A new genetic locus for X linked progressive cone-rod dystrophy

R Jalkanen, F Y Demirci, H Tyynismaa, T Bech-Hansen, A Meindl, M Peippo, M Mäntyjärvi, M B Gorin, T Alitalo

X linked progressive cone-rod dystrophy (COD) is a retinal disease primarily affecting the cone photoreceptors. The disease is genetically heterogeneous and two loci, COD1 (Xp21.1-11.4) and COD2 (Xq27.2-28), have been previously identified. COD1 was recently shown to be caused by mutations in RPRG exon ORF15 (Xp2.1), the gene that is also responsible for RP3 type retinitis pigmentosa. In this study, we performed a linkage study to map the disease gene in a large Finnish family with X linked cone-rod dystrophy, using a panel of 39 X chromosomal markers. Several recombinations between the disease gene and markers in the Xp21.1-p11.4 region have excluded COD1 as a candidate locus in this family. Consistent with the linkage results, no mutation was detected by direct PCR sequencing of the coding region of RPRG, including exon ORF15. The COD2 locus has been also excluded as the site of the gene on the basis of negative lod score values obtained for COD2 linked markers. The disease causing gene of the studied COD family has been localised between the markers DXS10042 and DXS8060 on Xp11.4-q13.1. Positive pairwise lod scores >3 were obtained for markers DXS993, MAOB, DXS1055, and DXS1194. Since this locus is distinct from the previously identified two loci, COD1 and COD2, our results establish a new third genetic locus for X linked progressive cone-rod dystrophy and further expands our knowledge about the genetic heterogeneity underlying this disease entity.
shown in fig 1. Clinical studies of the family members have been published elsewhere, including a complete pedigree of the family. The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants in accordance with the requirements of the University of Kuopio Ethics Committee. DNA was extracted from the collected blood samples using a non-enzymatic method.

DNA markers and linkage analysis
A total of 50 family members, including seven affected males, were genotyped using 37 microsatellite markers from the Xp22.32-q28 region and two intragenic SNPs from the RPGR gene. Primer sequences for most of the microsatellite markers were obtained from the Genome Database (http://www.gdb.org). The following two primer pairs were used to obtain the PCR amplified genomic fragments that currently harbour four intronic SNPs (rs3896245, rs3888228, rs3891252, and rs3015258) from the NCBI SNP database: rs1111401F, 5′-GCATGTCCATTTGAGTACAAAG-3′; rs1111401R, 5′-CATGTCTTTGCTTGGTGTTG-3′; rs1075939/rs1079728F, 5′-CCAGGTTCAAGCGATTCTC-3′; rs1075939/rs1079728R, 5′-ATGAAGGCCCTGAAATTACC-3′. The genomic fragment, which was amplified with rs1111401-primers, is located in intron 17 and contains the SNP rs3896245. The fragment amplified with rs1075939/rs1079728 primers lies in intron 18 and contains SNPs rs3888228, rs3891252, and rs3015258. The SNP rs3888228 was used in the linkage analysis, as well as the previously undescribed SNP A/T (named here SNP1) which is located in the same PCR fragment as rs3896245, 101 bp upstream from it. Allele frequencies for SNP1 are 0.27 for A and 0.73 for T. PCR conditions were as follows: 94°C for 10 minutes, followed by 35 cycles of 94°C for one minute, 56°C for one minute, and 72°C for one minute, followed by a final extension at 72°C for 10 minutes. SNPs were evaluated using single strand conformational analysis (SSCA) and gels were visualised by silver staining.

Two new microsatellite markers (CA repeats) were identified from the Genbank sequence (accession number AC006121). Primers for these markers are: CA20F, 5′-GAAGGTAAGTTGTGATGTGAGCTG-3′; CA20R, 5′-AAACAACTCTCTTGGCTTTACTCC-3′; CA23F, 5′-GAAACAGCAACCAATACTCAA-3′; CA23R, 5′-GCCCTATGGTAATGCCCT-3′. Markers CA20 and CA23 are located 165 kb and 212 kb distal to DXS993, respectively. Expected heterozygosity, which was calculated using allele frequencies of 50 normal male controls, was 92% for CA23 and 92.7% for CA20. Markers were amplified by PCR and the products were separated on 6% polyacrylamide gels. PCR conditions were similar to those described above, except that the annealing temperature was 59°C and the number of extension cycles was 32. Two point linkage analysis was performed using the MLINK option of FastLink package, version 4.1P. X linked inheritance with full penetrance and a disease allele frequency of 0.00001 were assumed. Allele frequencies for microsatellite markers were obtained from the healthy members of the Finnish COD family.

Mutation analysis
An affected family member and an obligate carrier female were included in the mutation analysis. A total of nine genes, mapping to the Xp21.1-Xq12 region, were analysed, including
ABI PRISM (Germany). Sequencing reactions were performed using the QIAquick PCR Purification kit (Qiagen GmbH, Hilden, Germany). Sequencing all the exons. PCR fragments were purified with Edge (Applied Biosystems, Foster City, CA) and purified with Edge (Applied Biosystems, Foster City, CA) and purified with Edge

In eight of our patients the onset of X linked progressive cone-rod dystrophy was in childhood and in two patients evidently in adulthood. All of the patients showed decreased visual acuities, moderate to high myopia, red or red-green colour vision defects, central scotomas (in some patients also concentric constriction in visual fields), affected cone and rod thresholds in dark adaptation, and diminished cone or cone-rod responses by full field ERG. The eye fundi showed irregular pigmentation or myopic changes in the macular area.

The 12-14 year follow up study of four patients did not show significant progression of the disease. The six obligate carriers who were examined had normal vision.

### Linkage data

Linkage analysis was performed using 39 markers covering both the COD1 and COD2 regions (Xp22.32-q28). Results of the two point linkage analysis are summarised in table 1. Close linkage with no recombinations was observed between cone-rod dystrophy and markers CA23, CA20, DXS993 (Zmax=3.90 at \( \theta \approx 0.00 \)), DXS8012, DXS1201, MAOB (Zmax=3.48 at \( \theta \approx 0.00 \)), DXS1055 (Zmax=3.18 at \( \theta \approx 0.00 \)), DXS1194 (Zmax=4.10 at \( \theta \approx 0.00 \)), DXS1275, and DXS559. The closest flanking markers, which showed recombinations with respect to the disease locus, were DXS10042 on the distal side and DXS8600 on the proximal side. The distance between these flanking markers is approximately 35 cm (estimated from the Marshfield Comprehensive human genetic map in url: http://research.marshfieldclinic.org/genetics/). Recombinations clearly in adulthood. All of the patients showed decreased visual acuities, moderate to high myopia, red or red-green colour vision defects, central scotomas (in some patients also concentric constriction in visual fields), affected cone and rod thresholds in dark adaptation, and diminished cone or cone-rod responses by full field ERG. The eye fundi showed irregular pigmentation or myopic changes in the macular area. The 12-14 year follow up study of four patients did not show significant progression of the disease. The six obligate carriers who were examined had normal vision.

### RESULTS

#### Clinical studies

The results of thorough clinical examinations have been published elsewhere. The complete family pedigree included 10 affected males, of whom seven participated in genetic studies. In eight of our patients the onset of X linked progressive cone-rod dystrophy was in childhood and in two patients clearly in adulthood. All of the patients showed decreased visual acuities, moderate to high myopia, red or red-green colour vision defects, central scotomas (in some patients also concentric constriction in visual fields), affected cone and rod thresholds in dark adaptation, and diminished cone or cone-rod responses by full field ERG. The eye fundi showed irregular pigmentation or myopic changes in the macular area. The 12-14 year follow up study of four patients did not show significant progression of the disease. The six obligate carriers who were examined had normal vision.

### Linkage data

Linkage analysis was performed using 39 markers covering both the COD1 and COD2 regions (Xp22.32-q28). Results of the two point linkage analysis are summarised in table 1. Close linkage with no recombinations was observed between cone-rod dystrophy and markers CA23, CA20, DXS993 (Zmax=3.90 at \( \theta \approx 0.00 \)), DXS8012, DXS1201, MAOB (Zmax=3.48 at \( \theta \approx 0.00 \)), DXS1055 (Zmax=3.18 at \( \theta \approx 0.00 \)), DXS1194 (Zmax=4.10 at \( \theta \approx 0.00 \)), DXS1275, and DXS559. The closest flanking markers, which showed recombinations with respect to the disease locus, were DXS10042 on the distal side and DXS8600 on the proximal side. The distance between these flanking markers is approximately 35 cm (estimated from the Marshfield Comprehensive human genetic map in url: http://research.marshfieldclinic.org/genetics/). Recombinations clearly in adulthood. All of the patients showed decreased visual acuities, moderate to high myopia, red or red-green colour vision defects, central scotomas (in some patients also concentric constriction in visual fields), affected cone and rod thresholds in dark adaptation, and diminished cone or cone-rod responses by full field ERG. The eye fundi showed irregular pigmentation or myopic changes in the macular area. The 12-14 year follow up study of four patients did not show significant progression of the disease. The six obligate carriers who were examined had normal vision.

### RESULTS

#### Clinical studies

The results of thorough clinical examinations have been published elsewhere. The complete family pedigree included 10 affected males, of whom seven participated in genetic studies. In eight of our patients the onset of X linked progressive cone-rod dystrophy was in childhood and in two patients clearly in adulthood. All of the patients showed decreased visual acuities, moderate to high myopia, red or red-green colour vision defects, central scotomas (in some patients also concentric constriction in visual fields), affected cone and rod thresholds in dark adaptation, and diminished cone or cone-rod responses by full field ERG. The eye fundi showed irregular pigmentation or myopic changes in the macular area. The 12-14 year follow up study of four patients did not show significant progression of the disease. The six obligate carriers who were examined had normal vision.

### Linkage data

Linkage analysis was performed using 39 markers covering both the COD1 and COD2 regions (Xp22.32-q28). Results of the two point linkage analysis are summarised in table 1. Close linkage with no recombinations was observed between cone-rod dystrophy and markers CA23, CA20, DXS993 (Zmax=3.90 at \( \theta \approx 0.00 \)), DXS8012, DXS1201, MAOB (Zmax=3.48 at \( \theta \approx 0.00 \)), DXS1055 (Zmax=3.18 at \( \theta \approx 0.00 \)), DXS1194 (Zmax=4.10 at \( \theta \approx 0.00 \)), DXS1275, and DXS559. The closest flanking markers, which showed recombinations with respect to the disease locus, were DXS10042 on the distal side and DXS8600 on the proximal side. The distance between these flanking markers is approximately 35 cm (estimated from the Marshfield Comprehensive human genetic map in url: http://research.marshfieldclinic.org/genetics/). Recombinations clearly in adulthood. All of the patients showed decreased visual acuities, moderate to high myopia, red or red-green colour vision defects, central scotomas (in some patients also concentric constriction in visual fields), affected cone and rod thresholds in dark adaptation, and diminished cone or cone-rod responses by full field ERG. The eye fundi showed irregular pigmentation or myopic changes in the macular area. The 12-14 year follow up study of four patients did not show significant progression of the disease. The six obligate carriers who were examined had normal vision.
were observed in one patient and one obligate carrier (distal region) and in two patients and one obligate carrier (proximal region, fig 1).

Negative lod score values were obtained with all markers of the COD2 region, thus excluding the Xq27-q28 region (table 1). Recombinations (in one affected and one unaffected subject) were also found between the disease gene and markers of the COD1 locus, including an intragenic SNP SNP1, of the RPGR gene, also excluding the COD1 locus as the site of the causative gene in the Finnish cone-rod dystrophy family (table 1).

Based on the information obtained from church registers, it was previously concluded that the disease segregating in the Finnish family was most likely inherited from the female I.2. However, in this study we could observe two distinct non-recombinant haplotypes (and one recombinant haplotype), in addition to the disease haplotype, in obligate carriers of the second generation, indicating that the disease haplotype was inherited from the father I.1 (fig 1).

**Mutation screening results**

A total of nine candidate genes from the Xp21.1-Xq12 region was included in the mutation analysis: the disease genes for COD1 and retinitis pigmentosa 3 (RPGR), retinitis pigmentosa 2 (RP2), congenital stationary night blindness (NYX), Norrie disease (NDP), and five other genes, DDX3, GPR34, I-4, TIMP1, and ARR3 (fig 2). No disease causing mutations were identified in any of these genes. All the identified sequence alterations were located in intronic or untranslated regions and were also observed in 50 normal male control samples, indicating that the changes were polymorphisms instead of disease causing mutations (data not shown). The coding region (exons and flanking exon-intron junctions) of RPGR, including exon ORF15, was also sequenced in the DNA from one of the affected family members but no alterations were found.

**DISCUSSION**

In this study we have identified a new and third locus for X linked progressive cone-rod dystrophy. Linkage data suggest that the gene is localised to the centromeric region of the X chromosome, between markers DXS10042 and DXS8060. The critical interval corresponds to a 35 cm region localised to Xp11.4-Xq13.1. The data clearly exclude the COD1 and COD2 loci as sites of the causative gene for cone-rod dystrophy in the family evaluated in this study. In addition to RPGR, eight candidate genes were screened but no mutations were identified.

The primary symptoms of our patients were decreased visual acuity, moderate to high myopia, and red or red-green colour vision defects. In addition, the onset of the disease was usually in childhood and the progression of the disease was slow. Table 2 summarises the clinical characteristics of the X linked recessive COD families for which the genetic locus has been assigned. Meire et al reported similar symptoms and similar fundus and ERG findings in six related male patients with X linked cone-rod dystrophy. In dark adaptation, the cone-rod thresholds were missing and also in one of their patients rod threshold was slightly raised as in our patients. However, in the oldest patient of Meire et al (86 years), colour vision defect had progressed to acquired achromatopsia, while our oldest patient (81 years) had only red-green defect. The visual field defects were also different; the patients of Meire et al had central scotomas while some of our patients had both central and peripheral defects. Their candidate gene region (Xp21.1-p11.3) overlaps partly with ours, but because it also encompasses the RP3 region, it is still possible that their patients have a mutation in the RPGR gene. Our family also shows similarities to the COD1 families with known RPGR mutations, with respect to disease onset and progression and association with myopia.

The clinical features of COD2 (Xq27.2-q28) are also very similar to those of COD1, although the early stages in COD2 family members were characterised by peripheral cone...
Table 2  The genetic and clinical features of X linked recessive COD loci

<table>
<thead>
<tr>
<th>Locus</th>
<th>Xp21.1-11.3, may be either COD1 (left column) or the newly described locus (right column)</th>
<th>Xp11.4-q13.1 (new locus)</th>
<th>Xq27.2-q28 (COD2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>Bergen et al,¹² Meire et al,¹³ Mäntyjärvi et al,³ this study</td>
<td>Pinckers and Timmerman,¹⁴</td>
<td>Bergen and Pinckers¹⁵</td>
</tr>
<tr>
<td>General ophthalmological characteristics</td>
<td>Onset within the first three decades and usually before the age of 20</td>
<td>Onset within the first three decades and usually in childhood</td>
<td>Onset in early childhood</td>
</tr>
<tr>
<td></td>
<td>Gradual progression of visual loss, photophobia, moderate to high myopia</td>
<td>Gradual progression of visual loss, high myopia</td>
<td>Very slow progression of visual loss moderate to high myopia</td>
</tr>
<tr>
<td>Visual fields</td>
<td>Generalised depression in younger patients, central scotomas, peripheral dysfunction in few cases</td>
<td>Central scotomas</td>
<td>Central scotomas</td>
</tr>
<tr>
<td></td>
<td>Red-green defects, no colour perception in advanced cases</td>
<td>Red or type I red-green defects</td>
<td>Type I red-green defect, primarily red cones are affected, no colour perception in advanced cases</td>
</tr>
<tr>
<td>Electroretinogram (ERG)</td>
<td>Severe cone dysfunction early in life, moderately reduced rod responses in all ages groups</td>
<td>Severe cone dysfunction, reduced rod responses in later stages</td>
<td>Defective cone responses in all, diminished rod responses in some cases</td>
</tr>
<tr>
<td>Fundus</td>
<td>Ranges from granular macula in younger patients to bull’s eye and geographical atrophy of the RPE in older patients ± tapetal-like sheen, thin peripheral RPE, peripapillary atrophy</td>
<td>Ranges from granular macula in young patients to geographical atrophy in older patients, no tapetal reflex, myopic degeneration with prominent choroidal pattern</td>
<td>Only myopic changes and irregular pigmentation in the macular area, no tapetal reflex, no bull’s eye appearance</td>
</tr>
<tr>
<td></td>
<td>Central cone disease progressing to diffuse cone-rod dysfunction: early colour vision impairment with a severity parallel to the degree of visual acuity impairment</td>
<td>Central cone disease progressing to diffuse cone-rod dysfunction</td>
<td>Peripheral cone disease progressing to diffuse cone-rod dysfunction: colour vision becomes impaired later compared to visual acuity and ERG</td>
</tr>
<tr>
<td>Molecular defect (References)</td>
<td>Mutations in RPGR exon ORF15 (Demirci et al,¹⁰ Yang et al)</td>
<td>Not described yet</td>
<td>Not described yet</td>
</tr>
</tbody>
</table>

degeneration in contrast to the central cone disease observed in COD1 families. However, clinically it is still difficult to distinguish these two entities. In addition to COD1 and COD2, two studies described progressive cone dystrophy phenotypes associated with deletions either in the red cone pigment gene¹⁴ or near the 5’ end of the red cone pigment gene²⁰ on Xq28. These patients also showed decreased visual acuity, reduced cone responses in ERG, and colour vision defects, although no progression to cone-rod dystrophy was observed. Both of these cone dysfunctions are congenital and show either a protan (red) defect in colour vision¹⁰ or incomplete achromatopsia with a little function only in blue cones.¹⁰ No tendency to any particular refractive error could be found, and nystagmus was a common sign in blue cone monochromacy (BCM) patients. The diseases described by Reichel et al¹⁰ and Nathans et al²³ can thus be distinguished clinically from COD1 and our patients. The clinical features of Bornholm eye disease (BED) include impaired central vision, myopia, optic nerve pallor, and deuteranopia.²³ Linkage analysis has shown that the locus maps to the distal end of Xq²³ and the mutation responsible for BED could be allelic with the COD2 locus.

In general, it is difficult to distinguish progressive cone-rod dystrophies clinically, both X linked and autosomal forms, from each other. The clinical symptoms and findings in functional eye examinations, such as visual fields, dark adaptation, and electroretinogram, are very similar. Therefore, in spite of the present results suggesting a new locus for X linked progressive cone-rod dystrophy, it is not surprising that our patients have similar clinical symptoms and findings in functional tests to the patients with either COD1 or COD2. Of the previously described X linked COD families, the cone-rod dystrophy phenotype described by Meire et al¹³ most closely matches the clinical picture of our patients.

Allelic diseases, that is, different phenotypes that are the result of different mutations in a single gene, are not rare among retinal diseases. Another example, in addition to COD1 and RP3, is the spectrum of phenotypes associated with mutations in ABCA4 (MIM 601691). Mutations in ABCA4 can give rise to autosomal recessive retinitis pigmentosa,²⁴ cone-rod dystrophy,²⁴ Stargardt disease,²⁵ and fundus flavimaculatus.²⁵ The disease interval on Xpl1.4-q13.1 reported here contains many known genes that are either expressed in the retina or are already known to cause retinal diseases. The genes for retinitis pigmentosa 2 (RP2, MIM 312600), the complete form of congenital stationary night blindness, CSNB1 (NYX, MIM 300278) and Norrie disease (NDP, MIM 310600) were considered as potential candidates for our family. However, sequencing of the coding regions did not show any disease causing mutations. Because only a single family has been investigated, there remains the possibility of a novel mutation in one of the
genes outside the coding regions that could affect the splicing or stability of the RNA transcript. A number of other retinal diseases map to the candidate gene region; these include the incomplete form of congenital stationary night blindness (CSNB2, MIM 300071) caused by mutations in the CACNA1F gene (MIM 300010), Åland Island eye disease (AIED, MIM 300660), X linked optic atrophy (OPA2, MIM 311050), and primary retinal dysplasia (PRD, MIM 312550). The screening of additional candidate genes within the critical region is in progress in our laboratory. Although the gene responsible for the disease in this Finnish family remains to be identified, closely linked markers introduced in this study can already be used in carrier diagnosis. The characterisation of the gene in this new locus will expand our knowledge and further our understanding of the biology pertaining to cone-rod dystrophies.

ACKNOWLEDGEMENTS

We thank the members of the COD family for participation, Sinikka Lindh, Terttu Myöhänen, and Marjatta Sipponen for their help in collecting blood samples, and Johanna Tommiska for technical assistance. This work was supported by a grant (TYH1338) from the Finnish State (TA), by the NIH Grant EY13130 (MBG), by the Eye & Ear Foundation of Pittsburgh, Pittsburgh, PA (MBG), and by Research to Prevent Blindness, NY, NY.

Authors’ affiliations

R Jalkanen, H Tynismaa, T Allitalo, Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, Helsinki, Finland
F Y Demirci, M Gorin, Departments of Ophthalmology and Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA
T Bech-Hansen, Department of Medical Genetics, University of Calgary, Calgary, Alberta, Canada
A Meindl, Department of Medical Genetics, Ludwig-Maximilians University, Munich, Germany
M Peippo, Department of Medical Genetics, the Family Federation of Finland, Helsinki, Finland
M Mäntyläari, Department of Ophthalmology, University of Kuopio, Kuopio, Finland

REFERENCES

7 Barley JA, Gries C, Jacobson D. Cone dystrophy (X-linked) (COD1) maps between DXS7(L1.28) and DXS206(XJ1.1) and is linked to DXS84(754). Cytogenet Cell Genet 1989; 51:959.