Heliyon 10 (2024) e32506

Contents lists available at ScienceDirect

Heliyon

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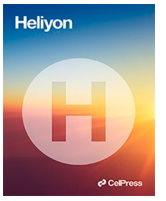

Case report

# Brittle cornea syndrome: A novel mutation

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ARTICLE INFO

*Keywords:*
Brittle cornea syndrome
Corneal opacity
Cornea
Impaired vision

ABSTRACT

*Purpose:* To report the clinical, tomographic, histopathological and genetic findings of a patient with brittle cornea syndrome and a novel mutation in the *ZNF469* gene likely implicated in the development of this disorder.

*Methods:* A 64-year-old man presented with a two-year history of worsening vision in both eyes. The patient and his son were examined by imaging and genetic analysis.

*Results:* The patient exhibited persistent ocular irritation, decreased vision, corneal epithelial defects and corneal stromal opacity. Confocal microscopy revealed that the anterior corneal stroma had a large amount of highly reflective and striated tissue. However, his son had no symptoms. Genetic analysis identified a heterozygous c.1781C > T:p.P594L variation in the *ZNF469* gene.

*Conclusions:* We reported a novel mutation in the *ZNF469* gene (c.1781C > T:p.P594L) in a patient with brittle cornea syndrome from China, which enriched the spectrum of *ZNF469* variants implicated in brittle cornea syndrome.

## 1. Introduction

Brittle cornea syndrome (BCS) is an autosomal recessive connective tissue disorder [1]. BCS often manifests as corneal thinning and fragility, leading to corneal rupture [2]. Mutation of the *ZNF469* gene leads to a decrease in collagen I (COL-I) expression and structural changes, which results in corneal stroma dysfunction and ultimately a decrease in corneal biomechanical strength [3,4]. Diagnosis of the disease mainly depends on the characteristic manifestations [5] or genetic analysis [6,7].

The first case of BCS was reported by Ticho U in 1968 [8]. To our knowledge, there are 69 reports in the literature on BCS; 33 mutations in the *ZNF469* gene and 15 mutations in the PRDM5 gene have been reported in these studies (Table 1). Here, we reported a novel mutation in the*ZNF469* gene (c.1781C > T:p.P594L) in BCS patient from China. We described the clinical appearances, imaging presentations, and histopathological and genetic findings of the patient.

## 2. Case report

A 64-year-old man presented with a complaint of red eyes, ocular irritation and decreased vision for 2 years at our ophthalmology clinic in December 2019. The patient was of Han Chinese ethnicity. His best corrected visual acuity (BCVA) was 0.5 (Snellen decimal conversion) with −1.00 dioptre sphere (DS) and −1.50 dioptre cylindering (DC) x 90° in the right eye (R) and 0.4 with −0.25 DS and −1.25 DC x 60° in the left eye (L). In 2022, the patient returned for follow-up due to further visual decline. At that time, the patient's

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https://doi.org/10.1016/j.heliyon.2024.e32506
Received 25 January 2024; Received in revised form 1 June 2024; Accepted 5 June 2024
Available online 6 June 2024

vision was 0.3 in the right eye and 0.04 in the left eye. In addition, corrected vision did not improve. There was no history of glaucoma, diabetes, hypertension or other immunodeficiency diseases. According to the patient, his grandmother, who had passed away, also had poor eyesight, but the exact cause was not known. His parents, sibling and other members of the family were all native Chinese and had no abnormalities of the eyes. The patient's family history revealed no consanguineous marriage and no known genetic disorders.

Mild conjunctival congestion (Fig. 1A), a rougher and partially defective corneal epithelium with patchy white deposits (Fig. 1B and D), and anterior corneal stroma opacity under a slit lamp (Fig. 1C) were observed. Anterior segment optical coherence tomography (AS-OCT) revealed a corneal thickness of approximately 550 μm and high reflected signal (Fig. 1I). On the basis of these clinical manifestations, confocal microscopy was performed, which revealed that the anterior corneal stroma had a large amount of highly reflective and striated tissue that was interwoven into a web or radial pattern. There was no obvious inflammatory cell infiltration in the cornea (Fig. 1J). The clinical manifestations were similar in both eyes. On the basis of the above results, our medical team initially

**Table 1**
Summary of *ZNF469* and *PRDM5* mutations in BCS patients reported to date.

| Gene | Origin | c-Notation | p-Notation | Patients | Ref |
|---|---|---|---|---|---|
| *ZNF469* | China | c.6727del | p.Asp2243Thr fs*8 | 3 | [17] |
| *ZNF469* | Mexico | c.2340delC | p.Arg781Glufs*19 | 3 | [10] |
| *ZNF469* | No | c:5716C > T | p.Arg 1906 | 3 | [12] |
| | | c.7220 del | p.Gln2407Arg fs*38 | | |
| *ZNF469* | Spain | c.2972 del | p.Pro991Hisfs62 | 4 | [20] |
| *ZNF469* | Belgium | c.1444delC | p.Leu482Cysfs*20 | 3 | [21] |
| *ZNF469* | Belgium | c.9876dupT | p.Ala3293Cysfs*6 | 1 | [21] |
| *ZNF469* | India | c.1081delG | p.Ala361Leufs*16 | 2 | [21] |
| | | c.1586delG | p.Gly529Aspfs*9 | | |
| *ZNF469* | Syria | c.3307C > T | p.Gln 1103* | 1 | [21] |
| *ZNF469* | No | c.10664delC | p.Pro3556Glnfs*136 | 3 | [6] |
| | | c.10240 delA | p.Arg3414Glyfs*59 | | |
| *ZNF469* | Czech/Polish | c.1402_1411del | p.Pro468Alafs*31 | 1 | [18] |
| | | c.1705C > T | P.Gln 569* | | |
| *ZNF469* | No | c.1963dupC | p.His655Profs*83 | 1 | [22] |
| | | c.6444dupG | p.Gln2149Alafs*42 | | |
| *ZNF469* | Pakistan | c.9831dupC | p.Arg3278GlnfsX197 | 4 | [23] |
| *ZNF469* | Saudi | c.8817_8830dup | p.E2944Gfs*50 | 1 | [1] |
| *ZNF469* | India | c.3476delG | p.Gly1159Alafs*105 | 1 | [19] |
| *ZNF469* | Britain | c.5788delC | p.Gln1930Argfs*6 | 1 | [19] |
| | | c.5788dupC | p.Gln1930Profs*133 | | |
| *ZNF469* | Pakistan | c.6444delG | p.Gln2149Serfs*51 | 1 | [19] |
| *ZNF469* | Syria | c.4174G > T | p.E1392X | 4 | [24] |
| *ZNF469* | Norway | c.10016G > A | p.Cys3339Tyr | 2 | [25] |
| *ZNF469* | Palestine | c.6027 delA | p.Gly2011Alafs*16 | 2 | [13] |
| *ZNF469* | Palestine | c.9615delG | p.Gln3206Argfs*23 | 6 | [13] |
| *ZNNF49* | Syria | c.6647 delA | p.Gln2216Argfs*19 | 2 | [19] |
| *ZNF469* | Saudi | c.8901_8914dup | p.Glu2972Glyfs*50 | 5 | [26] |
| *ZNF469* | Yemen | c.3304G > T | p.Glu 1102* | 1 | [19] |
| *ZNF469* | Saudi | c.5353C > T | p.Gln 1785* | 3 | [19] |
| *ZNF469* | Saudi | c.2029G > T | p.Gly 677* | 2 | [19] |
| *ZNF469* | Saudi | c.2150delT | p.Phe717Serfs*15 | 2 | [19] |
| *ZNF469* | Saudi | c.9483delG | p.His3162Thrfs*20 | 1 | [19] |
| *PRDM5* | Laos | c.1117_1123del | p.Asp373Phefs*57. | 3 | [27] |
| *PRDM5* | Albania | c.974delG | p.Cys325LeufsX2 | 3 | [28] |
| *PRDM5* | Pakistan | c.1768C > T | p. (Arg 590*) | 4 | [14] |
| *PRDM5* | Pakistan | c.1517_1527del | p. (Val506Glufs*5) | 2 | [14] |
| *PRDM5* | Pakistan | c.974delG | p. (Cys325Leufs*2) | 3 | [14] |
| *PRDM5* | Pakistan | c.713_716del | p. (Val238Alafs*35) | 1 | [14] |
| *PRDM5* | Pakistan | c.320A > G | p. (Tyr107Cys) | 2 | [14] |
| *PRDM5* | Pakistan | c.93+1G > A | | 1 | [14] |
| *PRDM5* | No | c.400G > T | p. (Glu 134*) | 1 | [29] |
| *PRDM5* | Pakistan | c.93+5G > A | | 4 | [30] |
| *PRDM5* | Saudi | c.93+2T > C | | 2 | [31] |
| *PRDM5* | No | c.247C > T | p. (Arg83Cys) | 3 | [32] |
| *PRDM5* | India | c.17T > G | p. (Val6Gly) | 2 | [21] |
| *PRDM5* | Caucasia | c.106G > A | p. (Gly36Arg) | 1 | [21] |
| *PRDM5* | India | c.1858delC | p. (His620Thrfs*8) | 1 | [21] |

No: Not available; Ref: reference.

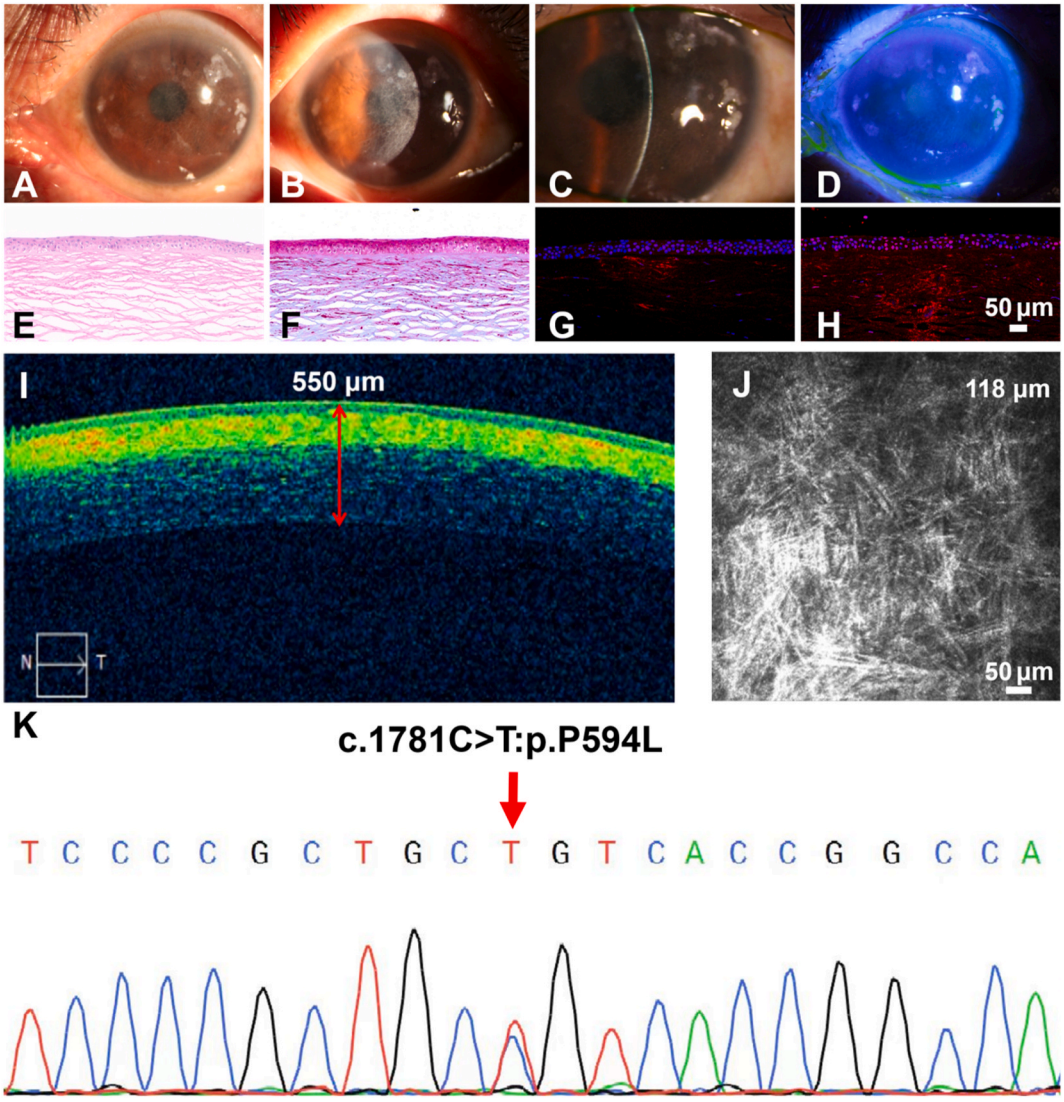


**Fig. 1.** Images of the anterior segment of the patient's left eye (A, B, C) and results of sodium fluorescein staining (D); A, B, D (10 × ), C (16 × ). Images of hematoxylin eosin staining (E) and Masson staining (F). Images of immunofluorescence staining of COL-I (G) and COL-III (H). AS-OCT (I) and confocal microscopy (J) images. DNA sequence chromatograms of the mutation in the *ZNF469* gene of the patient (K).

suspected a type of corneal dystrophy.

With the informed consent of the patient and his family, we drew the blood of the patient and his son for genetic testing to assist in the diagnosis. Then, a DNA library was constructed. Exons and adjacent splice regions (20 bp) of the target genes were captured by probe hybridization and sequenced *via* high-throughput sequencing. The relevant data were compared with the HG38 human reference sequence, and the variants were identified by GATK's Haplotype Caller. The criteria for the interpretation of the number of sequence variants were taken from the American College of Medical Genetics and Genomics (ACMG) Genetic Variation Classification Criteria and Guidelines [9]. A heterozygous c.1781C > T:p.P594L mutation in the *ZNF469* gene was detected in the patient, while this mutation was not detected in his son (Fig. 1K). The frequency of this mutation in the gnomAD database for the East Asian general population was 0.00041.

Penetrating keratoplasty was performed in the left eye to improve visual acuity. The corneal tissue was subsequently examined by histopathology. Hematoxylin-eosin (HE) staining revealed abnormal, disorganized collagen fibre accumulation in the corneal stroma (Fig. 1E). Masson staining revealed that the amount of collagen fibres was clearly reduced (Fig. 1F). Immunofluorescence histochemical staining revealed a decrease in COL-I but an increase in collagen III (COL-III) (Fig. 1G and H). After surgery, the patient received routine treatment to prevent infection and corneal graft rejection. The patient had a visual acuity of 0.2 in the left eye upon discharge.

## 3. Discussion and conclusion

BCS patients often present with a thin, spherical cornea and scleral discolouration [10,11]. In addition, some patients may be complicated with other systemic symptoms, such as frequent fractures and excessive flexion and extension of the joints [5,10,12]. Early diagnosis of BCS mainly depends on gene detection because of its occult onset. Currently, the mainstream view is that BCS is mainly related to mutations in *ZNF469* and *PRDM5* [13,14]. The *ZNF469* gene plays an important role in the synthesis of extracellular matrix (ECM). As a widely considered transcription factor, *ZNF469* regulates the synthesis and degradation of ECM. *ZNF469* mutations lead to a reduction in the synthesis of various ECM components, which reduces the thickness of the corneal stroma and cause significant thinning of the cornea [3,4]. However, our patient did not show significant thinning of the corneal stroma, which may be related to the heterozygous mutation status. This finding is consistent with reports in the literature. Homozygous mutations often result in corneal thinning, while patients with heterozygous mutations can exhibit normal corneal thickness [15].

In China, Wan et al. reported the case of a 6-year-old girl with blue sclera and extreme corneal thinning with anterior corneal protrusion who was diagnosed with BCS [16]. Wang et al. reported a 36-year-old Chinese female patient presented with significant bluish discolouration of the sclera in both eyes, extreme corneal thinning with increased corneal curvature, increased central corneal densitometry, scoliosis, severe osteoporosis, and thyroid disease [11]. Neither of these studies conducted further analyses of the types of mutations that might be present. Recently, Zhou et al. reported a homozygous *ZNF469* gene mutation (c.6727del) in a patient with BCS who presented with typical corneal thinning and joint hypermobility [17]. However, this present case report is the first to describe BCS-related heterozygous *ZNF469* gene mutation sites in China. However, we did not find any abnormalities other than corneal opacity. Since the patient's corneal thickness was normal, the corneal epithelium was not smooth and exhibited small local protrusions. Therefore, we did not perform corneal topography, which is a limitation of our research. Heterozygous gene mutations or different mutation sites may lead to varying degrees of tissue affects. In one study, both sisters of the proband had heterozygous mutations, but no ocular abnormalities were observed [18].

In addition to ocular signs such as decreased visual acuity, corneal fragility, and bluish sclera, BCS often manifests as widespread fragility of connective tissue throughout the body. Related studies have reported that BCS patients also exhibit joint hypermobility, susceptibility to fracture, joint dysplasia, and spinal deformities [12,19]. In this study, we examined the expression of corneal COL-I and COL-III in the BCS patient with a heterozygous *ZNF469* gene mutation. We observed abnormal corneal collagen synthesis without joint hyperplasia. The patient was admitted to the Department of Orthopedics Clinic in 2018 with "low back pain for 2 days", but no X-ray, computed tomography (CT) or magnetic resonance imaging (MRI) examination was performed. The detailed medical history was missing due to the COVID-19 pandemic. Therefore, this article does not describe in detail the patient's systemic connective tissue which is another limitation. We will focus on the patient's cardiovascular system abnormalities and skeletal system abnormalities in a subsequent study.

In our study, a novel pathological variant of the *ZNF469* gene, c.1781C > T:p.P594L, was discovered. To our knowledge, this variant has not yet been reported. In addition, this study is the first to report BCS-related heterozygous *ZNF469* gene mutations in East Asia. The results of this study broaden the mutation spectrum of BCS-related *ZNF469* gene mutations.

## Data availability statement

No applicable.

## CRediT authorship contribution statement

**Xingchen Geng:** Writing – original draft, Methodology, Investigation. **Lei Zhu:** Writing – review & editing. **Jingguo Li:** Writing – review & editing, Validation, Investigation. **Zhanrong Li:** Writing – review & editing, Validation, Funding acquisition.

## 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.

## Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (82371108 and 52173143), the Natural Science Foundation of Henan Province of China (242300421018 and 232300421176) and Basic Science Key Project of Henan Eye Hospital (23JCZD003).

## References

[1] H. Al-Hussain, S.M. Zeisberger, P.R. Huber, C. Giunta, B. Steinmann, Brittle cornea syndrome and its delineation from the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI): report on 23 patients and review of the literature, Am. J. Med. Genet. A 124A (2004) 28–34.

[2] S.A. Joshi, S. Uppapalli, P. More, M. Deshpande, Unusual case of globe perforation: the brittle cornea without systemic manifestations, BMJ Case Rep. 2016 (2016).

[3] C.M. Stanton, A.S. Findlay, C. Drake, M.Z. Mustafa, P. Gautier, L. McKie, I.J. Jackson, V. Vitart, A mouse model of brittle cornea syndrome caused by mutation in Zfp469, Dis Model Mech 14 (2021).
[4] J. Bao, X. Yu, X. Ping, X. Shentu, J. Zou, Znf469 plays a critical role in regulating synthesis of ECM: a zebrafish model of brittle cornea syndrome, Invest. Ophthalmol. Vis. Sci. 64 (2023) 29.
[5] K. Mandlik, R.A. Betdur, R. Rashmita, S. Narayana, Brittle cornea syndrome: a tale of three brothers, Indian J. Ophthalmol. 70 (2022) 2594–2597.
[6] T. Rolvien, U. Kornak, S.J. Linke, M. Amling, R. Oheim, Whole-exome sequencing identifies novel compound heterozygous ZNF469 mutations in two siblings with mild brittle cornea syndrome, Calcif. Tissue Int. 107 (2020) 294–299.
[7] M. Akbari, M. Dourandeesh, Spontaneous rupture of an infant's cornea, J. Pediatr. Ophthalmol. Strabismus 59 (2022) e42–e45.
[8] R. Stein, M. Lazar, A. Adam, Brittle cornea. A familial trait associated with blue sclera, Am. J. Ophthalmol. 66 (1968) 67–69.
[9] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H.L. Rehm, 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.
[10] R. Arce-González, O.F. Chacon-Camacho, V. Ordoñez-Labastida, E.O. Graue-Hernandez, A. Navas-Pérez, J.C. Zenteno, A novel homozygous ZNF469 variant causing brittle cornea syndrome is associated with corneal ectasias in heterozygous carriers, Int. Ophthalmol. 43 (2023) 807–815.
[11] H. Wang, X. Zhang, X. Gao, W. Li, Characteristics of brittle cornea syndrome by multimodal imaging modalities: a case report, BMC Ophthalmol. 23 (2023) 378.
[12] T. Cundy, A. Vincent, S. Robertson, Does brittle cornea syndrome have a bone fragility phenotype, Bone Rep 15 (2021) 101124.
[13] A. Abu, M. Frydman, D. Marek, E. Pras, U. Nir, H. Reznik-Wolf, E. Pras, Deleterious mutations in the Zinc-Finger 469 gene cause brittle cornea syndrome, Am. J. Hum. Genet. 82 (2008) 1217–1222.
[14] E. Burkitt Wright, H.L. Spencer, S.B. Daly, F. Manson, L. Zeef, J. Urquhart, N. Zoppi, R. Bonshek, I. Tosounidis, M. Mohan, C. Madden, A. Dodds, K.E. Chandler, S. Banka, L. Au, J. Clayton-Smith, N. Khan, L.G. Biesecker, M. Wilson, M. Rohrbach, M. Colombi, C. Giunta, G. Black, Mutations in PRDM5 in brittle cornea syndrome identify a pathway regulating extracellular matrix development and maintenance, Am. J. Hum. Genet. 88 (2011) 767–777.
[15] A.E. Davidson, E. Borasio, P. Liskova, A.O. Khan, H. Hassan, M.E. Cheetham, V. Plagnol, F.S. Alkuraya, S.J. Tuft, A.J. Hardcastle, Brittle cornea syndrome ZNF469 mutation carrier phenotype and segregation analysis of rare ZNF469 variants in familial keratoconus, Invest. Ophthalmol. Vis. Sci. 56 (2015) 578–586.
[16] Q. Wan, J. Tang, Y. Han, Q. Xiao, Y. Deng, Brittle cornea syndrome: a case report and review of the literature, BMC Ophthalmol. 18 (2018) 252.
[17] Q. Lin, X. Wang, T. Han, X. Peng, X. Zhou, Variants in the ZNF469 gene in families with Brittle cornea syndrome and keratoconus, Heliyon 10 (2024) e27052.
[18] P. Skalicka, L.F. Porter, K. Brejchova, F. Malinka, L. Dudakova, P. Liskova, Brittle cornea syndrome: a systemic review of disease causing mutations in ZNF469 and two novel variants identified in a patient followed for 26 years, Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc. Czech. Repub. 164 (2020) 183–188.
[19] M. Rohrbach, H.L. Spencer, L.F. Porter, E.M. Burkitt-Wright, C. Bürer, A. Janecke, M. Bakshi, D. Sillence, H. Al-Hussain, M. Baumgartner, B. Steinmann, G. C. Black, F.D. Manson, C. Giunta, ZNF469 frequently mutated in the brittle cornea syndrome (BCS) is a single exon gene possibly regulating the expression of several extracellular matrix components, Mol. Genet. Metabol. 109 (2013) 289–295.
[20] G. García de Oteyza, J. Fernández Engroba, V. Charoenrook, New ZNF469 mutations in Spanish siblings with brittle cornea syndrome, Cornea 00 (2023) 1–5.
[21] T. Dhooge, T. Van Damme, D. Syx, L.M. Mosquera, S. Nampoothiri, A. Radhakrishnan, P.O. Simsek-Kiper, G.E. Utine, M. Bonduelle, I. Migeotte, O. Essawi, S. Ceylaner, A. Al Kindy, B. Tinkle, S. Symoens, F. Malfait, More than meets the eye: expanding and reviewing the clinical and mutational spectrum of brittle cornea syndrome, Hum. Mutat. 42 (2021) 711–730.
[22] J. Menzel-Severing, R. Meiller, C. Kraus, R. Trollmann, D. Atalay, Brittle cornea syndrome type 1 caused by compound heterozygosity of two mutations in the ZNF469 gene, Ophthalmol. Times 116 (2019) 780–784.
[23] S. Micheal, S.N. Siddiqui, S.N. Zafar, I.T. Gabriël a Niewold, M.I. Khan, A. Bergen, Identification of a novel ZNF469 mutation in a Pakistani family with brittle cornea syndrome, Cornea 38 (2019) 718–722.
[24] A.O. Khan, M.A. Aldahmesh, J.N. Mohamed, F.S. Alkuraya, Blue sclera with and without corneal fragility (brittle cornea syndrome) in a consanguineous family harboring ZNF469 mutation, Arch. Ophthalmol. 128 (2010) 1376–1379.
[25] A.E. Christensen, P.M. Knappskog, M. Midtbø, C.G. Gjesdal, J. Mengel-From, N. Morling, E. Rødahl, H. Boman, Brittle cornea syndrome associated with a missense mutation in the zinc-finger 469 gene, Invest. Ophthalmol. Vis. Sci. 51 (2010) 47–52.
[26] M. Al-Owain, M.S. Al-Dosari, A. Sunker, T. Shuaib, F.S. Alkuraya, Identification of a novel ZNF469 mutation in a large family with ehlers-danlos phenotype, Gene 511 (2012) 447–450.
[27] B.A. Sklar, P. Pisuchpen, M. Bareket, T. Milman, R.C. Eagle Jr., J. Minor, R. Procopio, J. Capasso, A.V. Levin, K. Hammersmith, Identification and management of a novel PRDM5 gene pathologic variant in a family with brittle cornea syndrome, Cornea 42 (2023) 1572–1577.
[28] C. Incandela, F. D'Oria, L. Lapenna, A. Acquaviva, Penetrating keratoplasty in brittle Cornea syndrome: case series and review of the literature, Eur. J. Ophthalmol. 34 (2024) 11–17.
[29] L.F. Porter, R. Gallego-Pinazo, C.L. Keeling, M. Kamieniorz, N. Zoppi, M. Colombi, C. Giunta, R. Bonshek, F.D. Manson, G.C. Black, Bruch's membrane abnormalities in PRDM5-related brittle cornea syndrome, Orphanet J. Rare Dis. 10 (2015) 145.
[30] S. Micheal, M.I. Khan, F. Islam, F. Akhtar, R. Qamar, M.J. Tassignon, B. Loeys, A.I. den Hollander, Identification of mutations in the PRDM5 gene in brittle cornea syndrome, Cornea 35 (2016) 853–859.
[31] M.A. Aldahmesh, J.Y. Mohamed, F.S. Alkuraya, A novel mutation in PRDM5 in brittle cornea syndrome, Clin. Genet. 81 (2012) 198–199.
[32] L.F. Porter, G.G. Galli, S. Williamson, J. Selley, D. Knight, N. Elcioglu, A. Aydin, M. Elcioglu, H. Venselaar, A.H. Lund, R. Bonshek, G.C. Black, F.D. Manson, A role for repressive complexes and H3K9 di-methylation in PRDM5-associated brittle cornea syndrome, Hum. Mol. Genet. 24 (2015) 6565–6579.