Brittle Cornea Syndrome Associated with a Missense Mutation in the Zinc-Finger 469 Gene

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PURPOSE. To investigate the diverse clinical manifestations, identify the causative mutation and explain the association with red hair in a family with brittle cornea syndrome (BCS).

METHODS. Eight family members in three generations underwent ophthalmic, dental, and general medical examinations, including radiologic examination of the spine. Bone mineral density (BMD) and serum levels of vitamin D, parathyroid hormone, and biochemical markers for bone turnover were measured. Skin biopsies were examined by light and transmission electron microscopy. Molecular genetic studies included homozygosity mapping with SNP markers, DNA sequencing, and SNP genotyping.

RESULTS. At 42 and 48 years of age, respectively, both affected individuals were blind due to retinal detachment and secondary glaucoma. They had extremely thin and bulging corneas, velvety skin, chestnut colored hair, scoliosis, reduced BMD, dental anomalies, hearing loss, and minor cardiac defects. The morphologies of the skin biopsies were normal except that in some areas slightly thinner collagen fibrils were seen in one of the affected individuals. Molecular genetic analysis revealed a novel missense mutation of ZNF469, c.10016G>A, that was predicted to affect the fourth of the five zinc finger domains of ZNF469 by changing the first cysteine to a tyrosine (p.Cys3339Tyr). Both affected individuals were homozygous for the common red hair variant R151C at the MC1R locus.

CONCLUSIONS. BCS is a disorder that affects a variety of connective tissues. Reduced BMD and atypical dental crown morphology have not been reported previously. The results confirm that BCS is associated with mutations in ZNF469. The association with red hair in some individuals with BCS is likely to occur by chance. (Invest Ophthalmol Vis Sci. 2010;51:47–52) DOI:10.1167/iovs.09-4251

Brittle cornea syndrome (BCS; MIM 229200; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) is a rare autosomal recessive disorder characterized by extreme thinning of the cornea. Frequently, rupture of the cornea occurs as a result of minor trauma. Other ocular malformations include keratoconus, keratoglobus, and blue sclera. Systemic involvement is common, with manifestations such as joint hypermobility, skin hyperelasticity, kyphoscoliosis, hearing defects, and dental abnormalities. Hernias, syndactyly, and mental retardation have also been observed in patients with BCS. Red hair is associated with BCS in some families, although only 10 of 60 patients with BCS have been reported to have red hair.1 Most individuals with BCS have been born to consanguineous parents and are thus expected also to be homozygous for a chromosomal region adjacent to the BCS locus. Some of the nonocular features in patients with BCS could therefore be due to homozygosity for mutations in closely linked genes.

From the observed association of BCS with red hair, Zlotogora et al.2 suggested that the BCS locus was closely linked to a gene responsible for hair color.2 Abu et al.3 assigned the BCS gene to a 4.7-Mb region on chromosome 16, region q24, close to the melanocortin 1 receptor gene. MC1R. Recently, they identified mutations in ZNF469, associated with BCS in Jewish families of Tunisian origin and in a Palestinian family.4 The precise function of ZNF469 is not known. The protein has five predicted zinc-finger domains in its C-terminal part and also shares approximately 50% homology with the helical parts of several collagens. Thus, both a regulatory and a structural role in the assembly of collagen fibrils have been suggested.3

Most patients reported with BCS are from countries in the Middle East and North Africa. In 1968, a Norwegian family with two children presenting with clinical features of BCS was described by Bertelsen5 and called dysgenesis mesodermalis corneae et sclerae (MIM 224200). In the present study, we reexamined this family. In this report, we describe the clinical and morphologic features of the affected individuals, and we show an association between the disorder in the family and a missense mutation in the fourth zinc-finger domain of ZNF469.

In contrast to their parents and sibs, both affected individuals had red hair. Genotyping of the melanocortin 1 receptor revealed that the affected individuals were homozygous for a common variant of MC1R associated with red hair that cosegregated with the ZNF469 mutation in this family.

MATERIALS AND METHODS

Family Study

The family was first described in 1968, but was reexamined now as part of the present study. Since 1968, no other affected family members have been detected. Affected and unaffected family members were invited to participate, and all signed an informed letter of consent. The study was approved by the Regional Committees for Medical and Health Research Ethics, Western Norway (IRB 00001872), and adhered to the tenets of the Declaration of Helsinki. The parents, sibs,
and offspring of the affected family members underwent a general medical examination with particular attention to skin, bone, and joints. Ophthalmic examination included slit lamp biomicroscopy, gonioscopy, Goldmann and Icare (Espoo, Finland) tonometry, corneal topography (Pentacam; Oculus Wetzlar, Germany), and axial length measurements (IOLMaster; Carl Zeiss Meditec, Jena, Germany; or CineScan, Quantel Medical, Clermont-Ferrand, France). Radiologic examination of the vertebral column and complete dental examinations including orthopantomograms were performed. Bone mineral density (BMD) was measured in the lumbar spine (L2-L4) and both hips by dual x-ray absorptiometry (Prodigy; GE Healthcare-Lunar, Madison, WI). Biochemical markers for bone turnover in serum, osteocalcin, bone-specific alkaline phosphatase, and C-terminal cross-linking telopeptide of type I collagen were analyzed with kits from Immunodiagnostic Systems Nordic (Herlev, Denmark).

Morphologic Studies

Punch biopsies of upper arm skin were obtained from the affected individuals and from two age- and sex-matched healthy control subjects. For light microscopy, biopsies were fixed in 4% phosphate-buffered formaldehyde and embedded in paraffin, and 6-μm sections were stained with hematoxylin and eosin. For transmission electron microscopy, biopsies were fixed in 2% glutaraldehyde in 0.2 M cacodylate buffer, embedded in Epon and postfixed in OsO₄. Ultrathin sections were stained with 2% uranyl acetate and Reynold’s lead citrate before examination by transmission electron microscopy (JEM 1230; JEOL, Tokyo, Japan).

Homozygosity Mapping

Genomic DNA was isolated from whole blood (QiAmp kit; Qiagen, Hilden, Germany). A genome-wide single nucleotide polymorphism (SNP) scan was performed with a 50K chip (Affymetrix, Santa Clara, CA) and a search for regions of homozygosity was performed with the PLINK program.⁶

DNA Sequencing and Mutation Detection

PCR primers for amplification of exons and flanking intron sequences of ZNF469 were designed on computer (OLIGO software; National Bioscience, Plymouth, MN). DNA was amplified by PCR performed with standard procedures. After PCR amplification, the PCR products were treated with SAP/exonuclease I (Amersham, Chalfont St. Giles, UK) and sequenced with dye termination chemistry (Prism BigDye Terminator kit, ver 1.1; and a 3730 Genetic Analyzer; Applied Biosystems, CA) and a search for regions of homozygosity was performed with the PLINK program.⁶

MC1R Genotyping

Thirteen sequence variants in MC1R of which 11 were associated with red hair were examined by multiplex PCR single-base extension, and electrophoresis with multicolor fluorescence detection.⁷

RESULTS

The family pedigree is shown in Figure 1. Two of five siblings were affected. The parents were first cousins. In addition, genealogical studies revealed that the parents also were more distantly related, as more than 20 common ancestral couples appeared in the pedigree 6 to 13 generations back (data not shown).

Ocular History

Patient IV-4 was first seen at the age of 2 years when she sustained a rupture of the right eye after a minor trauma. She had blue sclerae and very thin corneae. Closure of the wound was unsuccessful, and the eye was enucleated. One year later, she had a rupture of the left eye after falling on the floor. The defect was sutured, and the cornea was covered with a Gundersen conjunctival flap. Visual acuity was light perception, the corneal diameter was 14 mm, and there was excessive thinning of the cornea. At the age of 8, she again had a rupture of her left eye when it was struck with a finger while she was playing. The wound was closed, and she retained light perception. She had varying eye pain, and was treated intermittently with antiglaucoma medication from the age of 8 to 14 years. However, light perception was eventually lost.

Renewed ocular examination at 48 years of age showed extensive scarring and band keratopathy (Fig. 2). In some areas, the cornea seemed to be without scars, but appeared extremely thin, and the inner surface seemed to be coated with a thin iris-like structure. Intraocular pressure measured by Icare tonometry was 14 mm Hg. Ultrasound examination revealed an enlarged eye with no evidence of retinal detachment.

Patient IV-6 had blue sclerae at birth. He had a fracture of the distal end of his right humerus after an injury a few days after birth. He was admitted for an eye examination when at the age of 1 month. He had a thin cornea with a diameter of 11 mm, and myopia of approximately −10 D. One year later, the corneal diameter was 12 mm. The corneal curvature was normal. At the age of 3 years, the corneal diameter was 13 mm, and there was myopia of −23 D. Intraocular pressure was normal. At the age of 3.5 years, he had a rupture of the left eye that was surgically closed. He then had two minor traumas to his right eye at the age of 4 and 8 years, respectively, with rupture of Descemet’s membrane and subsequent development of corneal edema, which persisted from then on. At 9 years of age, he suffered another rupture of the left eye, which again was sutured successfully, but visual acuity was gradually lost. By the age of 10 years, the left eye showed no perception of light. Corneal edema of the right eye varied from day to day, sometimes accompanied by a headache, and glaucoma was suspected. He was intermittently treated with topical timolol and pilocarpine. At the age of 28 years, he had a retinal detachment of his right eye. Despite repeated surgery, the retina remained detached, and vision was subsequently lost.

Renewed ocular examination at 42 years of age revealed a bulging, thin cornea on both eyes (Fig. 2). The right cornea was whitish, and the eye was filled with silicone oil which impaired intraocular examination. Intraocular pressure was between 15 and 21 mm Hg (Icare tonometry). The left cornea...
was scarred in its central part and seemed to be lined with a pigment layer on the internal surface, probably an atrophic iris. Ultrasound B-scan showed retinal detachment in both eyes. The axial length was 26.7 and 22.5 mm of the right and left eye, respectively.

Unaffected family members underwent a thorough ocular examination. Visual acuity, intraocular pressure (Goldmann tonometry), axial length, corneal diameter, and central corneal thickness were all within the normal range. Structurally, the eyes also appeared normal.

Nonocular Features

From childhood, both affected individuals were noted to have chestnut hair, as opposed to their siblings. They also had a more slender stature. Radiologic examination of the vertebral column was first performed at the age of 14 (IV-4) and 8 years (IV-6), respectively. Both had normal bone structure. In IV-4 there was a slight scoliosis with a right/left double curve, a thoracic curve of 9° Cobb angle, apex at T8, and a thoracolumbar curve of 10° Cobb angle, apex at L2. In IV-6, the Cobb angle of the right thoracolumbar single curve scoliosis was 65°, apex at T9. The unaffected individuals III-1, III-2, IV-1, and IV-5 did not have any evidence of structural scoliosis based on radiologic examination. Clinical examination of individuals IV-2 and V-1 produced normal findings with respect to scoliosis.

Involvement of different connective tissues is characteristic of BCS, and it was therefore of interest to examine BMD which had not been measured in patients with BCS previously. BMD of the hip and lumbar spine were in the range of osteopenia and osteoporosis, respectively for both of the affected individuals. Z-scores were lower in affected individuals than in unaffected family members, indicating a BMD lower than expected from age and sex (Table 1).

Dental examination revealed that both of the affected siblings had normal occlusion (Angle class I), but with anterior crowding in the mandible. The incisors showed mineralization disturbances. Linear enamel hypoplasia was especially prominent on the mandibular canines and incisors of the affected woman (Fig. 4). The second premolars in the mandible displayed atypical crown morphology with buccolingual compression and increased mesiodistal diameter (Fig. 4). The panoramic radiographs showed multiple pulp stones especially in molar teeth (data not shown). None of the healthy parents, siblings, or offspring had similar deviations.

Cardiac examination of individual IV-4 revealed aortic valve insufficiency grade I-II, and a slight mitral valve insufficiency. Individual IV-6 had been diagnosed with supraventricular...
tachycardia and had been treated with metoprolol. Cardiac ultrasonography showed a mitral valve insufficiency grade II, and a tricuspid valve insufficiency grade I. None of the valve insufficiencies were of functional importance.

Both affected individuals also presented with a hearing defect. IV-4 had a small conductive hearing loss with a small dip at 1000 Hz and a hearing level at 30 dB. IV-6 had a combined hearing loss with hearing level 40 and 65 dB for the left and right ear, respectively, and no hearing in the higher frequencies above 4000 Hz. The hearing had deteriorated over the years due to a sensorineural hearing loss. IV-6 also had additional problems with vertigo of probable inner ear origin.

Biochemical Analysis

Parathyroid hormone (PTH) and 25-OH vitamin D were normal in all individuals except for IV-6 who had a mild vitamin D deficiency (23 nM; normal range 50 to 113 nM), with slightly increased PTH (9.3 pM; normal range 1.3–6.8 pM). Biochemical markers of bone turnover, osteocalcin, bone-specific alkaline phosphatase and C-terminal cross-linking telopeptide of type I collagen were within the normal range in all individuals.  

Morphologic Studies

Light microscopy findings in the cornea from the enucleated eye of IV-4 have been published in detail. Light microscopy of skin biopsies did not reveal any gross abnormalities of the architecture of the skin. We did not observe the “holes” in the dermis reported by Royce et al. Transmission electron microscopy revealed collagen fibrils in IV-4 to have a normal diameter. In IV-6, we observed minor variation in the diameter of the fibrils, and slightly thinner fibrils could be seen in some areas (data not shown).

Genetic Analysis

Genome-wide testing with SNP markers identified two large candidate regions of homozygosity on chromosomes 9 and 10 and a 5.8-Mb (15 cM) region distal to the SNP marker rs962878 at the terminal end of the long arm of chromosome 16. This region encompassed 114 established and predicted genes, including ZNF469 and MC1R. DNA sequencing of ZNF469 revealed a missense mutation c.10016G>A (Fig. 5). This mutation was not seen in a panel of 185 blood donors (370 chromosomes). The mutation is predicted to alter the first cysteine in the fourth zinc-finger domain of the protein to a tyrosine (p.Cys3339Tyr).

To identify the genetic basis of red hair in this family, 13 sequence variants in MC1R of which 11 were associated with red hair were analyzed. Both affected individuals were homozygous, and both parents and one of the sibs were heterozygous for the most common red hair variant in the Caucasian population, R151C.

DISCUSSION

In the present report we have identified a novel mutation in ZNF469 in the family originally described with “mesodermal dysgenesis of the cornea and sclera” (MIM 224200). The clinical presentation and the morphologic features as well as the observed mutation show that the affected family members have the condition now known as BCS (MIM 229200). Heterozygous individuals appear to be indistinguishable from healthy individuals.
Rupture of the cornea either spontaneously or due to minor trauma is the most dramatic feature of BCS. In 31 of 60 patients with BCS, rupture of the cornea has been reported in 44 instances. The left eyes of IV-4 and IV-6 were surgically repaired after corneal rupture, but then visual function was gradually lost. At present, the atrophic iris appears to be attached to the posterior surface of the cornea, and impaired drainage from the anterior chamber may have occurred. Raised intraocular pressure has not been observed, but interpretation has been difficult due to scarring and the reduced rigidity of the extremely thin cornea. Secondary glaucoma after rupture has been reported in other patients with BCS and is the most likely cause of visual loss in the injured eyes of our patients. Development of corneal edema in patients with BCS has been observed as a consequence of detachment of Descemet's membrane, which is most likely the cause of corneal edema in the right eye of IV-6. Many patients with BCS are myopic. The right eye of IV-6 was myopic (~23 D) and had an axial length of 26.7 mm. The myopia cannot be explained only by the increase in axial length. Keratoglobus with thinning of the cornea and altered corneal curvature are also likely contributors. Patient IV-6 had retinal detachment that eventually led to blindness in his uninjured, right eye. Retinal detachment has rarely been reported in patients with BCS. Most patients, however, have been examined at a young age, and retinal detachments may therefore not have occurred yet.

Involvement of connective tissues in organs other than the cornea is common in BCS, including skin hyperelasticity, joint hypermobility, kyphoscoliosis, and dental anomalies. Clinically, the differential diagnosis of BCS and Ehlers-Danlos syndrome (EDS) type VI can be difficult. Type VI is the kyphoscoliotic form of EDS (MIM 225400) and is associated with mutations in the lysyl-hydroxylase gene. In general, the ocular features are more prominent and the systemic manifestations less severe in BCS than in EDS type VI. BCS was once thought to be a subtype of EDS (type VII), but after the identification of causative mutations in ZNF469, BCS must clearly be considered a separate entity.

BMD values >2 SD below age- and sex-specific mean values were found in the lumbar spine of the two affected persons. This is expected in less than 2% of the normal population. Scoliosis was mild in this part of the vertebral column and is not likely to have affected the results. Individual IV-6 was slightly deficient in vitamin D, and this could partly explain his low BMD. Sedentary lifestyle may predispose for bone loss and reduced peak BMD in adults, but it is not likely to be the cause of reduced BMD in the affected individuals, since they have had regular physical activity, both as children and adults. Reduced BMD measurements have not been reported previously in BCS, but BMD must be determined in additional patients before the conclusion can be reached that reduced BMD is part of BCS.

High levels of biochemical markers for bone turnover, indicating increased remodeling of the trabecular bone, are usually seen in patients with osteoporosis due to bone loss. There were no indications of increased bone turnover in the affected participants of our study. Whether the low BMD observed in BCS patients is due to structural changes in bone or increased bone loss remains to be determined.

Pathologic fractures, a typical feature of osteogenesis imperfecta, is usually not seen in BCS. Although IV-6 had a fracture of his right humerus a few days after birth, he has not had any fractures since. His affected sister IV-4 has never experienced any fracture. Thus, pathologic fractures do not seem to occur in the affected individuals, despite the presence of reduced BMD.

Dental anomalies (dentinogenesis imperfecta) have been described previously in 10 of 60 patients with BCS. The affected individuals examined in the present study both had dentinogenesis imperfecta-like mineralization disturbances, but few other characteristics. In human teeth, there is a broad spectrum of inherited dentin malformations with unknown etiology. The association of a dentin malformation with a mutation in the ZNF469 may therefore add to our understanding of these conditions. Atypical crown morphology is a common observation in many genetic disorders. In our patients, the lower second premolars are deviant, indicating that the defective gene is expressed in the series of epithelial-mesenchymal interactions before mineralization.

The cardiac abnormalities observed are common in healthy individuals. Valve deficiencies have been noted in other patients with BCS including pulmonary stenosis and mitral valve prolapse. In contrast to EDS type VI where arterial ruptures are common, patients with BCS appear to be less susceptible to cardiovasculard problems.

Hearing defects have been observed in 20 of 60 patients with BCS. Conductive hearing loss, as seen in IV-4, sensori-neural hearing loss, and combined hearing loss (individual IV-6) have been reported. The basis for the combined hearing loss is uncertain. A CT scan of IV-6 did not show otosclerotic foci.

The morphology of the skin biopsies was virtually identical with that of healthy controls, both at the light and electron microscopic level, except for minor variation in the diameter of collagen fibrils in one of the affected individuals. Several investigators have examined skin biopsies from patients with BCS. In one report, 40- to 60-μm holes were described in the dermis. Other investigators have observed minor variation in collagen fibril diameter, as seen in IV-6. In general, however, the morphology of the skin has been rather unremarkable with respect to both the gross architecture and the structure of the collagen fibrils.

Testing for homozygosity revealed a candidate region that encompassed the chromosome 16q24 region identified by Abu et al. They sequenced the genes in this region and identified two frameshift mutations in the ZNF469 that were associated with the disease. By sequencing the entire ZNF469, we observed homozygosity for a missense mutation that was predicted to affect the fourth of the five zinc finger domains of ZNF469 by changing the first cysteine to a tyrosine. This cysteine is conserved in many species, including chimpanzee, dog, mouse, and rat, suggesting that the missense mutation could alter a functionally important part of the protein. Our observation thus confirms that mutations in ZNF469 are the likely cause of BCS.

The first clues to the localization of a genetic locus for BCS was offered when red hair was associated with BCS in Tunisian Jews. Red hair is a highly unusual trait in this population. As one affected person did not have red hair, it was assumed that red hair was not a manifestation of a mutant BCS gene, but rather that the loci for the two traits were closely linked and a crossover had occurred. Red hair is not an uncommon trait in the Norwegian population. The sum of the highly penetrant allele variants for red hair R142H, R151C, R160W, D294H, and D84E in Caucasian populations is ~28%. Thus, the chance that a BCS mutation would occur on a chromosome with such a variant is correspondingly high. Since the affected sibs in this family are homozygous for the BCS-MC1R chromosomal region, the chance of observing red hair in BCS is also ~28%. Thus, it is likely that the association of BCS and red hair occurs by chance. Most of the other features seen in BCS are likely to be the result of connective tissue defects, and may represent a pleiotropic effect of the ZNF469 mutations. We cannot exclude, however, that variants in other syntenic genes...
cosegregating with ZNF469 may play a role in the development of the various facets of brittle cornea syndrome.

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References