhance the likelihood of corneal abnormalities, particularly keratoconus.

In summary, the present study identified a known variant in *KIF21A* and a novel variant in *TGFBR2*. While bioinformatic analysis and family-based studies suggest that these variants are likely causal for CFEOM1 and keratoconus, functional validation is imperative to confirm their pathogenicity in future investigations. The study underscores the importance of conducting comprehensive corneal topography assessments in patients with CCDDs, including CFEOM1, for early detection of corneal abnormalities before clinical manifestation. The findings emphasize that genetic abnormalities may significantly augment the risk of corneal abnormalities, notably keratoconus.

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Data availability statement

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

CRediT authorship contribution statement

Qinghong Lin: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Xuejun Wang:** Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Xin Zhan:** Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Xiaoliao Peng:** Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Yan Wang:** Software, Data curation. **Xingtao Zhou:** Writing – review & editing, Software, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28036.

References

[1] N.J. Gutowski, T.M. Bosley, E.C. Engle, 110th ENMC International Workshop: the congenital cranial dysinnervation disorders (CCDDs). Naarden, The Netherlands, 25-27 October, 2002, Neuromuscul. Disord. 13 (2003) 573–578.
[2] J.K. Chilton, S. Guthrie, Axons get ahead: Insights into axon guidance and congenital cranial dysinnervation disorders, Dev Neurobiol 77 (2017) 861–875.
[3] S. MacKinnon, D.T. Oystreck, C. Andrews, W.M. Chan, D.G. Hunter, E.C. Engle, Diagnostic distinctions and genetic analysis of patients diagnosed with moebius syndrome, Ophthalmology 121 (2014) 1461–1468.
[4] M.C. Whitman, B.J. Barry, C.D. Robson, F.M. Facio, C. Van Ryzin, W.M. Chan, et al., TUBB3 Arg262His causes a recognizable syndrome including CFEOM3, facial palsy, joint contractures, and early-onset peripheral neuropathy, Hum. Genet. 140 (2021) 1709–1731.
[5] D.M. Nowak, M. Gajecka, The genetics of keratoconus. Middle East, Afr J Ophthalmol 18 (2011) 2–6.
[6] S.S. Rong, S.T.U. Ma, X.T. Yu, L. Ma, W.K. Chu, T.C.Y. Chan, et al., Genetic associations for keratoconus: a systematic review and meta-analysis, Sci. Rep. 7 (2017) 4620.
[7] Y. Bykhovskaya, B. Margines, Y.S. Rabinowitz, Genetics in Keratoconus: where are we? Eye Vis (Lond) 3 (2016) 16.
[8] E. Loukovitis, K. Sfakianakis, P. Syrmakesi, E. Tsotridou, M. Orfanidou, D.R. Bakaloudi, et al., Genetic aspects of keratoconus: a literature review exploring potential genetic contributions and possible genetic relationships with comorbidities, Ophthalmol Ther 7 (2018) 263–292.
[9] M. Chen, R. Huang, Y. Zhang, D.J. Zhu, Q. Shu, P. Xun, et al., Phenotype, genotype, and management of congenital fibrosis of extraocular muscles type 1 in 16 Chinese families, Graefes Arch. Clin. Exp. Ophthalmol. 261 (2023) 879–889.
[10] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (2015) 405–424.
[11] Pantherdb.org. Available online: http://www.pantherdb.org/tools/csnpScore.do (accessed on 2 December 2022).
[12] S. Bianchi, W.E. van Riel, S.H. Kraatz, N. Olieric, D. Frey, E.A. Katrukha, et al., Structural basis for misregulation of kinesin KIF21A autoinhibition by CFEOM1 disease mutations, Sci. Rep. 6 (2016) 30668.
[13] M. Ali, C. Venkatesh, A. Ragunath, A. Kumar, Mutation analysis of the KIF21A gene in an Indian family with CFEOM1: implication of CpG methylation for most frequent mutations, Ophthalmic Genet. 25 (2004) 247–255.
[14] L.K. Lin, Y.H. Chien, J.Y. Wu, A.H. Wang, S.C. Chiang, W.L. Hwu, KIF21A gene c.2860C>T mutation in congenital fibrosis of extraocular muscles type 1 and 3, Mol. Vis. 11 (2005) 245–248.
[15] W.M. Chan, C. Andrews, L. Dragan, D. Fredrick, L. Armstrong, C. Lyons, et al., Three novel mutations in KIF21A highlight the importance of the third coiled-coil stalk domain in the etiology of CFEOM1, BMC Genet. 8 (2007) 26.
[16] S. Ramchandani, S.K. Bhattacharya, N. Cervoni, M. Szyf, DNA methylation is a reversible biological signal, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6107–6112.
[17] Y.C. Wang, O.B. Zolnik, S. Yasoda, L.K. Yeh, Y. Yuan, W. Kao, et al., Transforming growth factor beta receptor 2 (Tgfbr2) deficiency in keratocytes results in corneal ectasia, Ocul. Surf. 29 (2023) 557–565.

[18] L.M. Ittner, H. Wurdak, K. Schwerdtfeger, T. Kunz, F. Ille, P. Leveen, et al., Compound developmental eye disorders following inactivation of TGFbeta signaling in neural-crest stem cells, J. Biol. 4 (2005) 11.
[19] A.I. Iglesias, A. Mishra, V. Vitart, Y. Bykhovskaya, R. Höhn, H. Springelkamp, et al., Cross-ancestry genome-wide association analysis of corneal thickness strengthens link between complex and Mendelian eye diseases, Nat. Commun. 9 (2018) 1864.
[20] T. Zhu, X. Ye, P. Xu, J. Wang, H. Zhang, H. Ni, et al., Changes of corneal tomography in patients with congenital blepharoptosis, Sci. Rep. 7 (2017) 6580.
[21] T. Kim, B. Khosla-Gupta, C. Debacker, Blepharoptosis-induced superior keratoconus, Am. J. Ophthalmol. 130 (2000) 232–234.
[22] J. Sugar, M.S. Macsai, What causes keratoconus? Cornea 31 (2012) 716–719.
[23] S. Sahebjada, H.H. Al-Mahrouqi, S. Moshegov, S.M. Panchatcharam, E. Chan, M. Daniell, et al., Eye rubbing in the aetiology of keratoconus: a systematic review and meta-analysis, Graefes Arch. Clin. Exp. Ophthalmol. 259 (2021) 2057–2067.