Bothnia Dystrophy Caused by Mutations in the Cellular Retinaldehyde-Binding Protein Gene (RLBPI) on Chromosome 15q26

Marie S. I. Burstedt, Ola Sandgren, Gösta Holmgren, and Kristina Forsman-Semb

PURPOSE. To determine the chromosomal location and to identify the gene causing a type of retinitis punctata albescens, called Bothnia dystrophy, found in a restricted geographic area in northern Sweden.

METHODS. Twenty patients from seven families originating from a restricted geographic area in northern Sweden were clinically examined. Microsatellite markers were analyzed in all affected and unaffected family members. Direct genomic sequencing of the gene encoding cellular retinaldehyde-binding protein was performed after the linkage analysis had been completed.

RESULTS. Affected individuals showed night blindness from early childhood with features consistent with retinitis punctata albescens and macular degeneration. The responsible gene was mapped to 15q26, the same region to which the cellular retinaldehyde-binding protein gene has been assigned. Subsequent analysis showed all affected patients were homozygous for a C to T substitution in exon 7 of the same gene, leading to the missense mutation Arg234Trp. Analysis of marker haplotypes suggested that all cases had a common ancestor who carried the mutation.

CONCLUSIONS. A missense mutation in the cellular retinaldehyde-binding protein gene is the cause of Bothnia dystrophy. The disease is a local variant of retinitis punctata albescens that is common in northern Sweden due to a founder mutation.

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal dystrophies characterized by early night blindness and later loss of peripheral and central vision. Pigment deposition in the retina and attenuation of the retinal blood vessels are observed. The diagnosis is confirmed by an abnormal or extinguished electroretinogram (ERG). Retinitis pigmentosa loci have been mapped to numerous chromosomal locations, and mutations in many different genes have been found in patients. An updated list of disease loci is available on the RetNet web site: http://utsph.sph.uth.tmc.edu/retnet.

A unique atypical variant of RP, known among clinicians in northern Sweden for decades as Västerbotten dystrophy or Bothnia dystrophy (refers to the area adjacent to the Gulf of Bothnia), is described in this report. Affected individuals have had night blindness since early childhood, retinitis punctata albescens (RPA), and macular degeneration. Retinitis punctata albescens is associated with RP and characterized by numerous punctate whitish-yellow spots in the fundus. We demonstrate by linkage analysis that the disease gene is localized to 15q26. Subsequently we show that a missense mutation in the cellular retinaldehyde-binding protein gene (RLBPI) is present in a homozygous state in 20 patients from seven Västerbotten families with Bothnia dystrophy.

METHODS

Ophthalmologic Examinations

Inclusion criteria for the families in this study were ophthalmologic records of retinal disease and more than one family member showing early onset night blindness, fundus appearance similar to RPA with small white dots in central fundus, macular degeneration, and lack of for RP typical bone spicules in peripheral retina. Standard ophthalmologic examination and fundus photography were carried out in all affected individuals and selected siblings. Dark adaptation tests and full-field ERGs were performed in selected cases. The study followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all subjects.

Genotyping

Extraction of genomic DNA and analysis of microsatellite markers were performed as described by Balciuniene et al.
Linkage Analysis

Linkage analyses were performed using the LINKAGE computer package, version 5.10, under the assumption of an autosomal recessive gene with 95% penetrance and a population frequency of 0.0158. Pedigrees 004 and 013 were each separated into two parts for linkage analysis. Pedigree 004a contained individuals IV:1-2 and V:1-4, and pedigree 004b contained II:6-7 and III:8-15. Pedigree 013a comprised individuals III:1-2 and IV:1-4, and pedigree 013b contained IV:7-8, V:1-4, and VI:1-4.

Mutation Analysis

Polymerase chain reaction (PCR) fragments for single-strand conformation polymorphism (SSCP) and RNase cleavage assays were amplified from genomic DNA. All primer sequences have been previously described. Nonisotopic RNase cleavage assays (NIRCA) were performed according to the manufacturer's instructions (Ambion). RNase cleavage of a 1044-bp fragment amplified with primers 7F and 8S indicated a mismatch in the heteroduplex formed between patient and control templates. SSCP was therefore performed on PCR fragments specific for exons 7 and 8. PCRs were performed in a final volume of 20 μl by mixing 20 ng DNA with 0.15 pmol to 0.5 pmol 32P end-labeled primers and 0.5 U of Dynazyme DNA polymerase (Finnzymes) in Dynazyme buffer containing 200 μM of each nucleotide. The standard temperature profile was 1 cycle at 95°C, 4 minutes, followed by 35 cycles at 95°C, 30 seconds, 55°C, 30 seconds, 72°C, 30 seconds and one cycle at 95°C, 30 seconds, 55°C, 30 seconds, and 72°C for 5 minutes. An equal volume of formamide dye mix was added and the DNA denatured at 95°C for 4 minutes. The PCR products were separated on 6% non-denaturating polyacrylamide-bis (49:1) gels using 0.5× Trisborate EDTA as gel buffer. DNA sequencing of exon 7 was performed on PCR fragments amplified with primers 7F and 8S. Fragments were purified by ammonium sulfate precipitation followed by spin column purification with Microcon concentrators (Amicon) before sequencing with primers 7F and 7R on an ABI 377 sequencer (Applied Biosystems, Perkin Elmer).

RESULTS

Seven families from a restricted geographic area in northern Sweden were included in the study (Fig. 1). At least two affected individuals in each pedigree had a nonsyndromic type of retinal degeneration that was apparently inherited in an autosomal recessive way (Fig. 2). All patients reported night blindness as one of the first symptoms. Dark adaptation thresholds were markedly elevated in affected cases. RPA changes in fundus were predominantly found in younger individuals, and all families had one member or more with ophthalmoscopic findings of RPA (Fig. 3A). The oldest family member with RPA changes was a 55-year-old man (family 005, case IV:4). Macular degeneration most often presented as a central areolar atrophy and was observed in most patients older than 30 years of age (Fig. 3B). Visual acuity was reduced to legal blindness in most cases. The onset of visual loss could occur as early as before the teen years. However, one individual, a female, had a visual acuity of 0.7 RE and 0.9 LE and a mild central maculopathy at the age of 50 years (family 65, case IV:6). Disturbances in both peripheral and central vision were found. Visual field testing showed paracentral and central scotomas. A mottled appearance of the fundus could often be seen in the midperiphery (Fig. 3C). Peripheral retinal degeneration, with the appearance of circular confluent atrophic areas, was a common finding among adults, giving a generally atrophic picture of the fundus in advanced stages of the disease (Fig. 3D). Electrophysiological examinations typically showed initial impairment of scotopic ERG and progressively affected photopic ERG. A more detailed description of clinical findings in these patients will be published elsewhere.

All seven families with Bothnia dystrophy were screened for linkage to candidate gene regions, using 60 microsatellite markers located close to known loci for RP or other retinal genes. Several loci including the peripherin and rhodopsin genes could be excluded. Two-point lod score analyses initially showed positive lod scores with the marker FES, located on chromosome 15q26.1 close to the gene for cellular retinaldehyde-binding protein (RLBP1). Further genotyping was therefore performed with the markers D15S526, D15S116, and D15S202, all located within 1 cM of the gene and FES (Fig. 4). Recombinations in informative meioses restricted the disease gene to a 1.2-cM interval between the markers FES and D15S526. D15S202 and D15S116, which are located on the same yeast artificial chromosome contig as the RLBP1 gene, showed no recombination in relation to the disease phenotype. Lod scores for the marker D15S116 are shown in Table 1.

![Figure 1. Map of the county of Vasterbotten. Black dots show the birthplace of parents to the patients. The hatched area in inset map shows the location of Vasterbotten County in Scandinavia.](image-url)
FIGURE 2. Pedigrees of the seven families included in this study.

FIGURE 3. Fundus photographs of family 013. See pedigree in Figure 2. (A) Case VI:1, female, age 23 years. Central fundus with RPA. (B) Case V:4, female, age 39 years. Central fundus with RPA and maculopathy. (C) Case V:3, female, age 49 years. Midperiphery with pigment mottling. (D) Case IV:4, male, age 49 years. Midperiphery with atrophic areas.
The county of Västerbotten has the highest frequency of retinal dystrophies in Sweden according to the Swedish RP register (unpublished data). A large subset of RP patients in this region was found to have a distinctive phenotype known as Bothnia dystrophy. Patients report early night blindness, RPA, and macular degeneration with progressive reduction of visual acuity and visual fields. To identify the underlying genetic defect in this patient group, we screened for linkage to chromosomal regions already known to contain RP genes. Strong evidence for linkage (lod score 7.79 at \( \theta = 0 \)) was detected with the microsatellite marker D15S116, located close to the gene encoding cellular retinaldehyde-binding protein (RLBP1). Molecular genetic analysis showed that all affected subjects were homozygous for a C to T substitution in exon 7 of the RLBP1 gene, leading to an arginine to tryptophan substitution at amino acid 234 (R234W). There are currently no data available to demonstrate a functional role for this amino acid, but the conservation of the residue in a family of related proteins suggests that it has a functional significance. A missense mutation in another conserved residue of cellular retinaldehyde-binding protein (CRALBP; R150Q) abolished binding to 11-cis-retinaldehyde. The mutation was shown to be associated with an atypical form of autosomal recessive RP in a small consanguineous Indian family.

CRALBP is expressed in the retinal pigment epithelium (RPE) and the Müller cells in the retina, two cell types bordering the proximal and distal ends of photoreceptors. The RPE plays a crucial role in the visual cycle and the regeneration of the retinoid 11-cis-retinaldehyde. In RPE the esterification of the photoisomerization product all-trans-retinaldehyde produces all-trans-retinyl palmitate, which is converted to 11-cis-retinol and palmitate. In vitro studies show that the protein CRALBP binds either 11-cis-retinol or 11-a's-retinaldehyde. It promotes the oxidation of 11-cis-retinol to 11-cis-retinaldehyde to be recycled for visual pigment synthesis in photoreceptors. It also diminishes the regeneration of the retinoid 11-cis-retinaldehyde.

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### Table 1. Two-Point Lod Scores between Bothnia Dystrophy and D15S116

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A6CCN6  G T T C
A 6 C C T G G T T C

A
unaffected
sib
unaffected
parent
affected

B Family 013 *-*TQ Family 004
totransduction and the amount of 11-cis-retinyl ester stored in the RPE. An attractive model for the pathogenesis of Bothnia dystrophy is that the R234W mutation would lead to a lack of 11-cis-retinaldehyde and thereby an impairment of rod function manifested as early night blindness. In addition, a toxic accumulation of retinyl ester in the RPE could lead later to a defect of the RPE and photoreceptor cell death manifested as peripheral and central degeneration.

Previously, only one RLBP1 mutation in a single family has been described, and it is currently not known what proportion of patients have retinal dystrophy caused by RLBP1 mutations. The R234W mutation was also reported in one of our patients by an independent study. In addition, three novel RLBP1 mutations were detected in patients with RPA and a phenotype very similar to that seen in Bothnia dystrophy patients. In summary, five RLBP1 mutations have been described to date, and it is therefore clear that mutations in this gene are not a rare finding in isolated families. In this study we identified 20 familial cases, and the frequency of patients in Västerbotten would therefore be 1 in 12,000 (given a population of 240,000 individuals). Preliminary data suggest that there may be roughly 20 sporadic cases with the same phenotype in the region, giving an estimated frequency of 1 in 6000. However, this may not reflect the proportion of RLBP1 mutations in other populations but is probably the result of a founder effect. All patients were homozygous for the R234W mutation and for a given allele at the D15S202 locus. Furthermore, 36 of 40 patient chromosomes carried a rare allele at the D15S116 locus (found on 3/40 chromosomes in the general Västerbotten population), demonstrating a strong linkage disequilibrium between the two alleles ($P < 10^{-6}$). It will be of both scientific and diagnostic value to investigate how common this mutation is in a larger RP patient population in Sweden.

The possibility that recycling of retinoids in the retina might be impaired in Bothnia dystrophy patients might give clues to future therapeutic possibilities. Because of the large clinically well-characterized set of patients with this disease defined genetically in the present study, our patient population may constitute a suitable study group.

**Acknowledgments**

The authors thank Thaddeus Dryja and Sten Andreasson for sharing their unpublished data on one of our patients and Eleonora Westermark for excellent technical assistance. The Nordic microsatellite marker bank supported by the Nordic council of ministers supplied oligonucleotides.

**References**

Recessive Mutations in the RLBPI Gene Encoding Cellular Retinaldehyde-Binding Protein in a Form of Retinitis Punctata Albescens

Hiroyuki Morimura,1 Eliot L. Berson,2 and Thaddeus P. Dryja1,2

PURPOSE. To determine the frequency and spectrum of mutations in the RLBPI gene encoding cellular retinaldehyde-binding protein (CRALBP) in patients with hereditary retinal degeneration.

METHODS. The single-strand conformation polymorphism (SSCP) technique and a direct genomic sequencing technique were used to screen the coding exons of this gene (exons 2-8) for mutations in 324 unrelated patients with recessive or isolate retinitis pigmentosa, retinitis punctata albescens, Leber congenital amaurosis, or a related disease. Variant DNA fragments revealed by SSCP analysis were subsequently sequenced. Selected alleles that altered the coding region or intron splice sites were evaluated further through segregation analysis in the families of the index cases.

RESULTS. Four novel mutations were identified in this gene among three unrelated patients with recessively inherited retinitis punctata albescens. Two of the mutations were missense: one was a frameshift, and one affected a canonical splice donor site.

CONCLUSIONS. Recessive mutations in the RLBPI gene are an uncommon cause of retinal degeneration in humans. The phenotype produced by RLBPI mutations seems to be a form of retinitis punctata albescens. (Invest Ophthalmol Vis Sci. 1999;40:1000-1004)

The first step in vision occurs when a photon converts an 11-cis retinal chromophore, which is covalently linked to rod or cone opsins, to the all-trans isomer.1 In mammals, this reaction takes place in the outer segments of the photoreceptor cells of the retina. The all-trans chromophore subsequently leaves rod or cone opsin and travels as all-trans-retinol to a neighboring retinal pigment epithelial cell,2 where it is converted through a series of intermediates back to 11-cis-retinaldehyde. The protein CRALBP seems to play a role in this pathway. CRALBP is present in the retinal pigment epithelium and the Müller cells of the retina.3 The protein forms complexes with regenerated 11-cis-retinaldehyde and its immediate precursor 11-cis-retinol.4,5 Still uncertain is whether these complexes are essential intermediates in this pathway or whether CRALBP has another physiological role.

The requirement for CRALBP in the retina was highlighted by a recent report of the missense mutation Arg150Gln in the RLBPI gene (chromosome 15q26)6 in a family from India with a recessive retinal degeneration that was termed retinitis pigmentosa.7 The mutation was homozygous in the affected members of an 11-member sibship but not in the unaffected members of an 11-member sibship that was the product of a first-cousin marriage. Evidence for the pathogenicity of this mutation came from the observations that the mutant protein in vitro was less water soluble than normal and that it did not bind to 11-cis-retinaldehyde.7 In the present report, we provide additional evidence, through the identification of mutations in patients of European ancestry, that recessive defects in the human RLBPI gene are pathogenic. Furthermore, the clinical findings from our cases and those in the report by Burstedt et al8 in this issue suggest that the phenotype can be distinguished from typical retinitis pigmentosa.

METHODS

Ascertainment of Patients

This study, which involved human subjects, conformed to the tenants of the Declaration of Helsinki. The index patients in...