# More than meets the eye: expanding and reviewing the clinical and mutational spectrum of brittle cornea syndrome

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**Abstract**

Brittle cornea syndrome (BCS) is a rare autosomal recessive disorder characterized by corneal thinning and fragility, leading to corneal rupture, the main hallmark of this disorder. Non-ocular symptoms include hearing loss, but also signs of connective tissue fragility, placing it in the Ehlers-Danlos syndrome (EDS) spectrum. It is caused by biallelic pathogenic variants in ZNF469 or PRDM5, which presumably encode transcription factors for extracellular matrix components. We report the clinical and molecular features of nine novel BCS families, four of which harbor variants in ZNF469 and five in PRDM5. We also performed a genotype and phenotype-oriented literature overview of all (N=85) reported patients with ZNF469 (N=53) and PRDM5 (N=32) variants. Musculoskeletal findings may be the mean reason for referral, and often raise suspicion of another heritable connective tissue disorder such as kyphoscoliotic EDS, osteogenesis imperfecta or Marfan syndrome, especially when corneal rupture has not yet occurred. Our findings highlight the multisystemic nature of BCS and validate its inclusion in the EDS classification. Importantly, gene panels for heritable connective tissue disorders should include ZNF469 and PRDM5 to allow for timely diagnosis and appropriate preventive measures for this rare condition.

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Abstract

Brittle cornea syndrome (BCS) is a rare autosomal recessive disorder characterized by corneal thinning and fragility, leading to corneal rupture, the main hallmark of this disorder. Non-ocular symptoms include hearing

loss, but also signs of connective tissue fragility, placing it in the Ehlers-Danlos syndrome (EDS) spectrum. It is caused by biallelic pathogenic variants in *ZNF469* or *PRDM5* , which presumably encode transcription factors for extracellular matrix components. We report the clinical and molecular features of nine novel BCS families, four of which harbor variants in *ZNF469* and five in *PRDM5* . We also performed a genotype and phenotype-oriented literature overview of all (N=85) reported patients with *ZNF469* (N=53) and*PRDM5* (N=32) variants. Musculoskeletal findings may be the mean reason for referral, and often raise suspicion of another heritable connective tissue disorder such as kyphoscoliotic EDS, osteogenesis imperfecta or Marfan syndrome, especially when corneal rupture has not yet occurred. Our findings highlight the multisystemic nature of BCS and validate its inclusion in the EDS classification. Importantly, gene panels for heritable connective tissue disorders should include*ZNF469* and *PRDM5* to allow for timely diagnosis and appropriate preventive measures for this rare condition.

Keywords:

Brittle cornea syndrome; *ZNF469* ; *PRDM5* ; multisystemic disorder; Ehlers-Danlos syndrome

## Introduction

Brittle cornea syndrome (BCS) is a rare autosomal recessive disorder characterized by extreme corneal fragility. The first report of a familial brittle cornea trait dates back to 1968 (Stein, Lazar, & Adam, 1968). It described two brothers of Tunisian Jewish origin born to consanguineous parents with extreme corneal fragility and subsequent corneal ruptures at young age. Both had blue sclerae and red hair. Similar phenotypes were later described in other families but the red hair appeared to be linked to the Tunisian Jewish ancestry (Gregoratos, Bartsocas, & Papas, 1971; Ticho, Ivry, & Merin, 1980). Other ophthalmic findings such as keratoconus, keratoglobus, myopia and retinal detachment were more sporadically reported.

A very similar set of ocular features was also described in a group of patients with hearing loss and signs of more generalized connective tissue fragility, such as congenital bilateral hip dislocations, joint hypermobility, muscle hypotonia, kyphoscoliosis, deformities of hands and feet, skin hyperextensibility, a thin, translucent skin and easy bruising (Cameron, 1993; Macsai, Lemley, & Schwartz, 2000; Ogur et al., 1994). This phenotype shows a significant overlap with the Ehlers-Danlos syndromes (EDS), which are a group of heritable connective tissue disorders with joint hypermobility, skin hyperextensibility and generalized connective tissue fragility as their hallmarks. EDS is classified into different subtypes on the basis of mode of inheritance, biochemical and / or molecular defects and clinical features. One of the subtypes, the kyphoscoliotic type of EDS (kEDS), is distinguished by early-onset kyphoscoliosis and muscle hypotonia and was known as EDS VI in the 1997 Villefranche nosology (Beighton, De Paepe, Steinmann, Tsipouras, & Wenstrup, 1998). EDS VI was further subdivided in EDS VIA, in which lysyl hydroxylase 1 (LH1) activity was reduced, and EDS VIB, in which LH1 enzyme activity was normal (Krane, Pinnell, & Erbe, 1972). In 1992, EDS VIA was shown to result from biallelic pathogenic variants in*PLOD1* , the gene coding for LH1 (Hautala, Heikkinen, Kivirikko, & Myllyla, 1993; Hyland et al., 1992), whereas EDS VIB remained biochemically and molecularly unexplained at the time.

Part of the genetic etiology of BCS was discovered in 2006. The overrepresentation of red hair in the originally described families of Tunisian Jewish origin suggested linkage disequilibrium between the BCS locus and a locus for hair color (Zlotogora, BenEzra, Cohen, & Cohen, 1990). Therefore, haplotype analysis was done in the area surrounding the major gene associated with red hair color, *MC1R* . This mapped the BCS gene to 16q24 (Abu et al., 2006). Further narrowing of this region and genotyping revealed causal variants in *ZNF469* (MIM 612078) (Abu et al., 2008). This single-exon gene encodes ZNF469, a protein containing five C2H2 zinc finger motifs and an arginine-rich region located between two amino-terminal proline-rich domains (Rohrbach et al., 2013). Little is known about the function of ZNF469, but based on a limited homology (˜30%) to the helical domains of certain collagen chains strongly expressed in cornea (α1(I), α2(I), α1(IV) collagen), it was suggested that ZNF469 could act as a (transcriptional) regulator in the synthesis or assembly of collagen fibrils (Abu et al., 2008). To date 20 homozygous or compound heterozygous pathogenic variants have been identified in *ZNF469*(Abu et al., 2008; Al-Owain, Al-Dosari, Sunker, Shuaib, & Alkuraya, 2012;

Christensen et al., 2010; Khan, Aldahmesh, & Alkuraya, 2012; Khan, Aldahmesh, Mohamed, & Alkuraya, 2010; Menzel-Severing, Meiller, Kraus, Trollmann, & Atalay, 2019; Micheal et al., 2019; Ramappa, Wilson, Rogers, & Trivedi, 2014; Rohrbach et al., 2013; Rolvien, Kornak, Linke, Amling, & Oheim, 2020; Skalicka et al., 2019). Some of these were found in patients previously diagnosed with EDS VIB (Al-Hussain, Zeisberger, Huber, Giunta, & Steinmann, 2004; Rohrbach et al., 2013).

The absence of pathogenic variants in *ZNF469* later suggested locus heterogeneity for BCS (Aldahmesh, Mohamed, & Alkuraya, 2012; Burkitt Wright et al., 2011), and in 2011 *PRDM5* (MIM 614161) was uncovered as a second locus for BCS (Aldahmesh et al., 2012; Burkitt Wright et al., 2011). The gene codes for PRDM5 (PR domain zinc finger protein 5), containing 16 C2H2 zinc finger motifs and one PR-SET domain. PRDM5 is a transcription factor and has been identified as a tumor suppressor gene in different forms of epithelial cancer (Cheng, Chen, Cheng, Liu, & Lou, 2010; Deng & Huang, 2004; Galli et al., 2013; Meani, Pezzimenti, Deflorian, Mione, & Alcalay, 2009; Shu et al., 2011; Watanabe et al., 2009; Watanabe et al., 2007) and as an oncogene in other cancer types (Wang et al., 2016; Zhou et al., 2019). More recently, PRDM5 was shown to regulate extracellular matrix (ECM) development and maintenance in corneal and bone cells (Galli et al., 2012; Porter, Gallego-Pinazo, et al., 2015; Rolev, O'Donovan, Georgiou, Rajan, & Chittka, 2017) and apoptosis of neuronal and intestinal epithelial cells (Liu et al., 2016; Wu et al., 2017). Interestingly, studies in the bone of mouse embryos demonstrated that Prdm5 is a transcriptional regulator of multiple collagen genes and genes encoding small leucine-rich proteoglycans (SLRPs) (Galli et al., 2012). It can sustain type I collagen transcription by maintaining RNA polymerase II occupancy to *Col1a1,* and can bind to distal enhancer elements of the decorin gene. To date, 13 homozygous pathogenic variants in*PRDM5* have been associated with BCS (Alazami et al., 2016; Aldahmesh et al., 2012; Avgitidou et al., 2015; Burkitt Wright et al., 2011; Micheal, Khan, et al., 2016; Porter, Gallego-Pinazo, et al., 2015; Porter, Galli, et al., 2015).

The elucidation of these two genes has allowed for a better delineation of the associated phenotypes. Biallelic *ZNF469* or *PRDM5*pathogenic variants (Burkitt Wright et al., 2013) cause an undistinguishable but pleiotropic disorder mainly characterized by corneal fragility and hearing loss, but also signs of soft connective tissue fragility, such as joint hypermobility, kyphoscoliosis, deformities of the hands and feet, a soft or thin, mildly hyperextensible skin. The latter supported the inclusion of BCS in the revised 2017 international EDS classification (Malfait et al., 2017). However, many of the reported patients were presented as case reports or small case series in specialized ophthalmologic journals with the emphasis firmly on the ocular presentation. Apart from a 2013 review (Burkitt Wright et al., 2013), a comprehensive overview on the extra-ocular features and multisystemic phenotype is lacking. In recent years, multiple additional families have been identified and we further add to this by reporting 13 BCS patients from 9 novel families in whom biallelic pathogenic variants in *ZNF469* or *PRDM5* were found at our diagnostic center for heritable connective tissue disorders. We also reviewed clinical and molecular data of all BCS patients with pathogenic variants hitherto reported, and emphasize the multisystemic nature of BCS, thereby supporting its inclusion in the 2017 international EDS classification.

## Material & Methods

### *Editorial Policies and Ethical Considerations*

This study was approved by the Ethics Committee of the Ghent University Hospital, Ghent, Belgium. All patients and/or their parents signed informed consent forms.

### *Patients and samples*

Thirteen patients from nine independent families were included in this study. Genomic DNA (gDNA) was extracted from peripheral blood samples using the PureGene® DNA Purification Kit according to the manufacturer's instructions (Gentra Systems, Minneapolis, MN, USA).

### *Molecular analysis*

For index patients P5-III and P7-IV, whole exome sequencing (WES) was performed using Illumina's HiSeq 3000 system (Illumina, San Diego, CA, USA) after enrichment of the exome from gDNA by the SureSelec-

tXT Human All Exon V6 protocol (Agilent, Santa Clara, CA, USA). Read mapping and variant annotation was performed using the CLCBio pipeline (Aarhus, Denmark). Variants were analyzed with the in-house developed analysis platform SeqPlorer. For index patients P1-I, P4-II and P8-V, the exonic and flanking non-coding regions of *ZNF469* and *PRDM5* were PCR amplified on gDNA. The generated amplicons were then analyzed by Illumina's sequencing-by-synthesis (SBS) technology (MiSeq Personal Sequencer, Illumina). For index patients P9-VI, P10-VII and P11-VIII, sequencing analysis of *COL1A1* and *COL1A2* was performed before *ZNF469* and *PRDM5* were sequenced. For index patient P12-IX, sequencing analysis of an osteogenesis imperfecta (OI) and osteoporosis gene panel consisting of *COL1A1* , *COL1A2* ,*IFITM5* , *B3GALT6, CRTAP, LEPRE1, PPIB, CREB3L1, WNT1, TAPT1, SEC24D, P4HB, XYLT2, SPARC, LRP5, PLS3, BMP1, FKBP10, PLOD2, SERPINF1, SERPINH1, SP7* and *TMEM38B* preceded WES. The presence of the identified variants was confirmed by bidirectional fluorescence DNA sequencing (Sanger method). Amplicons were separated and sequenced using the BigDye® Terminator Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, USA) and analyzed on an ABI 3730XL DNA Analyzer (Life Technologies). The obtained sequences were compared with the reference sequences of *ZNF469* (RefSeq transcript NM_001367624.2) and*PRDM5* (RefSeq transcript NM_018699.4) using the SeqScape software package (version 2.5.0). Molecular analysis of *PRDM5* and*ZNF469* in family members was restricted to targeted mutation analysis. Nucleotide and protein position +1 correspond to the A of the start codon and the methionine translation initiator, respectively. Correct variant nomenclature was assessed using Mutalyzer name checker. Variants were classified according to the standards and guidelines of the American College of Medical Genetics and Genomics (Richards et al., 2015).

*Data Availability Statement*

The data of the in-house detected variants is submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) (Landrum et al., 2018), accession numbers SCV001437196 to SCV001437205.

*Statistical analysis*

Phenotypic data of all patients with pathogenic variants in*ZNF469* or *PRDM5* reported in this and earlier reports were summarized. For the comparison of qualitative variables in two groups, a chi-square test with continuity correction was used if the expected counts in the contingency table were high enough. If not, a Fisher's exact test was used. $p$ -values were reported as statistically significant if lower than 0.01. All statistical analyses were performed using Rstudio software (version 3.6.1).

Results

*Case reports*

The natural history of four families is reported below to illustrate the full clinical spectrum of BCS. The clinical synopsis for the other families is available in supplemental information. Table 1 summarizes the clinical information of all patients, including the presence or absence of the major and minor diagnostic criteria formulated in the 2017 international classification on EDS. Representative clinical images and pedigrees are shown in Figure 1.

**Family 1 (P1-I, P2-I and P3-I)**

P1-I is the second child of healthy consanguineous Omani parents. The initial working diagnosis was BCS. He was born with bilateral dislocation of the hip and bilateral calcaneovalgus deformity. He initially presented at the age of 6 months with muscle hypotonia and developmental delay. At the age of 21 months he was referred to a clinical geneticist with waddling gait and externally rotated hips and ankles. Length (88.5 cm; P50), weight (12 kg; P50) and head circumference (49 cm; P50) were all within normal range. Ophthalmic examination revealed thin cornea, low ocular rigidity, blue sclerae and mild myopic astigmatism. At later age he suffered a unilateral ocular rupture after minor trauma, necessitating corneal grafting. He developed a mild to moderate sensorineural hearing loss. Additional features included generalized joint hypermobility (Beighton score 8/9) and a history of repetitive dislocations of the thumb. His skin was normal. Motor, speech

and language development were all delayed. Mild facial dysmorphology included frontal bossing, hypertelorism, a depressed nasal bridge and thick lips (Fig. 1A). He is currently 12 years old and attends a school for the visually impaired. Echocardiography and a radiographic skeletal survey showed no abnormalities.

His younger sister (P2-I) presented with similar findings at the age of 6 months, displaying a developmental delay and marked muscle hypotonia, but without hip dislocation. She had blue sclerae and thin cornea. At the age of 6 years, moderate bilateral sensorineural hearing loss was noted, causing a mild speech and language delay. She had generalized joint hypermobility and pectus excavatum. Bone density was shown to be reduced.

The mother of the siblings had joint hypermobility and blue sclerae. The maternal grandfather did not show any signs of connective tissue disease, but two maternal aunts also had mild joint hypermobility and blue sclerae.

P3-I is the paternal cousin of P1-I and P2-I and the only child of a third-degree consanguineous couple (Fig. 1-III). She was born with bilateral hip subluxation and feet deformities. She presented at the age of 11 years with blue sclerae (Fig. 1C). Ophthalmic examination revealed no other abnormalities. Clinical examination showed a tall and slender girl (length > P97) with generalized joint hypermobility, long, slender fingers, flexion deformity of the fifth proximal interphalangeal joint, and bilateral hallux valgus. She had a history of elbow dislocations. There was no notion of skin hyperextensibility. The development appeared normal.

**Family 3 (P5-III and P6-III)**

P5-III is a 10-year-old girl born to non-consanguineous parents from India, in whom an initial clinical diagnosis of EDS VIB was made. She presented with blue sclerae, thin cornea, bilateral keratoglobus and myopia (R -16 D; L -18 D). She was seen by a physician at the age of 10 years. She was 134 cm tall (P25), weighed 24 kg (P3) and had macrocephaly (OFC 54 cm; > P97). At clinical examination, joint hypermobility was noted (Beighton 8/9). She did not have kyphoscoliosis or any pectus deformities. She had arachnodactyly, bilateral pedes plani and medial rotation of medial malleolus. Her skin was normal. Motor development was reported to be delayed. There was no history of dentition problems. Slit lamp examination revealed evidence of hydrops in both eyes, resulting in severe edema due to the rupture of the Descemet's membrane (Fig. 1G, Q, R). Anterior segment Optical Coherence Tomography (OCT) images (Fig. 1Q, R) showed bilateral global corneal thinning and edema, in addition to a deep left anterior chamber of 498 microns (normal range 250-350 microns).

Clinical examination of her 9-year-old brother (P6-III) showed blue sclerae, thin cornea and keratoglobus of the right eye. Following traumatic rupture of the left cornea, he displayed corneal scarring (Fig. 1 H, I, S, T). Bilateral high myopia and retinal detachment in the left eye were reported. Similar to his sister, he had joint hypermobility (Beighton 8/9). Arachnodactyly, bilateral pedes plani and medial rotation of medial malleolus were also observed. He had undergone surgical correction for a right side talipes deformity. His skin was normal and motor development was not delayed. His height, weight and OFC were 126 cm (P3-10), 20 kg (< P3) and 52 cm (P75) respectively. Anterior segment OCT revealed a central corneal thickness of 340 microns in the right eye (normal range 500-550 microns) and a deep right anterior chamber of 378 microns (normal range 250-350 microns) (Fig. 1 S). In the left eye, loss of the anterior chamber with adherence of the iris to the cornea was noted, in addition to degenerative changes including calcific keratopathy (Fig. 1 T).

**Family 4 (P7-IV)**

The index patient P7-IV of this family is a 14-year-old boy born to healthy consanguineous Syrian parents, and in whom Marfan syndrome was initially suspected (Fig. 1J-N). After an uneventful pregnancy, he was born with imperforate anus and bowel obstructions, both for which he was operated on in the first week of life. He also presented with congenital hip dysplasia. He quit school at the age of 7 years due to severe learning disorders. The patient was seen at the age of 14 years. He was 173 cm tall (P75) and weighed 41.3 kg (P3-10). He had blue sclerae (Fig 1J), high myopia and astigmatism. Otological problems included

recurrent otitis, bilateral tympanic membrane perforations and hearing loss. Generalized joint hypermobility was noted upon clinical examination. He displayed arachno-, campto- and clinodactyly in both hands (Fig. 1M), as well as pectus excavatum and genua valga (Fig. 1L). He had multiple feet deformities, including pedes plani, hallux valgus, camptodactyly of the fifth toe and syndactyly of the second and third toes (Fig. 1N). In addition, there was a difference in length between both legs. His skin was normal. He displayed hypertelorism, downslanting palpebral fissures, a high-arched palate and synophris (Fig. 1J). Audiometry measured a hearing loss of 30 dB with bone conduction and an air-bone gap of 20-45 dB. CT-scan of the inner ear bones showed bilateral absent long process of the incus and bilateral absent stapes. Echocardiography detected mitral valve prolapse and bicuspid aortic valve. The aorta was not dilated. He has an older brother and a younger sister, who are both healthy.

**Family 6 (P9-VI)**

P9-VI is a 41-year-old female and one of four children of a non-consanguineous couple of Caucasian origin. She was first referred for a suspicion of osteogenesis imperfecta. She presented with blue sclerae, bilateral keratoconus, scleral fragility and myopia. Mild bilateral hearing loss was noted. She was referred to a geneticist at the age of 41 years. Height, weight and arm span were 148 cm (< P3), 73.2 kg (P90) and 149 cm respectively. Clinical examination showed generalized joint hypermobility (Beighton score 6/9), kyphoscoliosis which progressed after rodding surgery, arachnodactyly, pedes plani, and muscle hypotonia. She had recurrent dislocations of knees and ankles. Her skin felt smooth and velvety and was hyperextensible. Motor development was delayed. She showed macrocephaly. She had a history of osteoporosis and a pelvic fracture and had had a bilateral total hip replacement at the age of 39 years.

*Molecular analysis*

Probands from families 1, 2 and 5 were referred because of a clinical suspicion of BCS. Direct sequencing of *ZNF469* and *PRDM5*identified biallelic pathogenic variants in *ZNF469* in families 1 and 2, and in *PRDM5* in family 5. In families 6, 7 and 8,*COL1A1* and *COL1A2* sequencing analysis was first performed in the probands as there was an initial clinical suspicion of osteogenesis imperfecta (OI). After exclusion of causal variants in these genes, *ZNF469* and *PRDM5* were sequenced. In family 9, suspicion of OI first led to sequencing of all genes that were known to be associated with the disorder at that time (*COL1A1* ,*COL1A2* , *IFITM5* , *B3GALT6, CRTAP, LEPRE1, PPIB, CREB3L1, WNT1, TAPT1, SEC24D, P4HB, XYLT2, SPARC, LRP5, PLS3, BMP1, FKBP10, PLOD2, SERPINF1, SERPINH1, SP7* and *TMEM38B)* . In the absence of pathogenic variants WES was performed, which led to the identification of a pathogenic variant in *PRDM5* . For the remaining families (family 3 & 4) the affected individuals were initially suspected to have EDS VIB (family 3) and Marfan syndrome (family 4) respectively, but their phenotype did not fully fit the clinical diagnosis. In these families, WES was performed, focusing on connective tissue genes. This allowed identification of biallelic pathogenic variants in *ZNF469* .

The variants identified in the patients are described below, listed in table 1 and mapped to the protein structures in figure 2 together with all previously reported variants in *ZNF469* and *PRDM5.*

Biallelic pathogenic variants in *ZNF469* were identified in family 1-4. They all result in a premature termination codon (PTC) and do not appear to localize to one or more specific domains of the protein. In P1-I, a homozygous 1-bp deletion (c.1444delC; p.(Leu482Cysfs*20)) was present (SCV001437196). P4-II was homozygous for a 1-bp duplication (c.9876dupT; p.(Ala3293Cysfs*6)) (SCV001437197). P5-III carried compound heterozygous variants (c.[1081delG];[1586delG]; p.[(Ala361Leufs*16)];[(Gly529Aspfs*9)]) (SCV001437198, SCV001437199). In P7-IV, a homozygous nonsense variant was identified (c.3307C>T; p.(Gln1103*)) (SCV001437200).

In the remaining families (5-9), pathogenic variants in *PRDM5*were identified. These include two frameshift variants in the zinc finger domains and three missense variants which are mapped in or near the PR-SET domain. A homozygous 1-bp deletion leading to a frameshift was identified in P8-V (c.974delG; p.(Cys325Leufs*2)) (SCV001437201). In P9-VI, a homozygous missense variant was present (c.106G>A; p.(Gly36Arg)) (SCV001437202). In P10-VII a homozygous c.17T>G; p.(Val6Gly) was detected

(SCV001437203). For P11-VIII, a homozygous 1-bp deletion resulted in a frameshift variant c.1858delC; p.(His620Thrfs*8) (SCV001437204). Finally, WES revealed the presence of a homozygous missense variant c.247C>T (p.(Arg83Cys)) in P12-IX (SCV001437205).

Segregation of the identified variants was studied where possible. In families 1, 3, 4 and 7, both parents were confirmed to be heterozygous carriers. In family 5, the father of the proband was shown to be a heterozygous carrier, but DNA from the mother was not available. In families 1, 3 and 9, affected siblings and the affected paternal cousin in family 1 were shown to harbor the biallelic variants. DNA was not available for unaffected members of family 2, 6, 8 and 9 (Fig. 1 panel III).

Three of the identified variants were previously reported in patients with BCS. These are the homozygous PRDM5 variants p.(Val6Gly), p.(Arg83Cys) and p.(Cys325Leufs*2) (Avgitidou et al., 2015; Burkitt Wright et al., 2011; Porter, Galli, et al., 2015). The latter two and the missense *PRMD5* variant c.106G>A (p.(Gly36Arg)) have a low allele frequency in the GnomAD database [c.106G>A (p.Gly36Arg)) in 3 of 251,332 alleles; c.247C>T (p.(Arg83Cys)) in 4 of 251,280 alleles; c.974delG (p.(Cys325Leufs*2)) in 2 of 250,490 alleles] which suggests that these are pathogenic variants. To the best of our knowledge, no other variants that we identified were hitherto reported in literature or public databases.

All variants introducing a PTC in *ZNF469* or *PRDM5* are presumed to be pathogenic, since it is a recurrent variant type in reported BCS patients. However, because the frameshift variant in P11-VIII introduces a PTC only two codons before the normal stop codon of the *PRDM5* mRNA and is thus predicted to generate a product only two amino acids shorter than the normal protein, the pathogenicity of c.1858delC (p.(His620Thrfs*8)) is uncertain. The three PRDM5 missense variants that were identified in the in-house patients (p.(Arg83Cys) in P12-IX, p.(Gly36Arg) in P9-VI and p.(Val6Gly) in P10-VII) affect highly conserved amino acids and are classified as variants of uncertain significance. For p.(Arg83Cys), p.(Gly36Arg) and p.(Val6Gly) respectively four, three and two out of four *in silico* prediction programs (PolyPhen2, SIFT, MutationTaster and Align-GVGD) suspect deleterious effects of the amino acid substitutions (variant classification given in supplemental table). In view of the combined information on the clinical phenotype, segregation, the absence or low frequency of the variants in population databases and the *in silico* predictions, we highly suspect that the identified variants are causative for BCS.

*Overview of all BCS patients with identified pathogenic variants*

The patients from our current study included, a total of 85 patients from 46 families with a biallelic pathogenic variant in *ZNF469* or*PRDM5* have been reported (Table 2). 18 different homozygous variants have been reported in the single-exon gene *ZNF469* , 3 of which were found in a second family. In addition, 6 families carry unique compound heterozygous variants in *ZNF469* . The majority of these *ZNF469* variants introduce a PTC (90%), which include 21 frameshift variants and 6 nonsense variants. Also, 2 homozygous missense variants located towards the carboxy-terminus and 1 whole gene deletion, in compound heterozygosity with a frameshift variant, were detected in*ZNF469* .

In *PRDM5* , 14 homozygous variants have been reported, five of which were found in a second family. These include four frameshift variants, two nonsense variants, one in-frame genomic deletion of multiple exons, three splice site variants and four missense variants located in or close to the PR-SET domain.

We summarized the clinical data of all patients with a pathogenic variant in *ZNF469* or *PRDM5* for whom clinical information was available in Table 3 & 4.

For the group of patients for whom the sex is reported, 45% is male. The mean age is 19 years old, with the youngest patient being a 6-week old baby and the oldest patient a 48-year-old female. Consanguinity was reported in 32 of 46 families.

The most frequently reported symptom was blue sclerae (72/78). Ocular ruptures (53/78) occurred in more than two thirds of the patients. Most were corneal ruptures, while a minority was undefined (Alazami et al., 2016). Keratoconus/keratoglobus (37/78), thin cornea (35/78) and myopia (35/78) were present in almost

half of the patients. Other ocular findings were more sporadic. Treatment of ophthalmic manifestations differed strongly between patients and surgery was frequently associated with complications. Multiple patients were advised to use protective eye goggles to prevent trauma to the eye (Avgitidou et al., 2015; Burkitt Wright et al., 2011; Khan et al., 2010; Menzel-Severing et al., 2019; Micheal, Khan, et al., 2016; Ramappa et al., 2014; Rohrbach et al., 2013). In one reported and three in-house patients, the ocular phenotype was limited to either blue sclerae or myopia (Rohrbach et al., 2013). Probands did not appear to be more severely affected than their family members with BCS (data not shown).

We further analyzed the occurrence of extra-ocular symptoms. In 8 of 78 patients, no systemic findings were reported. Hearing impairment was present in 32 of 78 patients and could be caused by conductive, sensorineural or combined defects. As for the EDS-associated symptoms, a high frequency of joint hypermobility was reported (64/78), which was often most pronounced in the small joints. In 13 patients, joint hypermobility was the only reported extra-ocular feature. Feet deformities (30/78) and an EDS-like skin (24/78) including soft, smooth skin in 23/78; thin, fragile or transparent skin in 11/78; hyperextensible skin in 9/78; easy bruising in 4/78 and delayed wound healing in 4/78 were also common. Other connective tissue signs, such as arachnodactyly (23/78) and (kypho)scoliosis (15/78) were reported as well. Bone fragility, defined as early osteopenia, osteoporosis or fractures upon minor trauma, was noted in 12 of 78 patients. In 16 of 78 patients, some (mild) facial dysmorphology was described. Pregnancy and birth were noted to be uneventful in a few cases (Alazami et al., 2016; Avgitidou et al., 2015; Porter, Gallego-Pinazo, et al., 2015; Ramappa et al., 2014). In one adult female patient, primiparous cervical incompetence caused pregnancy loss in the second trimester (Burkitt Wright et al., 2011). Premature ruptures of the membranes during pregnancy occurred in the mother of a patient (Burkitt Wright et al., 2011). Apart from one in-house patient who died at age 28 years of an unreported cause (P8-V, supplemental information), no premature deaths were described. Again, there were no apparent differences between probands and affected family members (data not shown).

Analysis of the clinical data between patients with pathogenic variants in *ZNF469* and *PRDM5* was performed (Table 3). Myopia is the only trait to reach a statistically significant difference, being more frequently reported in patients with *PRDM5* variants. In addition, there was a trend towards a higher number of corneal ruptures in patients with *ZNF469* variants, most pronounced in the probands (data not shown). Conversely, hearing loss was more common in patients with *PRDM5* variants. Traits that were more subjective such as soft skin and easy bruising, or reported in less than ten patients in total were not considered. Comparative analysis between the reported patients and the 13 in-house patients uncovered marked differences (Table 4). Multiple musculoskeletal manifestations were more frequently observed in the in-house families and reached statistical significance for joint dislocations, pectus deformities and delayed motor development.

While in many reported families, heterozygous carriers of a*ZNF469* or *PRDM5* pathogenic variant were noted to be asymptomatic (Abu et al., 2008; Alazami et al., 2016; Aldahmesh et al., 2012; Avgitidou et al., 2015; Burkitt Wright et al., 2011; Christensen et al., 2010; Khan et al., 2012; Menzel-Severing et al., 2019; Micheal, Khan, et al., 2016; Ramappa et al., 2014; Rohrbach et al., 2013; Skalicka et al., 2019), some carriers have been noted to have mild symptoms of BCS. Blue sclerae were present in the sister of a reported proband, who had a heterozygous *ZNF469* nonsense variant (Khan et al., 2010). In another family with a *ZNF469* frameshift variant, one carrier member had joint hypermobility and two carriers displayed myopia (Micheal et al., 2019). Multiple heterozygous members of the family in whom a deletion of exon 9-14 in *PRDM5* was identified had joint hypermobility and blue sclerae, and one carrier also displayed keratoconus and myopia (Burkitt Wright et al., 2011). Their central corneal thickness was also slightly below average. The father, carrier of a heterozygous *ZNF469* frameshift variant, of five affected children developed bilateral glaucoma (Al-Owain et al., 2012). Blue sclerae and joint hypermobility were noted in the mother of patients P1-I and P2-I, who had a heterozygous frameshift variant in*ZNF469* , as well as in two maternal aunts, although mutation analysis was not performed in the latter. No clinical information was available for the carrier members of other in-house families.

## Discussion

Brittle cornea syndrome as we now know it has a split history. On the one hand, early ophthalmologic reports

identified patients with a striking corneal fragility and little extra-ocular symptoms (Gregoratos et al., 1971; Stein et al., 1968; Ticho et al., 1980). On the other hand, patients with signs of generalized connective tissue fragility resembling the kyphoscoliotic type of EDS and additional marked brittleness of the cornea were clinically classified as EDS VIB (Cameron, 1993; Macsai et al., 2000; Ogur et al., 1994). It was not until the elucidation of the BCS genes *ZNF469* and *PRDM5*that the phenotypes were recognized to be part of a single clinical spectrum.

The molecular diagnosis of patients with BCS has allowed for a better clinical delineation of the disorder. To date, 53 patients have been reported with biallelic defects in *ZNF469* and 32 with biallelic defects in *PRDM5* . The most characteristic symptoms are blue sclerae, and thin and brittle cornea, often resulting in vision loss due to corneal rupture. In combination with hearing loss, which is reported in 41% of affected patients, this often results in severe sensory deprivation. While there are a few patients without any reported extra-ocular symptoms (Khan et al., 2012; Ramappa et al., 2014; Ticho et al., 1980), the majority of patients with pathogenic variants in*ZNF469* or *PRDM5* do present signs of generalized connective tissue fragility. Joint hypermobility (sometimes associated with joint dislocations) is the most common, being present in 82% of all reported patients. Other musculoskeletal signs include deformities of the hands, feet, pectus and spine, developmental hip dysplasia and dislocation, and bone fragility. Skin and craniofacial abnormalities are mild, if present. In some, but not all, family members carrying heterozygous pathogenic variants in *PRDM5* and *ZNF469* , a mildly reduced central corneal thickness can be observed, in addition to blue sclerae, keratoconus or joint hypermobility (Burkitt Wright et al., 2011; Khan et al., 2010; Lechner et al., 2014; Micheal et al., 2019; Rohrbach et al., 2013; Stein et al., 1968).

The multisystemic phenotype of BCS invokes a wide differential diagnosis (Table 5). This is illustrated by the fact that in six families identified by us, a diagnosis of EDS VIB, OI or Marfan syndrome was initially suspected. EDS VIB and OI were also the initial clinical diagnosis in some earlier reported cases (Cameron, 1993; Macsai et al., 2000; Rohrbach et al., 2013; Rolvien et al., 2020).

First and foremost, BCS shows considerable overlap with the kyphoscoliotic(-like) type of EDS, caused by biallelic pathogenic variants in *PLOD1* and resulting in LH1 deficiency, formerly known as EDS VIA, but currently classified as kEDS-*PLOD1* (Malfait et al., 2017). Affected patients typically present kyphoscoliosis, generalized joint hypermobility and muscle hypotonia, blue sclerae, osteopenia or osteoporosis and myopia. In a few patients, ocular ruptures have been reported, considered to be caused by scleral fragility (Beighton et al., 1998; Burkitt Wright et al., 2013), although the tissue is not specified in the original publications (Ihme et al., 1983; Kariminejad et al., 2010; Pinnell, Krane, Kenzora, & Glimcher, 1972; Sussman, Lichtenstein, Nigra, Martin, & McKusick, 1974). This is contrary to the obvious corneal fragility in BCS, which is further distinguished from kEDS-*PLOD1* by more moderate kyphoscoliosis and muscle hypotonia, and in which joint hypermobility is often limited to the small joints.

Multiple similarities are also observed with other EDS subtypes that were previously categorized as EDS VIB. One of those is a type of EDS caused by biallelic pathogenic variants in *FKBP14* and currently labeled as kEDS-*FKBP14* (Malfait et al., 2017). It clinically overlaps with kEDS-*PLOD1* , but, apart from blue sclerae and refractive errors, no ocular fragility has been reported in affected individuals. Instead, kEDS-*FKBP14* is associated with hearing loss. Other EDS types previously classified as EDS VIB, but now shown to be genetically distinct conditions include musculocontractural EDS (*CHST14* , *DSE* ) and spondylodysplastic EDS (*SL39A13* ,*B3GALT6, B4GALT7* ) (Malfait et al., 2017). Their phenotype is more easily distinguished from BCS, based on other clinical characteristics (Table 5).

Other disorders that show clinical overlap with BCS include OI and Marfan syndrome (Table 5). OI is commonly known as the brittle bone disorder and has (extreme) bone fragility and blue sclerae as main manifestations, in addition to hearing loss and joint hypermobility. Although thin cornea have been described in OI, extreme corneal brittleness is rare (Chau et al., 2014). In addition, fractures are more frequent than in BCS. Marfan syndrome is characterized by defects in the aorta and cardiac valves, combined with joint hypermobility and a marfanoid habitus (Meester et al., 2017). Some musculoskeletal signs can be present in both Marfan syndrome and BCS, such as feet and pectus deformities, arachnodactyly and (kypho)scoliosis.

Myopia is also common. However, cardiac examinations such as electrocardiography can reveal strong indicators when suspecting Marfan syndrome.

Overall, the clinical distinction between BCS and other connective tissue disorders seems to be more difficult in the absence of severe ocular manifestations. The identification of patients who present with marked musculoskeletal features that are generally, or at least initially, more prominent than the ophthalmic traits challenges the prevailing view of BCS as a disorder with corneal fragility as the major hallmark and the main reason for referral (Burkitt Wright et al., 2013). Possibly, BCS is therefore underdiagnosed in patients with signs of connective tissue defects but without pronounced ocular phenotype. To detect these patients, we recommend to include screening for*ZNF469* and *PRDM5* in gene panels for EDS and OI, and in general connective tissue panels.

Previous studies noted no clinical distinction between patients with pathogenic variants in *ZNF469* or *PRDM5* (Burkitt Wright et al., 2013). Our analysis revealed that myopia was significantly more frequent in patients with *PRDM5* variants. In addition, some trends were identified. Corneal ruptures were more likely to occur in patients with *ZNF469* variants, whereas hearing loss was more common in patients with *PRDM5* variants. However, it is difficult to assess the clinical relevance of these differences, due to limitations to our study. It was performed retrospectively on data reported by groups from different disciplines and is limited by missing information and confounders. Multiple factors might influence the results, such as age, gender, use of protective measures, as well as the specialty of the treating physician or the focus of the publishing journal. Moreover, it is possible that myopia has occurred in the patient's clinical history, but it is not recognized or reported in the presence of more severe visual problems. We conclude that additional reports and standardized studies are needed to verify these observations. The use of a clinical checklist during examination could result in more uniform, complete reports, and a patient registry collecting clinical and molecular information could keep a standardized record of the affected population.

At our genetic center, we identified nine families with a confirmed molecular defect, bringing the total number of molecularly confirmed BCS families to 46. Up to now, only one patient with BCS has been described in whom no *ZNF469* or *PRDM5* variant was identified (Al-Hussain et al., 2004; Rohrbach et al., 2013). However, the presence of deep intronic pathogenic variants or large deletions was not excluded (Rohrbach et al., 2013). The single case strongly suggests that the majority of BCS patients will have biallelic pathogenic variants in*ZNF469* or *PRDM5,* although additional locus heterogeneity cannot entirely be excluded.

The majority of *ZNF469* variants introduce a PTC. Because*ZNF469* is a single-exon gene, the mutant transcripts are believed to escape nonsense-mediated mRNA decay (NMD) and may give rise to truncated proteins (Rohrbach et al., 2013). Only two *ZNF469*variants are missense variants and both are located between the two last C2H2 zinc finger domains. Possibly, this region or these specific residues are crucial to exert a proper function and missense variants located in other domains do not result in a phenotype or remain subclinical.

More variation exists in the type of variants identified in*PRDM5* . Six variants introduce a PTC. However, the pathogenicity for the PRDM5 p.(His620Thrfs*8) variant identified in the in-house patient P11-VIII is unclear since it is predicted to result in a protein only two amino acids shorter than the wild-type protein. Nonetheless, the phenotype of the patient is consistent with BCS due to the combined presence of keratoconus, hearing loss and joint hypermobility. Functional analysis is needed to resolve this observation. Four missense variants are identified in PRDM5, all residing in or around the PR-SET domain, which mediates protein-protein interactions (Duan et al., 2007). For PRDM5 p.(Arg83Cys), computer modeling suggests that the arginine residue at this position may facilitate binding with its ligand S-adenosyl-methionine and that substitution with cysteine impairs the interaction (Porter, Galli, et al., 2015). Further, three splice site variants all located at the donor splice site of intron 1 and one in-frame exon deletion were detected.

The molecular mechanisms of *ZNF469* and *PRDM5* in BCS are unclear. Little is known of the function of the ZNF469 protein, but the*ZNF469* locus has been associated with central corneal thickness in several genome wide association studies (Hoehn et al., 2012; Lu et al., 2010; Ulmer et al., 2012; Vitart et al., 2010;

Vithana et al., 2011). Some studies describe a link between *ZNF469* and keratoconus, but others could not confirm this finding (Davidson et al., 2015; Karolak et al., 2020; Lechner et al., 2014; Lucas et al., 2017; Vincent, Jordan, Cadzow, Merriman, & McGhee, 2014; Yu, Chen, Zhang, & Shentu, 2017). A specific novel heterozygous *PRDM5* missense variant also segregated within a Pakistani family with Axenfeld-Rieger syndrome, a disorder mainly affecting the anterior segment of the eye and sometimes extending to systemic manifestations (Micheal, Siddiqui, et al., 2016). In addition, the PRDM5 protein is shown to work as a transcription factor targeting important ECM genes in mouse osteoblasts (Galli et al., 2012). Although $Prdm5^{LacZ/LacZ}$mice appear to have a normal gross morphology, they display reduced collagen levels and an osteopenic phenotype (Galli et al., 2012). This might reflect the bone fragility reported in 16% of the BCS patients. Along with the clinical observations in heterozygous family members of BCS patients, these studies indicate the involvement of *PRDM5* and*ZNF469* in both ocular and systemic phenotypes.

In conclusion, we added nine families with biallelic *ZNF469* or*PRDM5* variants to the existing group of patients with BCS, thereby expanding the current knowledge. Our analysis of all patients with a reported molecular defect in *ZNF469* or *PRDM5*highlights the multisystemic nature of the disorder and further validates its inclusion in the 2017 international EDS classification. Furthermore, we note that in some families, the musculoskeletal symptoms, and not corneal fragility, are the main reason for referral. Especially in patients without severe ocular manifestations, the distinction with (kyphoscoliotic) EDS, OI or Marfan syndrome can be daunting, but the presence of more pronounced generalized connective tissue symptoms might aid in making a diagnosis. We acknowledge that the expertise in EDS and OI of our genetic center infers a risk of referral bias. Nonetheless, our observation underscores the role for geneticists to be aware of BCS in patients presenting with a heritable connective tissue disorder, even without corneal rupture, since early diagnosis is critical to allow for an appropriate follow-up and to prevent potential corneal ruptures later in life. We also recommend to include*ZNF469* and *PRDM5* in gene panels for EDS and OI, and in general connective tissue panels. Conversely, ophthalmologists are more likely to diagnose patients with a severe ocular phenotype. They should be aware of the multisystemic presentation of BCS and its potentially substantial musculoskeletal symptoms associated with it, to improve the patients' quality of life.

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Figure legends

**Figure 1: Clinical images and pedigrees of in-house patients.***Panel I:* clinical images of in-house patients. **A, B** : patient P1-I at age 21 months with hypertelorism, a depressed nasal bridge, thick lips (A) and blue sclerae (B). **C** : patient P3-I at age 11 years with blue sclerae. **D-F** : patient P4-II at age 3 years with blue sclerae, ptosis of the right eye, full cheeks, a depressed nasal bridge (D), pectus excavatum (E) and joint hypermobility of the thumb (F). **G** : patient P5-III at age 10 years with abnormalities in the right eye. **H, I** : patient P6-III at age 9 years with calcific keratopathy in the left eye (I). **J-N** : patient P7-IV at age 14 years with blue sclerae, hypertelorism, downslanting palpebral fissures, synophris (J), genua valga (L) deformities of the feet (M) and hands (N). **O** : patient P12-IX at age 16 years with light blue sclerae. **P** : patient P13-IX at age 26 years with light blue sclerae. *Panel II:* Detailed clinical and anterior segment Optical Coherence Tomography (OCT) images of the eyes of the patients of family 3. **Q** : Right eye of P5-III with severe keratoglobus. Hydrops is observed in the central cornea. The edges of the rolled-up Descemet's membrane (DM) are seen as an inverted triangle. Anterior segment (OCT) shows reduced corneal thickness in the periphery. The area on the right side of the image where DM is separated from the cornea is edematous. **R** : Left eye of P5-III showing hydrops with vertical rip of DM. Anterior segment OCT displays global corneal thinning with a deep anterior chamber. DM scroll and adjacent corneal edema is visible in the center. **S** : Right eye of P6-III showing keratoglobus. Anterior segment OCT reveals significant corneal thinning and a deep anterior chamber. **T** : Left eye of P6-III with corneal scarring and calcific keratopathy. Anterior segment OCT image shows loss of anterior chamber depth with adherence of iris tissue to the cornea.*Panel III* : pedigrees of in-house families. Asterisks indicate individuals for whom mutation analysis was performed.

**Figure 2: Mapping of all pathogenic variants in ZNF469 and PRDM5 on the protein structure.** Presentation of all pathogenic variants in ZNF469 and PRDM5 identified in BCS patients, reported in this study (above structure, black-filled symbols) and previous studies (below structure, outline symbols). *ZNF469* : RefSeq transcript NM_001367624.2; *PRDM5* : RefSeq transcript NM_018699

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