Leber Congenital Amaurosis and Retinitis Pigmentosa with Coats-like Exudative Vasculopathy Are Associated with Mutations in the Crumbs Homologue 1 (CRB1) Gene

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Mutations in the crumbs homologue 1 (CRB1) gene cause a specific form of retinitis pigmentosa (RP) that is designated “RP12” and is characterized by a preserved para-arteriolar retinal pigment epithelium (PPRPE) and by severe loss of vision at age <20 years. Because of the early onset of disease in patients who have RP with PPRPE, we considered CRB1 to be a good candidate gene for Leber congenital amaurosis (LCA). Mutations were detected in 7 (13%) of 52 patients with LCA from the Netherlands, Germany, and the United States. In addition, CRB1 mutations were detected in five of nine patients who had RP with Coats-like exudative vasculopathy, a relatively rare complication of RP that may progress to partial or total retinal detachment. Given that four of five patients had developed the complication in one eye and that not all siblings with RP have the complication, CRB1 mutations should be considered an important risk factor for the Coats-like reaction, although its development may require additional genetic or environmental factors. Although no clear-cut genotype-phenotype correlation could be established, patients with LCA, which is the most severe retinal dystrophy, carry null alleles more frequently than do patients with RP. Our findings suggest that CRB1 mutations are a frequent cause of LCA and are strongly associated with the development of Coats-like exudative vasculopathy in patients with RP.

We have described mutations in the CRB1 gene (MIM 604210) in a severe, autosomal recessive form of retinitis pigmentosa (RP) that is designated “RP12” (MIM 600105) (den Hollander et al. 1999). The gene consists of 12 exons and exhibits alternative splicing at its 3’ end (A. I. den Hollander and F. P. M. Cremers, unpublished data). The CRB1 protein contains 19 epidermal growth factor (EGF)-like domains, 3 laminin A globular-like domains, a transmembrane domain, and a 37-amino acid cytoplasmic tail; in addition, it is homologous to the Drosophila crumbs protein. RP12 is a specific form of RP characterized by a preserved para-arteriolar retinal pigment epithelium (PPRPE) in the early-to-middle stages of disease. Patients experience night blindness and develop a progressive loss of their visual field at <10 years of age. Because of early macular involvement, patients have severe visual impairment at <20 years of age. Other features of this type of RP are hyperopia, nystagmus, optic-nerve–head drusen, vascular sheathing, and maculopathy (Heckenlively 1982; van den Born et al. 1994). Mutations have now been identified in 15 patients who have isolated or autosomal recessive of RP

Leber congenital amaurosis (LCA) is considered the earliest and most severe form of retinal dystrophy, causing blindness or severe visual impairment at birth or during the first months of life. Mutations that lead to LCA have been detected in *CRB1* (Freund et al. 1998; Sohocki et al. 2001), *AIPL1* genes (MIM 604392) (Sohocki et al. 2000), an indication that more LCA genes await discovery. Because of the early onset of symptoms in patients who have RP with PPRPE—and the observation that mutations in *RPE65* and *CRX* can lead to both LCA and RP (Gu et al. 1997; Marlhens et al. 1997; Morimura et al. 1998), *CRB1* (MIM 602225) (Freund et al. 1998; Sohocki et al. 2000), and *AIPL1* genes (MIM 604392) (Sohocki et al. 2000) in five patients with LCA, nine distinct sequence changes in the coding region or splice sites of *CRB1*, which contains several putative photoreceptor-gene regulatory sites (A. I. den Hollander and F. P. M. Cremer, unpublished data), with primers 5'-TAAATTCAACACCTTGTGACTTAGC-3' and 5'-TGGCATCAAAAAATACCAGAAAGTC-3'. Primers used to amplify exon 12 were 5'-CCTGAGTATCCATTGGTC-3' and 5'-ATTCAAGTGTTGGATCCC-3'. Products that migrated differently through the gel were analyzed by sequencing. When only one allele was identified in a patient, the patient’s sample was also subjected to sequence analysis of all 26 amplicons and of the promoter region of *CRB1*, but we replaced the primer set for exon 5 by primers 5'-TAAATTCAACACCTTGTGACTTAGC-3' and 5'-TGGCATCAAAAAATACCAGAAAGTC-3'.

| Table 1 |
|-----------------|-----------------|-----------------|
| **DISEASE AND PATIENT NUMBER** | **INHERITANCE** | **ALLELE 1** | **ALLELE 2** |
| **LCA:** | | | |
| 12831 | Isolated | 2978G→A | Cys948Tyr |
| 12859 | Recessive | 3434T→G | Ile1100Arg |
| 12862 | Isolated | 2536A→T | Lys801stop |
| 12872 | Recessive | 4127G→T | Arg1331His |
| 13067 | Isolated | 3466G→T | Glu1111stop |
| 16507 | Isolated | 2978G→A | Cys948Tyr |
| 16690 | Isolated | 748-754del | Frameshift |
| **RP with Coats-like exudates:** | | | |
| 9439 | Recessive | 1343C→G, 1433A→G | Ser403stop, Tyr433Cys |
| 16937 | Recessive | 2644G→C, 4195G→A | Asp837His, Ala1354Thr |
| 16968 | Isolated | 2978G→A | Cys948Tyr |
| 17658 | Recessive | 2536A→T | Lys801stop |

As described elsewhere (den Hollander et al. 1999), we obtained the primer set for exon 5 by primers 5'-TAAATTCAACACCTTGTGACTTAGC-3' and 5'-TGGCATCAAAAAATACCAGAAAGTC-3'. Primers used to amplify exon 12 were 5'-CCTGAGTATCCATTGGTC-3' and 5'-ATTCAAGTGTTGGATCCC-3'. Products that migrated differently through the gel were analyzed by sequencing. When only one allele was identified in a patient, the patient’s sample was also subjected to sequence analysis of all 26 amplicons and of the promoter region of *CRB1*, which contains several putative photoreceptor-gene regulatory sites (A. I. den Hollander and F. P. M. Cremer, unpublished data), with primers 5'-TAAATTCAACACCTTGTGACTTAGC-3' and 5'-TGGCATCAAAAAATACCAGAAAGTC-3'. Products that migrated differently through the gel were analyzed by sequencing. When only one allele was identified in a patient, the patient’s sample was also subjected to sequence analysis of all 26 amplicons and of the promoter region of *CRB1*, which contains several putative photoreceptor-gene regulatory sites (A. I. den Hollander and F. P. M. Cremer, unpublished data), with primers 5'-TAAATTCAACACCTTGTGACTTAGC-3' and 5'-TGGCATCAAAAAATACCAGAAAGTC-3'. Products that migrated differently through the gel were analyzed by sequencing. When only one allele was identified in a patient, the patient’s sample was also subjected to sequence analysis of all 26 amplicons and of the promoter region of *CRB1*, which contains several putative photoreceptor-gene regulatory sites (A. I. den Hollander and F. P. M. Cremer, unpublished data), with primers 5'-TAAATTCAACACCTTGTGACTTAGC-3' and 5'-TGGCATCAAAAAATACCAGAAAGTC-3'. Products that migrated differently through the gel were analyzed by sequencing. When only one allele was identified in a patient, the patient’s sample was also subjected to sequence analysis of all 26 amplicons and of the promoter region of *CRB1*, which contains several putative photoreceptor-gene regulatory sites (A. I. den Hollander and F. P. M. Cremer, unpublished data), with primers 5'-TAAATTCAACACCTTGTGACTTAGC-3' and 5'-TGGCATCAAAAAATACCAGAAAGTC-3'. Products that migrated differently through the gel were analyzed by sequencing. When only one allele was identified in a patient, the patient’s sample was also subjected to sequence analysis of all 26 amplicons and of the promoter region of *CRB1*, which contains several putative photoreceptor-gene regulatory sites (A. I. den Hollander and F. P. M. Cremer, unpublished data), with primers 5'-TAAATTCAACACCTTGTGACTTAGC-3' and 5'-TGGCATCAAAAAATACCAGAAAGTC-3'.
Figure 1  Cosegregation analysis of CRB1 mutations in (A) one family with LCA and (B) five families with RP and Coats-like exudates. A question mark (?) denotes the unidentified second allele; an asterisk (*) denotes patients with RP who have developed the Coats-like complication. Arrows indicate probands. IVS8 denotes the splice-site mutation of exon 8, 2978G→A.

Pathogenic CRB1 mutations were identified in 7 (13%) of 52 unrelated patients with LCA from the Netherlands, Germany, and the United States. Mutations in GUCY2D, RPE65, AIPL1, and CRX account for 6%–20%, 3%–16%, 7%, and 2%–3% of LCA cases, respectively (Morimura et al. 1998; Dharmaraj et al. 2000; Lotery et al. 2000; Perrault et al. 2000; Sohocki et al. 2000b; Thompson et al. 2000), which suggests that CRB1 mutations contribute significantly to the etiology of LCA.

To determine whether mutations in the CRB1 gene are a common cause of RP in the Dutch population, we screened 97 unrelated patients who had isolated or autosomal recessive RP for the presence of Cys948Tyr and Arg764Cys. These mutations had been previously identified in 5 and 3 CRB1 alleles, respectively, of a total of 30 CRB1 alleles of unrelated patients who had RP with PPRPE (den Hollander et al. 1999; U. Kellner, A. I. den Hollander, Y. J. M. de Kok, L. I. van den Born, F. P. M. Cremers, J. R. Heckenlively, unpublished data). The presence of the nucleotide alteration 2425C→T (Arg764Cys) was analyzed by allele-specific oligonucleotide (ASO) hybridization (Shuber et al. 1993), using wild-type primer 5′-AATATATCGTGTCTGG-3′ and mutant primer 5′-AATATATCGTGTCTGG-3′. The presence of 2978G→A (Cys948Tyr), was analyzed with the amplification-refractory mutation system (Newton et al. 1989) with sense primers 5′-ATTATACCTTCT-CTATTAGG-3′ (wild-type allele) or 5′-ATTATACCTTCTATTAGA1-A-3′ (mutant allele) and antisense primer 5′-GTGCCATCATTCTGACTG-3′. One of the 97 patients (patient 9439) carried the Arg764Cys mutation on one allele. Sequencing of the 12 exons of CRB1 revealed two more sequence changes (Ser403stop...
and Tyr433Cys), both of which are located on the second allele, as determined by allele-specific PCR (data not shown) and segregation analysis (fig. 1).

As a complication of RP, proband 9439 (individual II-4) (fig. 2) and his affected brother (II-5) had developed a Coats-like exudative vasculopathy, which caused additional loss of vision. Coats-like exudative vasculopathy, a relatively rare complication of RP, can develop in later stages of the disease and is characterized by vascular abnormalities (aneurysmal dilations and telangiectatic retinal veins), yellow extravascular lipid deposits, and retinal detachment. Patients with RP who develop Coats-like changes show a wide spectrum of disorders, ranging from mild visual difficulties or nystagmus, as observed in classical RP, to the other extreme, in which a proliferative vasculopathy develops. If untreated, this proliferative vasculopathy may result in a painful blind eye caused by rubeosis, retinal neovascularization, or serous retinal detachment. It has been suggested that genetic factors may be involved in RP with Coats-like exudative vasculopathy (Khan et al. 1988).

We therefore hypothesized that CRB1 mutations may be associated with the development of this complication of RP, and we ascertained eight additional isolated or autosomal recessive patients who had RP with Coats-like exudative vasculopathy. These patients were tested for mutations in CRB1 by sequence analysis of all 26 amplicons. Five of the eight patients had sequence changes, and clinical descriptions of these patients are summarized in table 2.

In one patient, we identified three sequence changes; in two patients, we found compound heterozygous mutations; and in one patient, we identified one allele (table 1). Screening of the promoter region of CRB1 in the patient with one allele revealed no sequence changes. Cosegregation analysis in family members of all five probands confirmed autosomal recessive inheritance of CRB1 mutations (fig. 1). Of the 10 different sequence changes identified in the five patients who had RP with Coats-like exudates, five mutations (Ser403stop, Arg764Cys, Lys801stop, Cys948Tyr, and 2978 + 5G->A) have been identified previously in patients who had RP.

Figure 2  Fundus photograph of the inferior part of the left eye of a patient who had RP with unilateral Coats-like exudative vasculopathy (proband 9439; individual II-4). Note the widespread subretinal yellowish-white deposits between the neural retina and RPE in the lower part. This region of the fundus is out of focus as a result of the elevation of tissue caused by subretinal accumulation of fluid. Triangles (▲) indicate retinal vessels with white sheathing that is indicative of vasculitis. The RPE shows some preservation near retinal arterioles as seen in patients who had RP with PPRPE (arrow).
with PPRPE. The other five changes (Tyr433Cys, Asp837His, Asn894Ser, Cys1181Arg, and Ala1354Thr) had not previously been identified in patients who had RP with PPRPE or in patients with LCA, and the changes were not found in 180 control chromosomes.

Coats-like exudative vasculopathy occurs in only 1.2%–3.6% of patients with RP (Khan et al. 1988). Among patients who had both RP and PPRPE and were described by Van den Born et al. (1994), 2 (8.3%) of 24 had Coats-like changes, and CRB1 mutations were found in these patients (den Hollander et al. 1999). At least one patient described in the present study (patient II-4 in family 9439) had RP with Coats-like changes and PPRPE. However, the disorders of two patients (patient II-2 in family 16894 and patient II-2 in family 16937) were clearly distinct from RP with PPRPE. In both patients, the onset of RP occurred when patients were >10 years old. Neither patient showed a preservation of the RPE surrounding the arterioles, and one patient was highly myopic.

Our findings show that CRB1 mutations are associated with Coats-like exudative vasculopathy in patients who have RP with or without the PPRPE phenotype. These findings demonstrate that patients with PPRPE should be checked regularly for the Coats-like complication. Furthermore, the routine screening of patients with autosomal recessive or isolated RP may be important because of its ability to reveal those patients who are at increased risk of developing exudative retinal detachment. If the process is detected before it becomes proliferative, cryotherapy can be used to prevent further progression.

Not all affected siblings of patients who have RP with Coats-like exudative vasculopathy develop the Coats-like complication (e.g., families 16894 and 17658 [fig. 1]). This finding, together with the observation that most patients with CRB1 mutations had developed unilateral Coats-like exudates, strengthens the idea that CRB1 mutations are an important risk factor for the development of the Coats-like reaction and that other genetic or environmental factors may be involved. Interestingly, no CRB1 mutations were identified in 13 (45%) of 29 patients who had RP with PPRPE (U. Kellner, A. I. den Hollander, Y. J. M. de Kok, L. I. van den Born, F. P. M. Cremers, J. R. Heckenlively, unpublished data), and in the present study we did not detect CRB1 mutations in four of nine patients who had RP with Coats-like exudates, which suggests that another gene may be involved in these two specific forms of RP.

We found no obvious genotype-phenotype correlation when we compared mutations in patients who had both RP and PPRPE with those in patients who had LCA or RP with Coats-like exudates. However, the absence of clear-cut null mutations on both CRB1 alleles of 15 patients who had RP with PPRPE and of 5 patients who had RP with Coats-like exudates, together with the presence of null mutations on both CRB1 alleles in 3 patients with LCA, suggests that LCA may be associated with complete loss of function of CRB1. In contrast, patients who have RP with PPRPE and patients who have RP with Coats-like exudates may have residual CRB1 function.

RP with PPRPE, RP with Coats-like exudative vasculopathy, and LCA represent different (but partly overlapping) clinical entities, as evidenced by the presence of the PPRPE characteristics in some patients with LCA and the higher-than-average incidence of Coats-like changes in patients who had RP with PPRPE. Because our genotype-phenotype comparison did not reveal conclusive evidence of qualitative or quantitative differences in CRB1 function in these patient groups, functional studies of CRB1 are necessary to shed light on this intriguing issue. We suggest that other genetic—and possibly environmental—factors influence the expression of CRB1 mutations, thereby contributing to the wide spectrum of features that have been described in the present study.

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Electronic Database Information

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for CRB1 [MIM 604210], RP12 [MIM 600105], GUCY2D [MIM 600179], RPE65 [MIM 180069], CRX [602225], and AIPL1 [MIM 604392])

References

den Hollander AI, ten Brink JB, de Kok YJM, van Soest S, van den Born LI, van Driel MA, van de Pol TJR, Payne AM,