Mapping of a Gene Causing Brittle Cornea Syndrome in Tunisian Jews to 16q24

Almogit Abu,1,2 Mosbe Frydman,1,3 Dina Marek,1,2 Eran Pras,3,4 Chaim Stolovitch,5,5 Ayala Aviram-Goldring,1,3 Sblomit Rienstein,1 Haike Reznik-Wolf,1 and Elon Pras1,3

PURPOSE. To map the gene that causes brittle cornea syndrome (BCS).

METHODS. Five patients from four families, all of Jewish Tunisian origin, were recruited into the study. Four of the five patients had red hair. DNA from the five patients and 104 control chromosomes was typed with seven 16q polymorphic markers surrounding the hair color gene, MCHR.

RESULTS. A common haplotype in the homozygous state, comprising five markers spanning 4.7 Mb on chromosome 16q24, was found in all five patients but in none of the control subjects (P < 0.00001).

CONCLUSIONS. The gene that causes BCS maps to a 4.7-Mb interval, between the markers D16S3425 and D16S342 on 16q24. (Invest Ophthalmol Vis Sci. 2006;47:5283–5287) DOI: 10.1167/iovs.06-0206

Brittle cornea syndrome (BCS) is an autosomal recessive disease characterized by a thin and fragile cornea that tends to perforate spontaneously or as a result of minor trauma to the eye. Despite the use of protective measures, patients have progressive visual deterioration that often leads to blindness.1–5 Keratoconus, keratoglobus, and a blue sclera are common associated findings.3– 8 BCS also manifests systemic features that include joint hypermobility with occasional dislocations, hyperlaxity of the skin, kyphoscoliosis, a congenital conductive hearing defect, dental abnormalities, and an increased incidence of hernias.7–11 Since the original description by Stein et al.5 more than 60 patients have been reported.

The disease is sometimes confused with Ehlers-Danlos (EDS) type VI also characterized by rupture of the eye, hypermobility of the joints, hyperlaxity of the skin, and autosomal recessive inheritance.1,2 However, reduced lysyl hydroxylase activity is found in most patients with EDS type VI, as opposed to normal enzyme activity in patients with BCS.9–13 EDS type VI is caused by mutations in the PLOD1 gene located on the short arm of chromosome 1, region 36.22.12 The etiology of BCS is unknown. No abnormality was found in types I and III collagens synthesized by cultured fibroblasts, but electron microscopy studies of dermis samples from patients with BCS revealed 20- to 60-μm-wide “holes,” filled with an unidentified amorphous material.1,2

In Israel, BCS has been described mainly among Jews of Tunisian origin. Five families with seven affected children have been reported.3,4,6,8 Of interest, all but one of the Tunisian Jewish patients with BCS had red hair. This finding is in contrast to patients from other ethnic origins who show a normal distribution of hair color. Zlotogora et al.8 have suggested that the BCS gene is closely linked to the locus of a gene responsible for hair color, with linkage disequilibrium in Tunisian Jews. On the basis of this hypothesis, we mapped the BCS gene. We assumed that because BCS is a rare autosomal recessive disorder, most or all the Tunisian Jewish patients are descendants of a common founder and are therefore homozygous for the same mutation. In such a case, examining polymorphic markers very close to the disease gene would reveal a common haplotype in the homozygous state in most or all the patients.

METHODS

Patients and DNA Extraction

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Helsinki committee at the Sheba Medical Center, Israel. The four families (Fig. 1) were recruited at the Sheba Medical Center. Informed consent was obtained from all participants after the nature and possible consequences of the study had been explained. Diagnosis in the patients was based on typical ophthalmic and extracocular BCS findings. One of the families (family B) has been described.5 Twenty milliliters of blood were drawn from each participant, and DNA was extracted by using a commercial kit (Genta System Inc., Minneapolis, MN).

Haplotype Analysis

All seven polymorphic markers were identified by electronically screening genomic clones located on 16q24.1–q24.3. Primers were designed with the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/ primer3/prime3 www.cgi; provided in the public domain by the Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA) and D segment numbers were obtained through the Genome Data Base. Primers and annealing temperatures are provided in Table 1.

Amplification of the polymorphic markers was performed in a 25-μl reaction containing 50 ng of DNA, 13.4 ng of each primer, and 1.5 mM dNTPs in 1.5 mM MgCl2 PCR buffer with 1.2 U Taq polymerase (Bio-Line, London, UK). After an initial denaturation of 5 minutes at 95°C, 30 cycles were performed (94°C for 2 minutes, 56°C for 3 minutes, and 72°C for 1 minute), followed by a final extension of 7 minutes at 72°C. PCR products were electrophoresed on an automated genetic analyzer (Prism 3100; Applied Biosystems, Inc. [ABI], Foster City, CA).

Comparative Genomic Hybridization

Comparative genomic hybridization (CGH) was performed by hybridization of differentially labeled test and normal DNA to normal metaphase chromosomes.13,15 Briefly, normal lymphocyte metaphase prep-
Preparations were denatured at 70°C for 2 minutes in a denaturation solution (70% formamide and 2× SSC [pH 7]), and dehydrated in an ethanol series (70%, 80%, 100%). Test DNAs were labeled with spectrum green dUTP and normal DNAs with spectrum red dUTP, using a nick-end translation-labeling kit (Spectrum; Vysis, Downers Grove, IL). After labeling, 0.5 μg of labeled patient’s and normal subject DNA were ethanol precipitated together, dissolved in 10× hybridization buffer (50% formamide, 10% dextran sulfate, and 2× SSC, [pH 7]), denatured at 75°C for 5 minutes and applied to normal lymphocyte metaphase preparations. The hybridization was performed at 37°C for 3 days in a humid chamber. After hybridization, slides were washed and counterstained with 4,6-diamidino-2-phenylindole (DAPI). Digital image analysis was used to facilitate the identification of chromosomal regions with abnormal fluorescence ratios. Images of the hybridized metaphases were evaluated with a fluorescence microscope (Carl Zeiss Meditec, GmbH, Dossenheim, Germany) interfaced to an image analysis system (Cytovision; Applied Imaging, Newcastle-upon-Tyne, UK). Calculation of test-to-normal fluorescence ratios was performed with the system software (Cytovision; Applied Imaging).

Screening Candidate Genes

For sequencing, the whole coding region and the exon–intron boundaries of the six candidate genes were amplified and sequenced using the genetic analyzer (Prism 3100; ABI). Sequencing included at least 100 bp upstream of the ATG initiation codon and the exon–intron boundaries. Primers and amplification conditions are available on request.

RESULTS

Initially, we obtained DNA samples from four patients with BCS from three unrelated families, all with red hair (Fig. 1, families A, B, and C). Two of the patients were siblings (family B, II:9, II:10). The major gene responsible for red hair color is the melanocortin 1 receptor (MC1R) located on 16q24. We therefore genotyped the four patients with a polymorphic marker (D16S3425) located very close to the MC1R gene. All patients were homozygous for the 204-bp allele. Encouraged by our initial findings, we genotyped the four patients with six additional markers all located on the long arm of chromosome 16. The results are presented in Table 2. All 4 patients were homozygous for a common haplotype comprising alleles of 216, 231, 326, 226, and 229 bp for the markers D16S3421, D16S3420, D16S3419, D16S3421, D16S3422, and D16S3423.

Table 1. Polymorphic Markers from 16q24 and Amplification Parameters

<table>
<thead>
<tr>
<th>Marker</th>
<th>Clone</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Annealing Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D16S3423</td>
<td>AC092275</td>
<td>5'-CACTGCTCAGAGAACAGAGG-3'</td>
<td>5'-GATAGAATTGGAGCTGAGCAGG-3'</td>
<td>56°C</td>
</tr>
<tr>
<td>D16S3424</td>
<td>AC135012</td>
<td>5'-TACTGCTCAGTCATCCCATGAA-3'</td>
<td>5'-CATGTAGATGTAGTCTGCCC-3'</td>
<td>56°C</td>
</tr>
<tr>
<td>D16S3422</td>
<td>AC136285</td>
<td>5'-GGTGCTATTGCTGACACTGTT-3'</td>
<td>5'-TTTTCTCTCTGCTGCCTCTC-3'</td>
<td>56°C</td>
</tr>
<tr>
<td>D16S3420</td>
<td>AC135782</td>
<td>5'-GGGCAACAGAGTGAGATTCTGG-3'</td>
<td>5'-TTAGGCTCATCTTCAGCTTACTGTT-3'</td>
<td>56°C</td>
</tr>
<tr>
<td>D16S3419</td>
<td>AC092125</td>
<td>5'-CCCACTCTACAGAGAGCAGCTT-3'</td>
<td>5'-GAGAGCCAGACCTCAGCTGAAGA-3'</td>
<td>56°C</td>
</tr>
<tr>
<td>D16S3421</td>
<td>AC095460</td>
<td>5'-CCACACTACACAGAGCAGACCT-3'</td>
<td>5'-GAGAGCCAGACCTCAGCTGAAG-3'</td>
<td>56°C</td>
</tr>
</tbody>
</table>

Table 2. Haplotypes of the Carrier Chromosomes from Families A, B, and C

<table>
<thead>
<tr>
<th>Location (Mb)</th>
<th>Cen.</th>
<th>Tel.</th>
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<tr>
<td>83.8</td>
<td>84.7</td>
<td>85.7</td>
</tr>
<tr>
<td>Markers</td>
<td>D16S3423</td>
<td>D16S3424</td>
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</table>

<table>
<thead>
<tr>
<th>A.C.</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C.C.F.</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

- The common ancestral chromosome is shaded. Cen, centromere; tel., telomere; A.C., ancestral chromosome; C.C.F., carrier chromosome in the family.
- The carrier chromosomes in this family were found in two affected siblings.
D16S3419, D16S3420, D16S3422, D16S3424, respectively. This haplotype was not found in any of the 52 control subjects (P < 0.00001). These results indicate that the gene causing BCS in Tunisian Jews maps to 16q24. The common haplotype extended in families A, B, and C to the telomeric end of the chromosome. On the centromeric side, a historic recombination was observed in family B, defining marker D16S3423 as the centromeric boundary of the linkage interval.

At this stage, we obtained DNA samples from family D. The patient in this family (II:17) was the only Tunisian Jewish patient with BCS without red hair. The ancestral chromosome was also found in this patient; however, a paternal recombination was observed distal to the marker D16S3421 (Fig. 2), setting D16S3425 as the new telomeric boundary. The distal recombination between D16S3421 and D16S3425 excludes MC1R from the linkage interval and accounts for the lack of red hair in this patient. The results map the BCS gene to a 4.7-Mb interval on 16q24 between the markers D16S423 and D16S425 (Fig. 3). A most unusual finding was that the patient inherited the carrier chromosome from her father but did not inherit a homologue chromosomal segment from her mother. This result is consistent with a paternal uniparental disomy (UPD) or with a deletion of a part of the long arm of chromosome 16. Typing the family with markers from other chromosomes and additional markers from the short arm of chromosome 16 ruled out nonmaternity and revealed that the deletion/UPD is limited to the long arm of this chromosome (data not shown). Comparative genomic hybridization revealed a normal amount of DNA at the long arm of chromosome 16, thus confirming the diagnosis of a partial chromosomal 16 UPD (Fig. 4).

We also typed the patients with three polymorphic markers located very close to the PLOD1 gene on 1p36.22. Each of the patients displayed a different haplotype in this region, none in the homozygous state, ruling out PLOD1 as the disease-causing gene (data not shown).

Six candidate genes from the interval were sequenced and several changes in the DNA sequence were detected (Table 3). As expected, all the patients displayed the same variants in the homozygous state. However, comparison to the databases or sequencing of control DNA revealed that all these changes are polymorphisms and not the disease-causing mutation.

**DISCUSSION**

We have mapped a gene causing BCS in Tunisian Jews to a 4.7-Mb interval on 16q24. Mapping a gene locus in such a small
group of patients was achievable thanks to a unique feature common to most of the patients: red hair. We took advantage of the fact that red hair cosegregated with the disease and showed that in these patients the two loci are located very close to each other. Of note, in another study from Israel, Levy and Glovinsky\(^2\) found an association between red and blonde hair color and pigmentary glaucoma (PG). Nineteen of 35 patients with PG had red or blonde hair compared with only 16 with black hair. The prevalence of red and blonde hair in Israel is between 3% and 5%. These results may reflect the close proximity between one of the genes responsible for hair color and the gene that causes PG, or a causative association.

Of the 86 genes expressed in the interval (http://www.ncbi.nlm.nih.gov/mapview/maps.cgi/ National Center for Biotechnology Information, Bethesda, MD), we sequenced six candidates. Procollagen type III N-endopeptidase is a matrix metalloproteinase. Metalloproteinases are a functionally diverse group of enzymes that are involved in critical stages of many biological processes, including collagen synthesis which in turn forms the fibrous scaffold of the extracellular matrix of tissues.\(^9\)\(^,\)\(^10\) Tubulin \(\beta-3\) is the major component of microtubules and elements of the cytoskeleton in eukaryotic cells.\(^2\) Microtubule-associated protein 1, light chain \(3\beta\) is involved in the filamentous cross-bridging between microtubules and other cytoskeletal elements.\(^2\)\(^,\)\(^2\) Solute carrier family 7, member 5 encodes a protein necessary for system L-amino acid transport that is thought to be a major route by which cells import large neutral amino acids with branched or aromatic side chains.\(^4\)\(^,\)\(^2\) Ribosomal protein L13 is a component of the 60S ribosomal subunit and is highly expressed in the cochlea and corneal epithelial cells.\(^5\) and KIAA0182 protein R1082R (nt : a\(\rightarrow\)t) is a hypothetical gene of unknown function with very high expression levels in the cornea.\(^2\) Even though we did not find any significant sequence alterations in these genes, we cannot completely rule out promoter or intrinsic variants in unsequenced regions, as the cause of the disease.

The single ethnic origin of all our patients, in combination with red hair was essential for the mapping process. However, the fact that all the patients will most probably have the same mutation, may impose severe limitations on future efforts to identify the disease-causing gene, especially if the mutation lies in the promoter or in an intronic sequence. Expanding the cohort of patients to other ethnic origins will most likely overcome this problem.

In a unique and surprising finding in the patient from family D, we detected a partial paternal UPD of chromosome 16. Partial UPDs are an uncommon finding, but have been described before.\(^2\)\(^,\)\(^2\) Of interest, in this family, only the father was a carrier, but duplication of the chromosomal segment containing one copy of the mutant gene left the patient without a normal copy, thus leading to a disease state.

Blue sclera, one of the characteristic features of BCS, is thought to be caused by reduced thickness, to one third or less of its normal size. Three other diseases have been described in association with blue sclera and fragile cornea. EDS, osteogenesis imperfecta, and Marfan syndrome manifest as connective tissue disorders with systemic signs similar to BCS. In all three, the underlying molecular basis has been revealed: EDS type VI is caused by deficient cross-linking of collagen fibers,\(^5\) osteogenesis imperfecta is caused by mutations in type I collagen,\(^3\) and Marfan syndrome is caused by mutations in the fibrillin gene.\(^2\) It is intriguing to speculate that BCS is caused by a defect in a biochemical pathway common to one or more of these diseases.

Cloning of the BCS gene will increase our understanding of the molecular pathways involved in the pathogenesis of corneal and connective tissue diseases and will enable prenatal diagnosis in affected families.

### References


