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

ARTICLE INFO

Keywords:
Brittle cornea syndrome
Ehlers-Danlos syndrome
Fractures
Bone density

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 bi-allelic 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.
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, MESS, LRP5, P3H1, P4HB, PLOD2, PL3, PP1B, SEC24D, SERPINF1, SERPINH1, SP7, SPARC, SUO, SGMS2, TMEM38B, WNT1, WNT4 and XL72.

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.

### 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               |
| Central corneal thickness μm (age) | 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             |
| Brightness score (age)   | 1/9 (39)      | 6/9 (35)        |
| Skin hyperelasticity     | Yes           | Yes             |
| Cardiac valve prolapse (age at detection) | Mitral - mild | 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.

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 687μm (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 0.7μm/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: PV51_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 p.Arg1906 term WT c.5716C > T; p.Arg1906 term and p.Arg1906 term WT are novel and both are predicted to be likely pathogenic (ACMG Class 4: PM1 – 2.1, PM2 – 2.1, PM3 – 2.1, PM4 – 2.1).

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 characteristic of BCS, with a phenotype dominated by ocular and auditory complications. They also had a significant history of fractures characteristic of BCS, with a phenotype dominated by ocular and auditory complications. They also had a significant history of fractures characteristic of BCS, with a phenotype dominated by ocular and auditory complications. They also had a significant history of fractures characteristic of BCS, with a phenotype dominated by ocular and auditory complications. They also had a significant history of fractures characteristic of BCS, with a phenotype dominated by ocular and auditory complications.

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

| Fractures          | Father | Mother | Older sibling | Younger sibling |
|--------------------|--------|--------|---------------|-----------------|
| Neonatal           | 0      | 0      | Both femora   | Skull, femur⁴   |
| 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   |        | WT     | c.5716C > T; p.Arg1906 term | c.5716C > T; p.Arg1906 term |
|                   | WT     |        | p.Gln2407Arg fs*38    | p.Gln2407Arg fs*38    |

WT = wild type.

⁴ 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.

**Acknowledgements**

We thank all the family members for their participation, and Cur-ekids for funding for Stephen Dray for help with the bone histology and to Emma Buckels for the micro CT images.

**References**

Ai, M., Heeger, S., Bartels, C.F., Schelling, D.K., 2005. Osteoporosis-pseudoglioma collaborative group. clinical and molecular findings in osteoporosis-pseudoglioma syndrome. Am. J. Hum. Genet. 77 (5), 741–753.

Basalom, S., Rauch, F., 2020. Bone disease in patients with ehlers-danlos syndrome. Curr. Osteoporos. Rep. 18 (2), 95–102.

Burkitt Wright, E.M.M., Spencer, H.L., Daly, S.B., Manson, F.D.C., Zeef, L.A.H., Urquhart, J., Zoppi, N., Bonshek, R., Tosounidis, I., Mohan, M., Madden, C., Dodds, A., Chandler, K.E., Banks, S., Au, L., Clayton-Smith, J., Khan, N., Biesecker, L. G., Wilson, M., Rohrbach, M., Colombi, M., Giunta, C., Black, G.C.M., 2011. Mutations in PRDM5 in brittle cornea syndrome identify a pathway regulating extracellular matrix development and maintenance. Am. J. Hum. Genet. 88, 767–777.

Burkitt Wright, E.M.M., Porter, L.F., Spencer, H.L., Clayton-Smith, J., Au, L., Munier, F. L., Smithson, S., Suri, M., Rohrbach, M., Manson, F.D.C., Black, G.C.M., 2013. Brittle cornea syndrome: recognition, molecular diagnosis and management. Orphanet J. Rare Dis. 8, 68.

Christensen, A.E., Knappskog, P.M., Midbø, M., Clara, G., Gjesdal, C.G., Mengel-From, J., Morling, N., Redal, E., Boman, H., 2010. Brittle cornea syndrome associated with a missense mutation in the zinc-finger 469 gene. Invest. Ophthalmol. Vis. Sci. 51, 47–52.

Dhoooge, T., Van Damme, T., Syl, D., Mosquera, L.M., et al., 2021. More than meets the eye: expanding and reviewing the clinical and mutational spectrum of brittle cornea syndrome al. Hum. Mutat. 1, 1–20. https://doi.org/10.1002/humu.24199.

Eller-Vannich, C., Bassotti, A., Imeraj, A., Canesi, E., Ullviver, F.M., Cortini, F., Dubini, M., Marinelli, B., Spada, A., Chiodieri, L. 2016. Bone involvement in adult patients affected with ehlers-danlos syndrome. Osteoporos. Int. 27 (8), 2525–2531.

Galli, G.G., Hommes de Lichtenberg, K., Carrara, M., Hans, W., Quwelling, M., Mertz, B., Multhaupt, H.A., Fog, C.K., Jensen, K.T., Rappapber, J., Vortkamp, A., Coulton, L., Fuchs, H., Gullus-Durner, V., Hrabe de Angelis, M., Calogero, R.A., Coachman, J.R., Lund, A.H., 2012. Pmrd5 regulates collagen gene transcription by association with RNA polymerase II in developing bone. PLoS Genet. 8 (5), e1002711 https://doi.org/10.1371/journal.pgen.1002711.

Hoehn, R., Zeller, T., Verhoeven, V.J., et al., 2012. Population-based meta-analysis in caucasians confirms association with COL5A1 and ZNF469 but not COL8A2 with central corneal thickness. Hum. Genet. 131, 1783–1793.

Lu, Y., Dimasi, D.P., Hysi, P.G., et al., 2010. Common genetic variants near the brittle cornea syndrome locus ZNF469 influence the blinding disease risk factor central corneal thickness. PLoS Genet. 6, e1000947.

Malfait, F., Francomano, C., Byers, P., Belmont, J., Berglund, B., Black, J., Bloom, L., Bowen, J.M., et al., 2017. The 2017 international classification of the ehlers-danlos syndromes. Am. J. Med. Genet. C: Semin. Med. Genet. 175C, 8–26.

Mazzotti, G., Deodono, C., Doga, M., Galderisi, F., Venturini, M., Calzavara-Pinton, P., Maroldi, R., Giuntini, A., Colombi, M., 2016. High prevalence of radiological vertebral fractures in adult patients with ehlers-danlos syndrome. Bone 84, 88–92.

Melsen, F., Melsen, B., Moseklede, L., Bergmann, S., 1978. Histomorphometric analysis of normal bone from the iliac crest. Acta Pathol. Microbiol. Scand. 86 (1), 70–81.

Rolvien, T., Kornak, U., Linke, S.J., Amling, M., Oehr, M., 2020. Whole-exome sequencing identifies novel compound heterozygous ZNF469 mutations in two siblings with mild brittle cornea syndrome. Calcif. Tissue Int. 107, 294–299.