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A novel locus (CORD12) for autosomal dominant cone-rod dystrophy on chromosome 2q24.2-2q33.1

Gaël Manes1,2,3*, Maxime Hebrard1,2,3, Béatrice Bocquet1,2,3, Isabelle Meunier4, Delphine Coustes-Chazalette4, Audrey Sénéchal1,2,3, Anne Bolland-Augé5, Diana Zelenika5 and Christian P Hamel1,2,3,4

Abstract

Background: Rod-cone dystrophy, also known as retinitis pigmentosa (RP), and cone-rod dystrophy (CRD) are degenerative retinal dystrophies leading to blindness. To identify new genes responsible for these diseases, we have studied one large non consanguineous French family with autosomal dominant (ad) CRD.

Methods: Family members underwent detailed ophthalmological examination. Linkage analysis using microsatellite markers and a whole-genome SNP analysis with the use of Affymetrix 250 K SNP chips were performed. Five candidate genes within the candidate region were screened for mutations by direct sequencing.

Results: We first excluded the involvement of known adRP and adCRD genes in the family by genotyping and linkage analysis. Then, we undertook a whole-genome scan on 22 individuals in the family. The analysis revealed a 41.3-Mb locus on position 2q24.2-2q33.1. This locus was confirmed by linkage analysis with specific markers of this region. The maximum LOD score was 2.86 at θ = 0 for this locus. Five candidate genes, CERKL, BBS5, KLHL23, NEUROD1, and SF3B1 within this locus, were not mutated.

Conclusion: A novel locus for adCRD, named CORD12, has been mapped to chromosome 2q24.2-2q33.1 in a non consanguineous French family.

Background

Retinitis pigmentosa (RP, [MIM 268000]) is a genetically heterogeneous group of retinal photoreceptor degeneration characterized by night blindness and loss in the peripheral visual field, slowly progressing towards total blindness after several decades [1]. RP accounts for about 2/3 of the inherited retinal dystrophy cases [2]. In contrast to typical RP, also called rod-cone dystrophies (RCDs) because of primary involvement of rods, inverse RP or cone-rod dystrophies (CRDs) are pigmentary retinopathies characterized by first decrease in visual acuity and loss in the central visual field and lately by night blindness and loss in the peripheral visual field. CRDs are due to the primary degeneration of cone photoreceptors, followed by the secondary, or, sometimes, concomitant loss of rod photoreceptors [3]. Forty nine genes and loci are responsible for non syndromic RP and 18 for non syndromic CRD (including 6 in common with RP and 4 with Leber congenital amaurosis) http://www.sph.uth.tmc.edu/Retnet. The three types of Mendelian inheritance are encountered in both RP and CRD.

Among the 18 CRD genes, ten (GUCY2D, PITPNM3, GUCA1A, HRG4/UNC119, CRX, AIPL1, RIMS1, SEMA4A, PROM1 and PRPH2/RDS) are found in autosomal dominant (ad) CRD, six (ABCA4, RPGRIP1, RAX2, CORD8, ADAM9 and CERKL) in autosomal recessive (ar) CRD and two (RPGR and CACNA1F) in X-linked recessive (ar) CRD and two (RPGR and CACNA1F) in X-linked CRD http://www.sph.uth.tmc.edu/Retnet. The prevalence of mutations for each gene in the CRD population is highly variable. ABCA4, which causes Stargardt macular dystrophy, is also a major gene for CRD, being responsible for 30-60% of arCRD cases [4–6]. In contrast, the overall prevalence of adCRD genes remains low, many of them being described in only one or a few

* Correspondence: gael.manes@inserm.fr
1INSERM U1051, Institute for Neurosciences of Montpellier, 80 rue Augustin Fliche, Montpellier, 34091, France
Full list of author information is available at the end of the article

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cases. Only CRX, GUCY2D and PRPH2/RDS have been consistently reported in adCRD [7-10]. Yet, CRX was estimated to account for only 5-10% of adCRD cases and the prevalence of GUCY2D and PRPH2/RDS is unknown [11,12]. Therefore, there are probably other genes remaining to be discovered in adCRD.

In search for new genes responsible for pigmentary retinopathies, we recruited one large non-consanguineous French family with adCRD. This family was unlinked to any known adRP or adCRD locus and SNP genotyping revealed that it was linked to a new locus on chromosome 2, designated CORD12.

Methods
Clinical examination
Members of this large French non-consanguineous family (RP470) were identified with CRD which segregated as a dominant trait (adCRD). There were 9 affected patients out of 22 in 4 generations (Figure 1). Examination included assessment of visual acuity, slit lamp biomicroscopy, direct funduscopy and full field electroretinography. There was no evidence of extraocular signs of disease indicating that CRD was non-syndromic.

Genotyping of microsatellite markers and linkage analysis
Informed written consent and peripheral blood samples were obtained from 22 examined family members. The investigators followed the tenets of the Declaration of Helsinki. Genomic DNA was isolated from 10 ml peripheral blood leucocytes using standard salting out procedure [13]. The DNA samples were quantified by spectrophotometer and diluted to 25 ng/μl for PCR amplification. PCR was carried out in a 25 μl final volume containing 50 ng genomic DNA, 5 picomoles of each primer, 0.2 mM dNTPs (MP Biochemicals), 2 mM...
MgCl₂, PCR buffer and 1 unit of DNA polymerase (AmpliTaq Gold; Applied Biosystems, Foster city, CA). Initial denaturation at 95°C for 10 minutes was followed by 35 cycles of denaturation at 94°C for 30 seconds, specific annealing temperature for 30 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 10 minutes. The PCR products were diluted and mixed with Genescan 400HD ROX size standard and subsequently analysed on an Applied Biosystems 3130xL genetic analyser (Applied Biosystems, Foster city, CA).

Genotyping was performed using 2 to 3 polymorphic commercially available microsatellite markers from ABI PRISM Linkage Mapping Set version 2.5 (Applied Biosystems, Foster city, CA), within or contiguous to known adRP and adCRD genes, and within the locus CORD12. Results were analysed with GeneMapper software (version 4.0, Applied Biosystems, Foster city, CA). Segregation of the markers among the family members was examined.

Two-point LOD scores were calculated with Superlink-online http://bioinfo.cs.technion.ac.il/superlink-online/. The phenotype was analyzed as an autosomal dominant and fully penetrant trait with an affected allele frequency of 0.0001. Family and haplotype data were generated using Cyrillic software (version 2.1.3; Cherwell Scientific, Oxford, UK).

SNP genotyping and analysis

To map the disease locus, a genome-wide scan was performed by the Centre National de Génotypage (CNG, http://www.cng.fr) by genotyping 262,264 SNPs (GeneChip Mapping 250 K Nsp Array, Affymetrix, Santa Clara, CA). Results were analyzed using TASE (Transmitted Allele to All Children) a home-made software which compared every SNP between each individuals in the family.

The first test, named Common Allele to All Affected individuals (C3A), highlighted the common allele to all affected patients within the family. The second test, Transmitted Allele to All Children (TAAC), estimated the specific allele carried by the affected parent in a nuclear family (parents + child) and transmitted to the affected child. Two consecutive mismatched SNPs limited the size of the locus. Only the regions longer than 1 Mb were considered.

Mutation screening

Coding exons and adjacent intronic sequences of candidate genes were sequenced with an Applied Biosystems 3130xL genetic analyser (Applied Biosystems, Foster city, CA) following manufacturer's instructions. Primer pairs and PCR conditions are available on request. Sequence analysis and mutation identification were performed using Collection and Sequence Analysis software package (Applied Biosystems, Foster city, CA).

Ethics Committee

Statement about Conformity with Author Information: Informed and written consent was obtained for all patients participating to the study. The study was done in adherence to the tenets of the Declaration of Helsinki.

The authors confirm that they are in compliance with their Institutional Review Boards (IRBs) as the Department of Ophthalmology of the Hospital of Montpellier has the authorization # 11018S from the French Ministry of Health for biomedical research in the field of physiology, pathophysiology, epidemiology and genetics in ophthalmology.

Results

Clinical description

The pedigree of the four generations family is shown in Figure 1. The 9 affected patients revealed features of adRP with intra-familial variable phenotype including progressive loss of the visual acuity, typical bone spicule-shaped pigmentary deposits in the macular area or macular atrophy, moderate nightblindness and reduced electroretinogram (ERG) responses (Table 1). The proband (III:1) showed patches of atrophy in the macular area with a few pigment deposits, attenuation of retinal arterioles and temporal pallor of the optic disc (Figure 2).

Mapping to CORD12

Microsatellite markers for the 21 adRP genes http://www.sph.uth.tmc.edu/Retnet, the 3 most frequent adCRD genes (CRX, GUICY2D and PRPH2/RDS) [7-10] and a fourth adCRD gene, GUCA1A, were used to genotype family members, and to search for co-segregation of the markers with the disease phenotype. All these candidate genes were excluded. We then performed a genome wide scan using Affymetrix 250 K microarrays and genotypes were analysed with the TASE software. No linkage was found for most chromosomal regions except for a large region located on chromosome 2q42.2-2q33.1. The boundaries of the locus were determined by SNP exclusion between SNPs rs174240 and rs4619591 and encompassed a 41.3-Mb region (Figure 3).

Microsatellite markers were then used to confirm linkage with the locus. We genotyped all 22 members of the family with 8 microsatellite markers located on 2q42.2-2q33.1 (Figure 1). All affected patients had a common haplotype and the boundaries of the region were determined by recombination events that occurred in affected individuals III:1, III:4, III:9, IV:4, IV:9 and healthy individual III:7. The proximal boundary was defined by the
| Patient | Sex | Age at onset | Symptoms | Visual acuity OD/OS | Fundus | Visual field | ERG OD/OS | Scotopic dim blue | Photopic single white | Light adapted 30-Hz flickers |
|---------|-----|--------------|----------|---------------------|--------|--------------|-----------|------------------|-----------------------|------------------------|
| II:2    | F   | None         | 64       | 20/20 20/16         | Mild attenuation of retinal vessels | NA      |             | 40 μV/23 μV    | 181 μV/175 μV          | 90 μV/94 μV            |
| II:3    | F   | 40           | Night blindness | 70 | 20/40 20/32 | Mild attenuation of retinal vessels. Macular atrophy | OD:relative 20° central scotoma OS:absolute 20-30° central scotoma | N/A 124 μV/173 μV | 56 μV/60 μV | 46 μV/56 μV |                  |
| III:1   | F   | 32           | Nystagmus | Night blindness | Severe macular atrophy | Rare bone spicule-shaped pigment deposits | Absolute 30° central scotoma and normal PVF on both eyes | 48 μV/35 μV | 44 μV/42 μV | 32 μV/41 μV |                  |
| III:2   | M   | None         | 38       | 20/25 20/20         | Normal | Normal |             | 253 μV/275 μV | 30 μV/41 μV | 70 μV/84 μV |                  |
| III:3   | F   | 35           | Nystagmus | Night blindness | Photo phobia | No night blindness | Mild attenuation of retinal vessels | Normal |             | 130 μV/121 μV | 34 μV/46 μV | 41 μV/40 μV |                  |
| III:10  | M   | Early childhood | Night blindness | Mild photo phobia | 38 | 20/32 20/32 | Posterior pole atrophy Mild attenuation of retinal vessels | Absolute 10° central scotoma and normal PVF on both eyes | 157 μV/160 μV | 51 μV/45 μV | 88 μV/77 μV |                  |
| IV:1    | M   | Photophobia | 1        | NA                  | Mild attenuation of retinal vessels. Abnormal pigmentation of the macular area | NA      |             | NA             | NA                   | NA                    |
| IV:4    | M   | No photo phobia | No night blindness | 11 | 20/32 20/50 | Posterior pole atrophy Attenuated retinal vessels | Normal |             | NA/139 μV | NA/24 μV | NA/66 μV |                  |
| IV:10   | M   | Early childhood | Night blindness | Photo phobia | No night blindness | 19 | 20/200 20/200 | Moderate pallor of the optic discs, and macular atrophy | Relative 20° central scotoma and normal PVF on both eyes | 91 μV/89 μV | 20 μV/13 μV | 39 μV/42 μV |                  |

PVF = Peripheral Visual Field
OD/OS = oculus dexter/oculus sinister
NA = Not available

Normal value ranges are:
Scotopic dim blue: 160 μV - 250 μV
Photopic single white: 70 μV - 150 μV
Light adapted 30-Hz flickers: > 110 μV

![Fundus photographs of the patient III:1](http://www.biomedcentral.com/1471-2350/12/54)
recombination event between markers D2S142 and D2S306 in affected patient IV:9 and distal boundary by the recombination event between markers D2S117 and D2S325 in healthy individual III:7 (Figure 3). Using Superlink software, we found a maximum LOD score of 2.86 at θ = 0 for the marker D2S118, defining a new locus named CORD12. The markers D2S142, D2S325, D2S2361 and D2S126, outside the locus, gave negative LOD scores (Table 2).

The CORD12 41.3-Mb interval contains 280 genes. None of them were previously reported in adCRD or adRP. However the interval does contain two previously
Table 2 Two-point LOD score for microsatellite markers of family RP470 calculated at different recombination fractions 0

| Marker | Position (Mb) | 0.00 | 0.01 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 |
|--------|---------------|------|------|------|------|------|------|------|
| D2 S142 | 156,283,230  | -∞   | -0.9396 | -0.3168 | -0.1121 | -0.0002 | 0.0069 | -0.0000 |
| D2 S306 | 160,562,440  | 2.6182 | 2.5720 | 2.3834 | 2.1389 | 1.6185 | 1.0548 | 0.4722 |
| D2 S364 | 183,034,534  | 2.3172 | 1.2273 | 2.1047 | 2.1884 | 2.1468 | 0.9190 | 0.4192 |
| D2 S118 | 191,606,469  | 2.8588 | 2.8094 | 2.6077 | 2.3459 | 1.7872 | 1.1789 | 0.5432 |
| D2 S117 | 195,618,799  | 2.0280 | 1.9949 | 1.8600 | 1.6860 | 1.3181 | 0.9208 | 0.4865 |
| D2 S325 | 208,270,870  | -∞   | 1.1956 | 1.6867 | 1.7184 | 1.4494 | 1.0188 | 0.5190 |
| D2 S361 | 216,478,443  | 3.8589 | 2.5393 | 0.1857 | 0.4554 | 0.5836 | 0.4992 | 0.2977 |
| D2 S126 | 222,016,968  | -∞   | 0.2379 | 0.7858 | 0.8926 | 0.7898 | 0.5512 | 0.2719 |

Discussion

In this study, a novel locus, CORD12, for autosomal dominant cone-rod dystrophy (adCRD) was identified and localized to chromosome 2q24.2-2q33.1. With CORD8 assigned to chromosome 1q23.1-q23.3, it is the second CRD locus for which the causative gene remains unknown [23]. To date, the total number of known adCRD genes and loci, including CORD12, is eleven.

A maximum two-point LOD score of 2.86 at \( \theta = 0 \) for the marker D2S118, close to theoretical significance, was obtained. The common haplotype for affected patients in the family was flanked by SNPs between rs174240 and rs4619591, which defined the 41.3-Mb CORD12 locus. Two other retinal dystrophy loci are mapped on chromosome 2. R5P4, a 19.98-Mb autosomal recessive RP interval flanked by D2S149 and D2S367 on chromosome 2p22.3-p24.1 [24] and RP28, a 14-Mb autosomal recessive RP interval flanked by D2S1337 and D2S286 on chromosome 2p11-p15 [25,26]. The causative genes have recently been reported for both regions in September 2010, respectively ZNF513 [27] for R5P4 and FAM161A for RP28 [28,29]. A third gene, C2ORF71, was identified earlier this year next to ZNF513, by homozygosity mapping in two independent studies in an 8-Mb locus on chromosome 2p24.1-p23.1 and in a 6.8-Mb locus on chromosome 2p23.1-p24.1 [30,31]. None of these 3 regions overlap with CORD12.

The CORD12 41.3-Mb interval contains 280 annotated genes. We sequenced five possible candidate genes. CERKL and BBS5, which cause autosomal recessive RP and Bardet-Biedl syndrome, respectively [14,15]. All exons and flanking intron regions were sequenced but no mutation was found. Within the CORD12 locus, three other candidate genes were also sequenced. KLHL23 has strong similarities with the recently described gene KLHL7 responsible for adRP [16]. NEUROD1 regulates development and maintenance in the visual system [17]. SF3B1 is a splicing factor [18]. Other essential components of the spliceosome, PRPF31, PRPF3, PRPF8, PAP1 and SNRNP200, have been associated with adRP [19-22]. No disease causing mutations were detected in KLHL7, NEUROD1 and SF3B1.

Conclusions

In summary, we report on the identification of a novel locus for adCRD in chromosome 2q24.2-2q33.1. Identification of the disease causing gene in the interval will increase our understanding of the causes of cone-rod dystrophy.
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Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

GM carried out the molecular genetic studies and the sequence alignment, participated in the design of the study and drafted the manuscript. MH performed the genotyping analysis. BB participated in the molecular genetic studies. IM participated in the medical examinations. DCC participated in the molecular genetic studies. AS participated in the sequence alignment. ABA performed the genotyping analysis. DZ performed the genotyping analysis. CPH carried out the medical examinations, conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.
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