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Case Report

Does brittle cornea syndrome have a bone fragility phenotype?

Tim Cundy [a,*], Andrea Vincent [b], Stephen Robertson [c]

[a] Department of Medicine, Faculty of Medical & Health Sciences, University of Auckland, Auckland, Aotearoa-New Zealand
[b] Department of Surgery, Faculty of Medical & Health Sciences, University of Auckland, Auckland, Aotearoa-New Zealand
[c] Department of Women's and Children's Health, Dunedin School of Medicine, University of Otago, Dunedin, Aotearoa-New Zealand

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ABSTRACT

Brittle cornea syndrome is a rare recessively inherited disorder (a sub-type of Ehlers-Danlos syndrome) with a clinical presentation dominated by corneal fragility and deafness. There have been suggestions that it may also have a bone fragility phenotype, but there has been little detailed description. We describe two siblings with brittle cornea syndrome due to compound heterozygous mutations in *ZNF469* who had sustained ten or more fractures, the majority before the age of 15. When investigated as adults they had osteopenia, with lower z-scores than their parents who each carried one mutation. A bone biopsy from one sibling showed reduced cortical porosity. Both parents, who were heterozygous mutation carriers, had also suffered fractures but had normal bone density. This data supports the view that brittle cornea syndrome may have a bone fragility phenotype.

1. Introduction

Brittle cornea syndrome (BCS) is a rare recessively inherited disorder with a phenotype dominated by extreme corneal fragility, and mixed conductive/sensorineural deafness. It results from biallelic mutations in either *ZNF469* or *PRDM5*, causing respectively, BCS-1 and BCS-2 (OMIM 22920; 614170). The phenotypes of BCS-1 and BCS-2 are indistinguishable (Burkitt Wright et al., 2013).

BCS is classified as a subtype (#9) of the Ehlers-Danlos syndromes (Malfait et al., 2017). As in other disorders in the Ehlers-Danlos group, joint hypermobility, skin hyperelasticity and abnormal scarring are common and the teeth are normal. Scoliosis, pectus deformity, foot deformity and developmental hip dysplasia are well recognized in BCS (Burkitt Wright et al., 2013; Christensen et al., 2010), but not so osteoporosis and fracture. In 2010 Christensen *et al* reported low bone density in two siblings with BCS-1 whose heterozygous parents had normal bone density, suggesting a potential osteoporotic phenotype (Christensen et al., 2010). In a review of published cases with confirmed bi-allelic mutations of either *ZNF469* or *PRDM5* Dhooge *et al* suggested that BCS may have a bone fragility phenotype (Christensen et al., 2010). However, the clinical data is inadequate, with little information on frequency and age distribution of fractures, or bone density measurements. In this report we describe two siblings with BCS-1 who had both low bone mass and fractures.

2. Clinical histories

The main features and their chronology are described in Table 1. In childhood, both siblings lost sight in one eye through corneal rupture (Fig. 1A). In their mid-thirties both retain adequate vision in the other eye although the central cornea is markedly thin. The central corneal thickness measured by pachymetry (Galilei G4 Scheimpflug tomography; Ziemer Ophthalmology, Denzlingen, Germany) was 167 and 149 μm, respectively (normal 540–550 μm; Fig. 1B). Deafness, predominantly sensorineural but with some conductive element, was recognized in infancy. Other features of BCS included mild cardiac valve prolapse, keloid scarring, skin hyperelasticity, joint laxity with recurrent ligamentous injuries and dislocations (Fig. 1B), dysplasia of the hip and umbilical hernia. Both siblings had fractures in the neonatal period and continued to fracture until adolescence. The rate of fracture fell after reaching skeletal maturity (Table 2). There were no dental abnormalities.

The siblings' mother had early onset hearing loss (confirmed age 11) and two failed stapedectomy surgeries. She wore hearing aids from age 30. Her ankle joints were loose and unstable. She sustained 11 fractures between the ages of 4 and 59 (including humerus, radius, elbow, metatarsal, carpal bones and clavicle). The siblings' father had hearing loss in middle age, attributed in part to industrial noise exposure. He wore hearing aids from the age of 50. He had sustained two fractured wrists at age 7 and a femoral fracture after major trauma at age 8.

* Corresponding author.
E-mail address: t.cundy@auckland.ac.nz (T. Cundy).

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Table 1
Clinical history and findings in two siblings with Brittle Cornea Syndrome-1.

	Older sibling	Younger sibling
Ocular phenotype		
Blue sclerae	Yes	Yes
Astigmatism (age diagnosed)	4	1
Corneal rupture (age)	7, 17	2
Central corneal thickness μm (age)[a]	167 (39)	149 (35)
Best corrected visual acuity (age)	6/6 (39)	6/7.5 (35)
Auditory phenotype		
Hearing loss first detected (age)	2	2
Hearing aids (age at first use)	5	2½
Cochlear implant (age)	27	–
Other features		
Keloid scarring	Yes	Yes
Hernia – type (age)	None	Umbilical (1), Inguinal (33)
Joint instability/dislocation	Yes	Yes
Beighton score (age)	1/9 (39)	6/9 (35)
Skin hyperelasticity	Yes	Yes
Cardiac valve prolapse (age at detection)	Mitral - mild (9)	Mitral and tricuspid - mild (5)
Skeletal features		
Developmental dysplasia of the hip	No	Yes
Pectus carinatum	Yes	Yes
Scoliosis – severity (age detected)	Mild (14 1/2)	Mild (10½)
Adult height (m)	1.806	1.776

All ages given as years.
[a] Normal values 540–550 μm.

Neither parent had joint hypermobility or skin hyperelasticity. Measures of central corneal thickness were not available.

3. Methods

Leukocyte DNA from the younger sibling was examined by a gene panel for the known brittle cornea genes *ZNF469* and *PRDM5* (Connective Tissue Gene Tests, Allentown, PA, USA). Following identification of bi-allelic *ZNF469* mutations, customized variant testing in the older sibling and the parents was undertaken (Genetics Group, Canterbury Health Laboratories, Christchurch, New Zealand).

DNA from the older sibling was further examined using a 28-gene bone fragility gene panel (Dept of Molecular Genetics, The Children's Hospital, Westmead, Sydney, Australia). The genes examined were: *ANO5, BMP1, COL1A1, COL1A2, CREB3L1, CRTAP, TENT5A, FKBP10, IFITM5, MBTPS2, MESD, LRP5, P3H1, P4HB, PLOD2, PLS3, PPIB, SEC24D, SERPINF1, SERPINH1, SP7, SPARC, SUCO, SGMS2, TMEM38B, WNT1, WNT4* and *XYLT2*.

Bone density was measured using a Lunar-DPX densitometer with the results expressed as the age and gender-standardized standard deviation (z-score). The older sibling had a transiliac bone biopsy after tetracycline double-labelling. Undecalcified sections were examined by bone histomorphometry (Melsen et al., 1978). The biopsy was also imaged by micro computed tomography (Bruker, Karlsruhe, Germany).

4. Results

In both siblings, plasma calcium and phosphate and vitamin D concentrations were normal, as were bone turnover markers. Bone radiographs were unremarkable (Fig. 1C).

Bone density at the lumbar spine and femoral neck was in the osteopenic range in both siblings. Forearm bone density was normal.

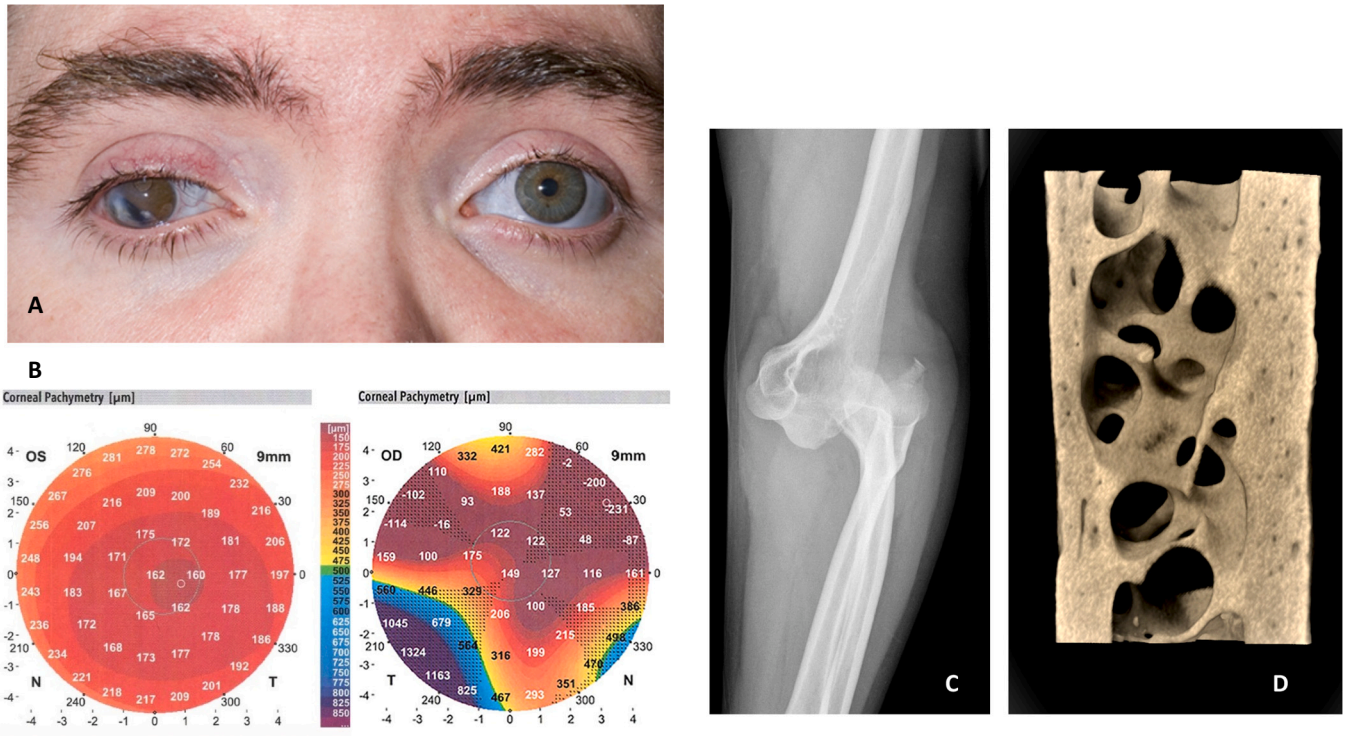

Fig. 1. A. Appearance of the eyes in adulthood in the older sibling. He has a poorly seeing exotropic right eye after suffered corneal rupture at ages 7 and 17 years with subsequent retinal detachment. The sclera of the left eye is slightly blue in colour.
B. Galilei G4 pachymetry imaging of the best functioning eye of the older (left) and the younger sibling (right) showing very thin corneas (normal central corneal thickness values 540–550 μm).
C. Fracture dislocation of the elbow in the younger sibling at age 31.
D. Micro CT image of the transiliac bone biopsy from the older sibling illustrating low cortical porosity.

Both parents had z-scores above average at both the lumbar spine and femoral neck (Table 2).

4.1. Quantitative histology

On the bone biopsy from the older sibling trabecular bone volume was increased at 30.2% (22.5 ±3.5%), but the mean cortical thickness was below average at 687um (NR 909 ±98). Cortical bone porosity was markedly low at 1.3% (NR 6.3 ±0.6%) (Fig. 1D). The trabecular osteoid surface was low 5.3% (NR 19.3 ±3.0%). Only short runs of osteoid, mainly woven, were seen. The trabecular resorptive surface was normal at 5.7% (NR 5.1 ±0.6%). A few osteoclasts are noted. The bone apposition rate, assessed from tetracycline double labelling, was normal at 0.7um/day.

DNA from the younger sibling disclosed compound heterozygous mutations in exon 2 of *ZNF469*: c:5716C > T; p.Arg1906 term and c.7220del; p.Gln2407Arg fs*38). Both mutations are novel and both are predicted to be likely pathogenic (ACMG Class 4: PVS1_Strong PM3, PM2_supp, PP1, PP4). Customized variant testing confirmed that his older sibling carried the same compound heterozygous mutations and that each parent was heterozygous for one mutation (Table 2).

We considered the possibility of a blended or modified bone phenotype, so in the older sibling undertook screening for mutations in 28 genes associated with bone fragility, using a next generation sequencing platform. No pathogenic variants were detected, though the technique used is less sensitive for detecting deletions and duplications >15 bp, repeat expansions and copy number variants. As no likely pathogenic mutations were found, we did not pursue testing of the younger sibling.

5. Discussion

The two siblings we describe had most of the symptoms and findings characteristic of BCS, with a phenotype dominated by ocular and auditory complications. They also had a significant history of fractures in childhood, beginning in the neonatal period. Their fracture rate slowed after adolescence, as is commonly seen in other pediatric disorders. Bone histology in one sibling showed normal to high trabecular volume but with narrow cortices with low porosity. There was no evidence of abnormal bone turnover. The low cortical porosity could potentially be related to the propensity to fracture, but we know of no other reports of bone histology in this condition. The radiographs as adults were unremarkable, but we have been unable to access radiographs from childhood. As adults, their bone density was in the osteopenic range, but not particularly low.

The siblings' parents who were both carriers of heterozygous *ZNF469* mutations also had significant fracture histories. Bone density measurements were higher than those of their adult children, suggesting that the predisposition to fracture may not be directly linked to bone density, but may reflect a problem with bone quality. The history of hearing problems in both parents, and fractures in the mother raises the question of whether the heterozygous carrier state has a phenotype. There is no literature to suggest this with regard to hearing problems, but heterozygous *ZNF469* mutations are associated with reduced central corneal thickness (Hoehn et al., 2012; Lu et al., 2010).

The 28-gene panel found no abnormalities in any of the known genes linked to bone fragility, thus there was no evidence to suggest blended or modified inheritance impacted on the phenotype, arguing that the *ZNF469* mutations might be causative. Fractures and osteopenia have been described in a number of the rarer subtypes of EDS including the arthrochalasia, dermatosparaxis, kyphoscoliotic and spondylodysplastic subtypes (# 6,7,8 and 10, respectively) (Malfait et al., 2017; Basalom and Rauch, 2020) and possibly in classical and hypermobile types too (# 1 and 5) (Eller-Vainicher et al., 2016; Mazziotti et al., 2016).

Burkitt Wright *et al* described a family with BCS-2 due to homozygous *PRDM5* mutations in which 3 of 4 family members had fractures (Burkitt Wright et al., 2011) and Dhhoge *et al* recently described 5 of 8 cases of BCS as having bone fragility (Dhooge et al., 2021b). In both these papers there is very limited data on the number of fractures or their evolution over time, and none on bone density. In two families with BCS-1 a possible bone phenotype has been reported in more detail. Christensen *et al* described adult two siblings with homozygous *ZNF469* mutations who had no fractures, but osteopenia on bone density scanning. Their parents had normal bone mass (Christensen et al., 2010). In contrast, Rolvien *et al* found no evidence for a low bone mass or fracturing phenotype in two siblings with a mild form of BCS-1 (Rolvien et al., 2020). In a recent review Dhooge *et al* have estimated that bone fragility may affect 16% of patients with BCS and suggested that screening for *ZNF469* and *PRDM5* mutations should be added to bone fragility gene panels (Dhooge et al., 2021b). This could be important because blue sclerae are present in early life in BCS and can be mistaken as a sign of osteogenesis imperfecta. Another important differential diagnosis is osteoporosis pseudoglioma syndrome caused by biallelic mutations in *LRP5*. However, the timing of visual loss is much earlier in the latter and the cause (retinal detachment *vs* rupture of the globe) is quite different (Ai et al., 2005).

The molecular mechanisms of *ZNF469* and *PRDM5* in BCS are not known. Both genes encode proteins with multiple zinc fingers, suggesting roles in transcription. The similarity of the BCS-1 and BCS-2 phenotypes suggests they are involved in a common pathway. Mutation in either *ZNF469* or *PRDM5* causes downregulation of genes encoding molecules involved in extracellular matrix development and maintenance. These include fibrillar collagens, connective tissue components, and molecules regulating cell migration and adhesion (Burkitt Wright et al., 2011). In murine bone PRDM5 can bind to the exonic DNA of collagen I genes and to upstream enhancer elements of proteoglycans with key roles in the extracellular matrix (Galli et al., 2012).

In conclusion we found a distinctive fracture history and osteopenia

Table 2
Fracture history, bone density measurements and *ZNF469* genotype.

	Father	Mother	Older sibling	Younger sibling
Fractures				
Neonatal	0	0	Both femora	Skull, femur[a]
Age 3–15 years (n)	3	5	8	7
Age ≥ 16 years (n)	0	6	1	1
Bone density - DXA				
Age at measurement	73	58	29	25
Lumbar spine z-score	+0.5	+1.8	−1.5	−1.0
Femoral neck z-score	+0.6	+1.5	−2.1	−1.7
Radius (total) z-score	–	–	–	+0.3
ZNF469 genotype	c:5716C > T; p.Arg1906 term WT	WT c.7220del; p.Gln2407Arg fs*38	c:5716C > T; p.Arg1906 term c.7220del; p.Gln2407Arg fs*38	c:5716C > T; p.Arg1906 term c.7220del; p.Gln2407Arg fs*38

WT – wild type.
[a] Fracture of the femur at age 7 weeks related to use of Pavlik harness for hip dysplasia.

in two siblings with compound heterozygous *ZNF469* mutations. The findings support the idea that bone fragility may be a feature of BCS, though the mechanism remains to be defined.

CRediT authorship contribution statement

Tim Cundy: wrote and revised the manuscript. **Andrea Vincent** and **Stephen Robertson:** data collection and interpretation, clinical care, methodology and writing.

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