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CRB1 mutations in inherited retinal dystrophies

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Abstract

Mutations in the *CRB1* gene are associated with variable phenotypes of severe retinal dystrophies, ranging from Leber Congenital Amaurosis (LCA) to rod-cone dystrophy (also called retinitis pigmentosa (RP)). Moreover, retinal dystrophies resulting from *CRB1* mutations may be accompanied by specific fundus features: preservation of the para-arteriolar retinal pigment epithelium (PPRPE) and retinal telangiectasia with exudation (also referred to as Coats-like vasculopathy). In this publication we report seven novel mutations and classify over 150 reported *CRB1* sequence variants that were found in more that 240 patients. The data from previous reports was used to analyse a potential correlation between *CRB1* variants and the clinical features of respective patients. This meta-analysis suggests that the differential phenotype of patients with *CRB1* mutations is due to additional modifying factors rather than particular mutant allele combination.

Keywords

CRB1; LCA; Retinitis Pigmentosa; rod-cone dystrophy

Background

Mutations in the *CRB1* gene (MIM# 604210) are associated with variable phenotypes of severe retinal dystrophies, ranging from Leber Congenital Amaurosis (LCA) to rod-cone

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dystrophy (also called retinitis pigmentosa (RP)) (Azam, et al., 2011; Benayoun, et al., 2009; Bernal, et al., 2003; Booij, et al., 2005; Clark, et al., 2010; Coppieters, et al., 2010; den Hollander, et al., 2004; den Hollander, et al., 2001a; den Hollander, et al., 2007; den Hollander, et al., 1999; Galvin, et al., 2005; Gerber, et al., 2002; Hanein, et al., 2004; Henderson, et al., 2010; Henderson, et al., 2007; Jacobson, et al., 2003; Khaliq, et al., 2003; Li, et al., 2011; Lotery, et al., 2001a; Lotery, et al., 2001b; Riveiro-Alvarez, et al., 2008; Seong, et al., 2008; Siemiatkowska, et al., 2011; Simonelli, et al., 2007; Tosi, et al., 2009; Vallespin, et al., 2007; Walia, et al., 2010; Yzer, et al., 2006a; Yzer, et al., 2006b; Zernant, et al., 2005). LCA is a group of the most severe and the earliest occurring retinal dystrophies resulting in congenital blindness (den Hollander, et al., 2008). The onset of the disease occurs at birth and the characteristic features include non-recordable electroretinogram (ERG), nystagmus, sluggish or absent pupillary responses and oculo-digital reflexes, a distinctive eye-rubbing also called the Franschetti sign (den Hollander, et al., 2008; Franceschetti and Dieterle, 1954; Leber, 1869). RP is a clinically heterogeneous disorder characterised by a progressive degeneration of the photoreceptors and leading to a visual impairment of variable severity that can end in complete blindness. The disease onset is highly variable: it may commence in the first decade of life or much later. There is a considerable clinical overlap between LCA and early-onset RP and in some cases/reports the diagnosis is ambiguous. Early-onset RP, however, is considered as a relatively milder form, where patients do not have a congenital onset of visual impairment.

LCA and RP resulting from *CRB1* mutations may be accompanied by specific fundus features: preservation of the para-arteriolar retinal pigment epithelium (PPRPE) (Bernal, et al., 2003; den Hollander, et al., 2004; den Hollander, et al., 1999; Heckenlively, 1982; Henderson, et al., 2010; Khaliq, et al., 2003; Simonelli, et al., 2007; Yzer, et al., 2006b) and retinal telangiectasia with exudation (also referred to as Coats-like vasculopathy) (Coppieters, et al., 2010; den Hollander, et al., 2004; den Hollander, et al., 2001a; Henderson, et al., 2010; Yzer, et al., 2006b). PPRPE is characterized by a relative preservation of retinal pigment epithelium (RPE) adjacent to retinal arterioles despite a panretinal RPE degeneration (Heckenlively, 1982). This is, however, not consistent in *CRB1*-associated RP and the absence of PPRPE in a severe RP should not exclude *CRB1* as a potential causal gene (Lotery, et al., 2001b). Retinal telangiectasia is a condition of abnormally permeable blood vessels, leading to exudation and retinal detachment (Cahill, et al., 2001). Some patients with *CRB1* mutations show macular atrophy (Henderson, et al., 2010), similar features were found for other LCA causing genes (*GUCY2D* MIM# 600179, *AIPL1* MIM# 604392 and *RPGRIP1* MIM# 605446), which lead to classification of LCA into cone-rod LCA and rod-cone LCA (Hanein, et al., 2004). Patients with *CRB1* mutations belong to both categories. Predisposition of the *CRB1* patients to keratoconus (McKibbin, et al., 2010; McMahon, et al., 2009) and implication for pigmented paravenous chorioretinal atrophy (McKay, et al., 2005) and nanophthalmos (Zenteno, et al., 2011) have also been reported.

CRB1 is a human homologue of the *Drosophila melanogaster* gene coding for protein crumbs (crb) and it is expressed in the retina and the brain (den Hollander, et al., 1999). *CRB1* consists of 12 exons and exhibits alternative splicing at the 3′ end, yielding two proteins of 1376 and 1406 amino acids (den Hollander, et al., 2001b). Both proteins contain 19 epidermal growth factor (EGF)-like domains, three laminin A globular (AG)-like domains and a signal peptide sequence. In addition, the longer isoform contains transmembrane and cytoplasmic domains (den Hollander, et al., 2001b; Gosens, et al., 2008). The cytoplasmic domain includes conserved FERM and PDZ binding motifs, through which CRB1 participates in the formation of adherens junction and links to the actin cytoskeleton (Gosens, et al., 2008).

In *Drosophila*, crb determines the polarity of the embryonic epithelium and peripheral neurons; it is important for the maintenance of zonula adherens (ZA) and it is localized in the apical membrane (Tepass, et al., 1990). In the mouse retina, Crb1 is present in the apical membranes of the epithelial cells, in Muller cells and in photoreceptor inner segments, where it concentrates in the vicinity of the outer limiting membrane (den Hollander, et al., 2002; Mehalow, et al., 2003; Pellikka, et al., 2002; van de Pavert, et al., 2004). A similar distribution was found in the human retina (van de Pavert, et al., 2004). Crumbs and its mouse homolog Crb1 is involved in the photoreceptor morphogenesis (Pellikka, et al., 2002; Tepass, et al., 1990). Analysis of the naturally occurring *Crb1*^{rd8} mouse mutant, suggests a developmental defect of the retina, where disruption of the outer limiting membrane and formation of retinal folds (pseudorosettes) are observed (Mehalow, et al., 2003). Disorganization of the retinal layers was also noted in other *Crb1* mouse models (van de Pavert, et al., 2004; van de Pavert, et al., 2007). These findings are in accordance with clinical features of the patients carrying *CRB1* mutations, whose retinas are thickened and show an altered laminar organization, resembling an immature normal retina (Jacobson, et al., 2003). The latter further supports the importance of CRB1 in the development of the retina.

This study presents an overview of the previously published *CRB1* variants and novel mutations identified in a French cohort of simplex and autosomal recessive RP (arRP) patients. Based on the available genetic and phenotypic data from the literature and on our original findings, we classify all variants into one of the three groups (likely pathogenic, unclassified variants and unlikely pathogenic, Supp. Tables S1-S3). We discuss the clinical variability of patients harboring *CRB1* mutations and analyse the phenotype-genotype correlation of likely pathogenic changes. Identification of novel mutations in the French cohort is described (Supp. Methods and Results) and precise clinical characterisation is given.

Novel *CRB1* Variants

Eleven unrelated patients with ar or isolated RP in the French cohort carried likely pathogenic variants of *CRB1* (Table 1). Seven mutations were novel: three missense changes (p.Ser740Phe, p.Tyr1198Cys and p.Cys1223Ser), one nonsense mutation (p.Cys423*), one in-frame deletion (p.Asn789del) and two frameshift deletions (p.Leu655Trpfs*10, p.Ser1220Asnfs*62) (Table 1). Mutations identified in this study were not present in the SNP databases nor listed as non-pathogenic variants in the literature. None of the novel mutations was present in at least 362 control alleles and the mutations co-segregated in available family members (Supp. Figure S1). In all but one patient (547) two mutated *CRB1* alleles were found.

The three novel missense mutations are in the conserved domains of the CRB1 protein. The p.Ser740Phe exchange replaces a highly conserved serine in the second laminin AG-like domain, the p.Tyr1198Cys mutation replaces a conserved tyrosine with a cysteine in the 16th calcium binding EGF-like domain and the p.Cys1223Ser is a replacement of a conserved cysteine with a serine in the 17th calcium binding EGF-like domain (Figure 1). The in-frame deletion p.Asn789del is also located in the second laminin AG-like domain. Other novel mutations (p.Cys423*, p.Leu655Trpfs*10, p.Ser1220Asnfs*62) result in premature stop codons, which most likely lead to nonsense mediated decay (Chang, et al., 2007) and therefore these alleles are considered as null alleles. Five novel mutations are within exons 7 and 9, which are the most frequently mutated (Figure 1).

Clinical Characterisation of Patients with *CRB1* Mutations

Clinical findings of French patients with *CRB1* mutations are summarized in Tables 2 and 3. The average age at time of diagnosis was 17. Visual acuity was decreased in all patients ranging from 20/50 to light perception with no clear correlation with age or duration of the disease. Hyperopia was noted for 6/11 patients including three for whom spherical equivalent was equal or above +5 diopters. Night blindness was present in all patients but three, for whom a decrease of central vision and photophobia dominated. None of the patients had nystagmus. Most patients (9/11) had a clear lens; in the remaining two, one had undergone cataract surgery and one had significant lens opacities. These two patients were over 40 years of age. Two patterns of fundus pigmentary changes were present in this cohort: 7/11 had typical bone spicule-shaped pigment migration within the peripheral retina whereas 4/11 had widespread clumped pigmentary changes of nummular appearance at the level of the retinal pigment epithelium (Figure 2). Clumped pigmentation is therefore highly suggestive of *CRB1* mutations but it is not specific since it has also been associated with mutations in *NR2E3* (Schorderet and Escher, 2009; Sharon, et al., 2003), *NRL* (Nishiguchi, et al., 2004) or *TULP1* (Mataftsi, et al., 2007). None of the patients displayed preservation of the para-arteriolar retinal pigment epithelium as previously described in association with *CRB1* mutations (Bernal, et al., 2003; den Hollander, et al., 2004; den Hollander, et al., 1999; Heckenlively, 1982; Henderson, et al., 2010; Khaliq, et al., 2003; Simonelli, et al., 2007; Yzer, et al., 2006b). In addition, none of the patients displayed Coats-like changes in the periphery. All patients had macular involvement. Six of the patients displayed cystoid macular edema whereas the other five had macular thinning with loss of the outer retinal layers and corresponding loss of autofluorescence (Figure 2). Color vision was normal in four patients or showed either tritan deficit or a dyschromatopsia with no clear axis when visual acuity allowed color vision testing. Full field electroretinogram showed severe generalized retinal dysfunction with no detectable responses in all patients except three for whom some residual rod and cone function was detectable. Among those three, the best responses on ERG were obtained in the youngest patients. Residual responses on ERG were correlated with better preservation of the visual field.

All patients displayed severe retinal involvement with early macular changes, half of them had cystoid macular edema, a higher percentage than the usually reported prevalence of about 30% in overall RP (Hajali, et al., 2008). This higher prevalence could at least be in part related to vascular abnormalities with Coats-like changes encountered in patients with *CRB1* mutations (Coppieters, et al., 2010; den Hollander, et al., 2004; den Hollander, et al., 2001a; Henderson, et al., 2010; Yzer, et al., 2006b). Alternatively, these changes could be related to abnormal laminar structure associated with *CRB1*-mutations (Jacobson, et al., 2003). None of our patients developed Coats-like changes or para-arteriolar retinal pigment epithelium suggesting that these changes are not consistant in *CRB1*-related RP (Lotery, et al., 2001b). Four subjects displayed clumped retinopathies reinforcing that *CRB1* should be considered as a potential causal gene for this specific phenotype along with *NR2E3* (Sharon, et al., 2003) or *NRL* (Nishiguchi, et al., 2004).

CRB1 Variants and Their Classification

Over 240 patients with *CRB1* mutations and more than 150 gene variants have been described in the literature (Azam, et al., 2011; Benayoun, et al., 2009; Bernal, et al., 2003; Booij, et al., 2005; Clark, et al., 2010; Coppieters, et al., 2010; den Hollander, et al., 2004; den Hollander, et al., 2001a; den Hollander, et al., 2007; den Hollander, et al., 1999; Galvin, et al., 2005; Gerber, et al., 2002; Hanein, et al., 2004; Henderson, et al., 2010; Henderson, et al., 2007; Jacobson, et al., 2003; Khaliq, et al., 2003; Li, et al., 2011; Lotery, et al., 2001a; Lotery, et al., 2001b; Riveiro-Alvarez, et al., 2008; Seong, et al., 2008; Siemiatkowska, et

al., 2011; Simonelli, et al., 2007; Tosi, et al., 2009; Vallespin, et al., 2007; Yzer, et al., 2006a; Yzer, et al., 2006b; Zenteno, et al., 2011; Zernant, et al., 2005). The most frequently occurring of the known mutations is the p.Cys948Tyr in exon 9 (96 alleles reported, 24% of known *CRB1* mutations) (Bernal, et al., 2003; Booij, et al., 2005; Clark, et al., 2010; Coppieters, et al., 2010; den Hollander, et al., 2004; den Hollander, et al., 2001a; den Hollander, et al., 2007; den Hollander, et al., 1999; Galvin, et al., 2005; Hanein, et al., 2004; Henderson, et al., 2010; Henderson, et al., 2007; Jacobson, et al., 2003; Lotery, et al., 2001a; Riveiro-Alvarez, et al., 2008; Tosi, et al., 2009; Vallespin, et al., 2007; Yzer, et al., 2006a; Zernant, et al., 2005). In general most of the mutations are in exons 9 (41%) and 7 (27%), therefore as a screening strategy these exons can be tested in the first instance (Figure 1, Supp. Table S1). Exons 7 and 9 encode second and third laminin AG-like domains respectively, implying that these domains are particularly important for CRB1 function. Missense mutations constitute 66% of all known mutations, the remaining being frameshift, truncation and splice site mutations.

We have attempted to classify all the reported mutations in three groups: 1) likely pathogenic, 2) unclassified variants, 3) unlikely pathogenic. This classification was based on the genetic data available from the literature, amino acid conservation and bioinformatic pathogenicity prediction tools (Supp. Tables S1-S3). An important criterion was the presence of two mutant alleles and co-segregation in the family. Approximately 30% of cases were reported with only one mutant allele, assuming that the second mutation is within the intronic region. For these patients however, one cannot exclude the possibility that there is another molecular cause of the pathology. The lack of the second mutant *CRB1* allele is sometimes explained by a digenic inheritance, however so far it has not been proven by co-segregation analysis (Li, et al., 2011; Vallespin, et al., 2007).

Pathogenicity is easier to asses in deletions and frameshift variants than in the case of missense changes, hence the importance of the bioinformatic analysis of the pathogenicity, amino acid conservation and functional analysis of the variants. On this basis we have not considered two changes identified in our cohort as pathogenic (p.Gly959Ser and p.Ala1354Thr) (den Hollander, et al., 2004; den Hollander, et al., 2001a)). The respective patients did not carry a second *CRB1* mutation and we did not consider the p.Gly959Ser and p.Ala1354Thr substitutions as likely pathogenic, based on poor conservation of the residues and low pathogenicity predictions using online bioinformatic tools: PolyPhen-2 and SIFT (Supp. Tables S2 and S3). One report suggests involvement of *CRB1* in autosomal dominant pigmented paravenous chorioretinal atrophy (McKay, et al., 2005), though the reported mutation p.Val162Met has a questionable pathogenicity, since valine is not conserved and methionine is present in this position in other mammals (Supp. Table S2).

Prevalence

In the investigated cohort, at least 2.5% of arRP patients carry *CRB1* gene defects, which lies within the previously published range of 0-6.5% (Bernal, et al., 2003; den Hollander, et al., 2004; Vallespin, et al., 2007), or 2.7% after cohort averaging (Table 4). The high preponderance of novel *CRB1* mutations in our cohort suggests, however, that probably more arRP patients carry *CRB1* pathogenic defects, which are novel and therefore undetectable by arRP microarray. Much higher prevalence is observed in LCA/EORD cohorts and RP with additional features like PPRPE and retinal telangiectasia, representing 10.1%, 74.1%, 53.3% respectively in averaged cohorts (Table 4) (Bernal, et al., 2003; Coppieters, et al., 2010; den Hollander, et al., 2004; den Hollander, et al., 2001a; den Hollander, et al., 2007; den Hollander, et al., 1999; Hanein, et al., 2004; Henderson, et al., 2010; Henderson, et al., 2007; Lotery, et al., 2001a; Seong, et al., 2008; Simonelli, et al., 2007; Vallespin, et al., 2007; Walia, et al., 2010).

Genotype-Phenotype Correlation

We were not able to establish a clear genotype/phenotype correlation for our cohort, which might be due to the small number of patients with *CRB1* mutations and their variable phenotype. In addition, the nature of existing published data makes it difficult to correlate the recurring *CRB1* mutations with different phenotypes for a number of reasons. First, the phenotyping of patients is complex and distinguishing between early-onset RP and LCA is often arbitrary and depends on the guidelines of a particular clinical center. Second, precise clinical data is often omitted in the publications and therefore it is difficult to adjust for these diagnostic differences in a cross-paper analysis. Despite these inconsistencies, we attempted to analyse data from previous reports in order to find the relationship between the *CRB1* variants and the clinical features of respective patients. In this meta-analysis we used 171 patients, who carried two likely pathogenic mutations in trans (Benayoun, et al., 2009; Bernal, et al., 2003; Booij, et al., 2005; Clark, et al., 2010; Coppieters, et al., 2010; den Hollander, et al., 2004; den Hollander, et al., 2001a; den Hollander, et al., 2007; den Hollander, et al., 1999; Galvin, et al., 2005; Hanein, et al., 2004; Henderson, et al., 2010; Henderson, et al., 2007; Jacobson, et al., 2003; Khaliq, et al., 2003; Li, et al., 2011; Lotery, et al., 2001a; Lotery, et al., 2001b; McKibbin, et al., 2010; Riveiro-Alvarez, et al., 2008; Seong, et al., 2008; Simonelli, et al., 2007; Tosi, et al., 2009; Vallespin, et al., 2007; Yzer, et al., 2006a). Combination of two mutant alleles was analysed in relation to clinical characteristics of the published cases. Based on the reports we distinguished the following phenotypes: LCA, early onset retinal degeneration (EORD), RP, presence of PPRPE and Coats-like vasculopathy. The mutations were classed as null mutations (all mutations leading to a premature stop codon) or as variants leading to an altered protein (missense and in frame deletions). The likely pathogenic mutations were plotted on a graph, where affected codons on allele 1 and allele 2 served as coordinates (codon 0 was assigned to null mutations). The results show that we cannot assign a specific allele combination to a particular phenotype, e.g. homozygous null alleles or homozygous p.Cys948Tyr alleles are found in LCA, EORD and RP patients (Figure 3 A). Null alleles are however more frequent in LCA cohorts (Figure 3 B) as previously suggested (den Hollander, et al., 2004). The presence/absence of PPRPE or Coats-like vasculopathy did not reveal a particular mutation pattern (Figure 3 C). These findings suggest the involvement of additional modifying factors (genetic and/or environmental), which are responsible for the modulation of the phenotype in patients harboring *CRB1* mutations.

Future Directions

The above analysis of the phenotype-genotype correlation suggests that the disease severities associated with *CRB1* mutations are in fact a continuum of the same clinical entity with possible additional modifying factors influencing disease onset and progression. There is increasing evidence of the involvement of multiple alleles in the patient's phenotype, as has been shown for the Bardet-Biedl patients (Katsanis, et al., 2001) and more recently for a *PRPH2*-associated macular dystrophy family, where the phenotype has been modulated by additional heterozygous mutations in *ABCA4* (MIM# 601691) and *ROM1* (MIM# 180721) (Poloschek, et al., 2010). It is likely that the new next generation sequencing (NGS) technology will help to shed light on the potential genetic modifiers that influence disease phenotype. One has, however, to analyse the data with caution since NGS will reveal large numbers of polymorphic changes, which do not modulate the disease. The potential new modifying changes will have to be confirmed by appropriate genetic and functional analysis. The certainty of the molecular cause of a disease is particularly important in the era of gene therapy trials. Genetic treatment of recessive disorders should not be undertaken before obtaining proof that both alleles of a given gene are dysfunctional. In-depth genetic analysis, as presented here, is necessary to provide a basis for conducting such therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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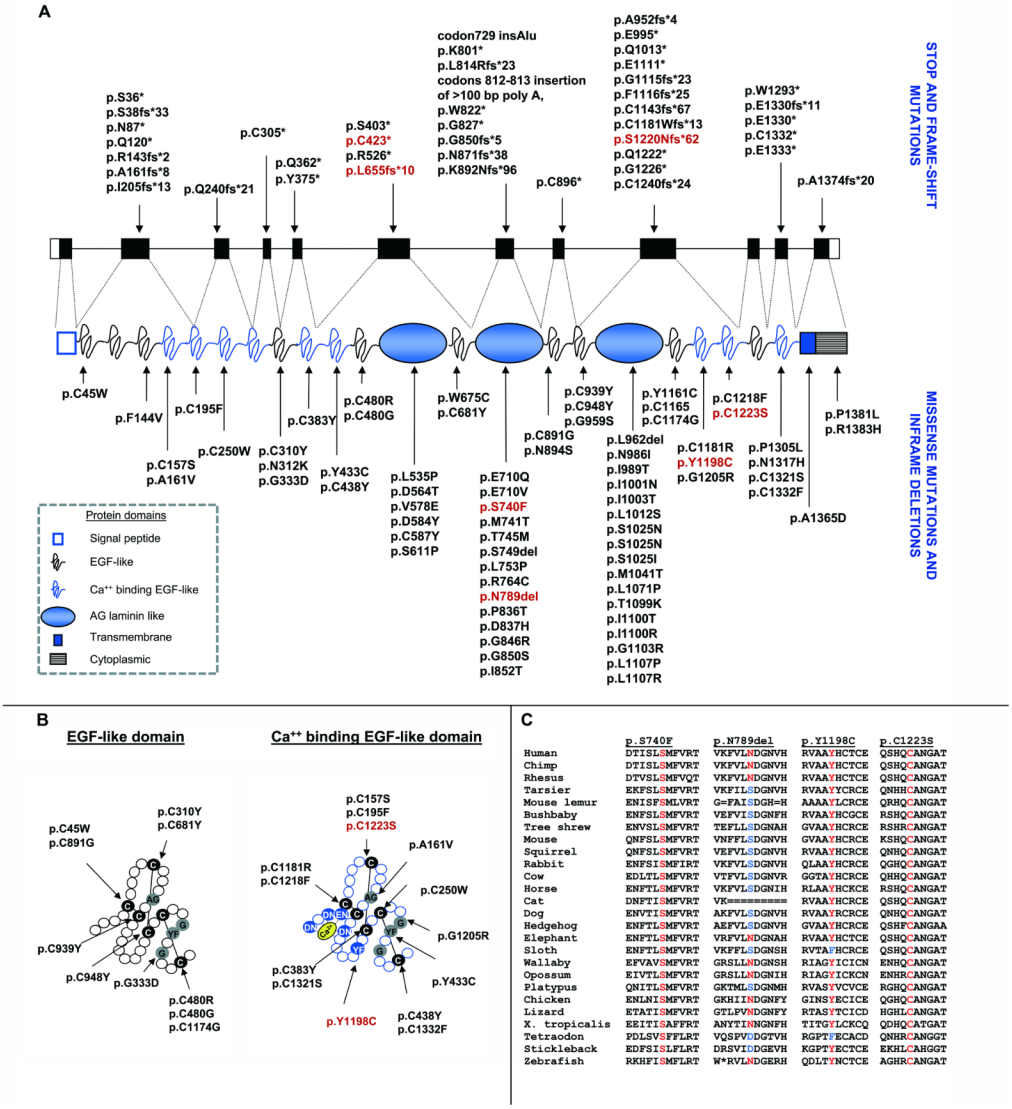

Figure 1.

Distribution of *CRB1* mutations in the gene and protein. A) Nucleotide numbering is based on cDNA sequence of *CRB1* (Ref. NM_201253.2) where A of the ATG initiation codon is 1. The stop and frameshift mutations are indicated above the structure of the gene and the position of the missense mutations are drawn in relation to protein domains. The novel mutations are indicated in red. B) The structures of EGF-like and Ca^{++} binding EGF-like domains with indications of conserved residues and recurrent mutations. The highly conserved cysteine residues are in black, the conserved residues between both domains are in grey and the conserved amino acids specific to the Ca^{2+} binding domain are in blue. C) Evolutionary conservation of the likely pathogenic CRB1 residue changes identified in this work.

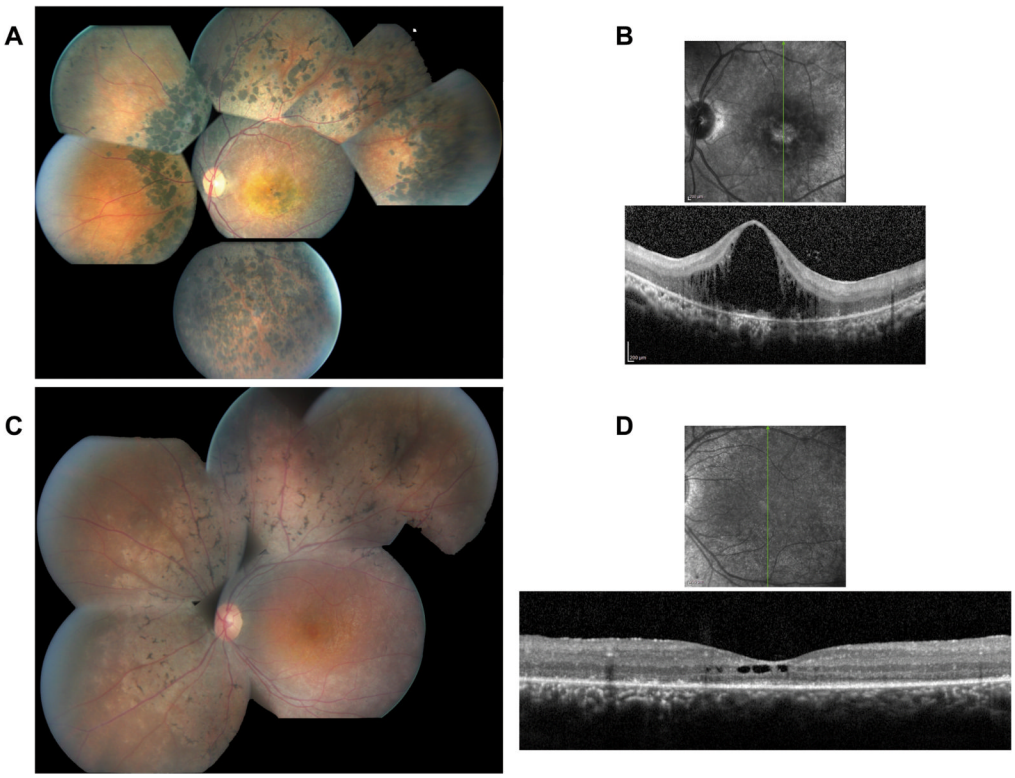

Figure 2.
Fundus color photographs and Optical Coherence Tomography (OCT). A) Color fundus photograph of the left eye of 3969 showing nummular pigmentary migration in the mid periphery in addition to pigmentary changes within the macula. B) Vertical scan OCT of the left eye of 3969 showing cystic changes in the macular region. C) Color fundus photograph of the right eye of 547 showing bone spicules pigmentary migration in the periphery in addition to atrophic changes within the macula. D) Vertical scan OCT of the right eye of 547 showing atrophic changes in the macular region after resolution of episodes of cystoid changes.

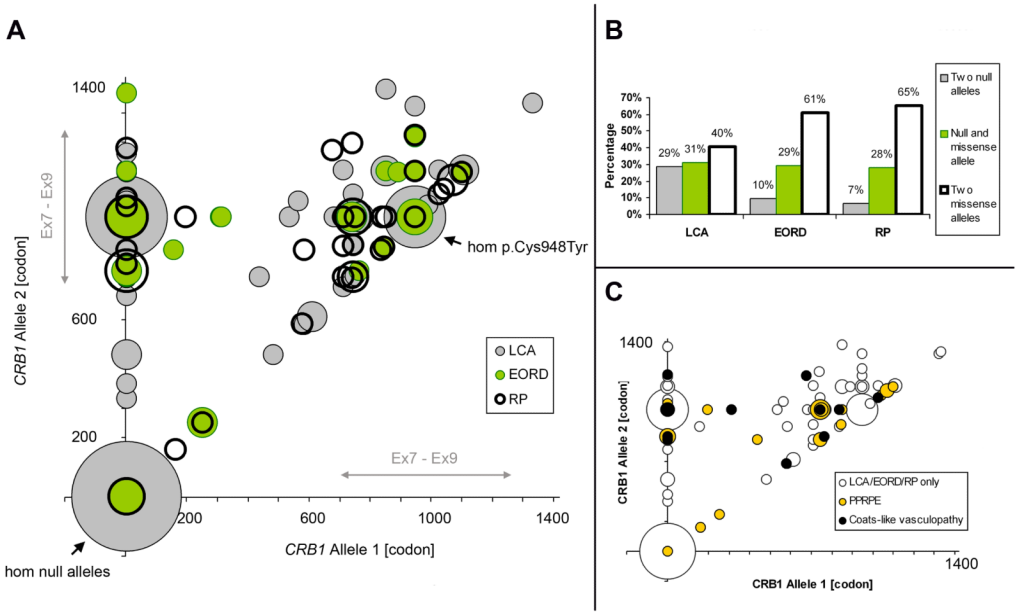

Figure 3.

Genotype-phenotype correlation of patients with *CRB1* mutations. A) Distribution of *CRB1* mutations in LCA, EORD and RP. XY axes represent allele 1 and 2 of the patients, the affected codons serve as xy coordinates, null allele coordinate is designated as 0. The size of the circles is proportional to the number of the *CRB1* patients with a given genotype. B) Frequency of null and missense allele combinations in LCA, EORD and RP patients. C) Distribution of *CRB1* mutations in patients with/without additional features: PPRPE and Coats-like vasculopathy.

Table 1

Patients with *CRB1* mutations identified in this study

Patient number	Family	Allele 1			Allele 2		
		Exon	Nucleotide change	Protein change	Exon	Nucleotide change	Protein change
229	159	2	c.613_619del	p.Ile205Aspfs*13	7	**c.2365_2367delAAT**	**p.Asn789del**
53	No family members	**6**	**c.1269C>A**	**p.Cys423***	7	c.2506C>A	p.Pro836Thr
368	249	6	c.1750G>T	p.Asp584Tyr	7	c.2506C>A	p.Pro836Thr
547	372	**6**	**c.1963delC**	**p.Leu655Trpfs*10**		?	
4240 [a]	2025	**7**	**c.2219C>T**	**p.Ser740Phe**	**7**	**c.2219C>T**	**p.Ser740Phe**
54	39	7	c.2222T>C	p.Met741Thr	**9**	**c.3593A>G**	p.Tyr1198Cys
3969	No family members	7	c.2506C>A	p.Pro836Thr	7	c.2506C>A	p.Pro836Thr
409	281	9	c.2843G>A	p.Cys948Tyr	**9**	**c.3668G>C**	**p.Cys1223Ser**
1183 [b]	709	**9**	**c.3659_3660delinsA**	**p.Ser1220Asnfs*62**	**9**	**c.3659_3660delinsA**	**p.Ser1220Asnfs*62**
1731	1008	9	c.2843G>A	p.Cys948Tyr	9	c.2843G>A	p.Cys948Tyr
3144	1302	9	c.2843G>A	p.Cys948Tyr	7	c.3307G>A	p.Gly1103Arg

[a] mutation in this patient was identified by NGS

[b] mutation in this patient was found through homozygosity mapping novel mutations are in bold

Table 2

Clinical data

Patient	Age at time of testing	Age at time of diagnosis	Sex	Relevant medical and ophthalmology history	Family history	Symptoms	BCVA OD/OS Refraction	Lens	Fundus examination	OCT	FAF
53	27	20	M	none	From Ivory Coast, 10 brothers and sisters, 1 sister affected	Night blindness at 6 then photophobia then decreased vision	LP 20/500 +2(−1.50)60° +1.75(−1.5)125°	Clear	Widespread clumped pigment migration with no pale optic disc or narrowed retinal vessels	Macular thinning with loss of ONL	Loss of AF at the posterior pole and periphery
54	41	25	F	none	From French descent One affected brother	Night blindness	20/640 20/100 Prior to lens surgery: +5.50(−1)5° +5.50(−1)165°	IOL	Peripheral RPE changes with bone spicules, perifoveal atrophy, pale optic disc, narrowing of retinal vessels	Thinning of the ONL within the macular region	Loss of AF in the perifoveal region and outside the vascular arcades
229	29	20	F	none	From French descent	Night blindness	20/80 20/50 +2(−0.75)5° +2.50(−1.50)5°	Clear	Peripheral RPE changes, little bone spicules, no pale optic disc or narrowed retinal vessels, CME	CME, thinning of ONL	Patchy loss of AF in the periphery; foveal modification of AF due to the CME
368	13	12	F	Seizure in infancy	From Turkish descent maternal grand-mother said to be blind	photophobia	20/80 20/63 +6.50(−1.25)160° +6.50(−1)7°	Clear	Peripheral RPE changes with bone spicules, perifoveal atrophy, pale optic disc, narrowing of retinal vessels, CME	CME with relative preservation of foveal architecture	Patchy loss of AF outside the vascular arcades, foveal AF changes due to CME
409	43	Teenage years	F	none	From Italian descent	Night blindness then photophobia	20/160 20/100 Plano Plano	Clear	Peripheral bone spicules with perifoveal atrophy	Thinning of the ONL	Loss of AF outside the vascular arcades and in the perifoveal area
547	57	39	M	Recurrent anterior uveitis, which delayed the diagnosis of RP	From French descent, no family history of RP	Night blindness then photophobia and decreased vision	20/80 20/63 +0.25(−0.50)110° −2(−1.25)65°	Bilateral nuclear cataract	Peripheral bone spicules with CME	Bilateral CME, perifoveal thinning	Loss of AF in the perifoveal region and outside the vascular arcades
1183	38	15	F	none	From Tunisian descent; consanguinity among parents	Night blindness and photophobia	20/640 20/640 Emetropia	Clear	Widespread clumped pigment migration with no pale optic disc or narrowed blood vessels; OD asteroides hyaloids	Macular thinning with loss of ONL	Loss of AF at the posterior pole and periphery

Patient	Age at time of testing	Age at time of diagnosis	Sex	Relevant medical and ophthalmology history	Family history	Symptoms	BCVA OD/OS Refraction	Lens	Fundus examination	OCT	FAF
1731	23	17	M	Deafness since age 9	From Spanish descent; parents first cousins; one brother affected	Low vision since early childhood	HM 20/80 Emetropia	Clear	Widespread clumped pigment migration with relative sparing of the macula, with no pale optic disc or narrowed blood vessels	Macular thinning with loss of ONL	Loss of AF at the posterior pole and periphery
3144	20	9	F	none	From French descent	Night blindness since early childhood	20/80 20/80 +9(−1.50)170° +7.50	Clear	Some RPE changes in the periphery, normal disc color and no narrowing of blood vessels; CME	CME with relatively spared foveal structure	Patchy loss of AF outside the vascular arcades, foveal AF changes due to CME
3969	28	12	F	none	From Mali	Night blindness then photophobia	20/125 20/320 +0.50(−1.50)90° +1.75(−1.25)95°	Clear	Widespread clumped pigment migration in the posterior pole and periphery CME	CME Thinning of ONL	Diffuse patchy loss of AF within the posterior pole and periphery
4240	7	6	M	none	One sister affected, from Turkish descent	Decreased vision	20/63 20/80 −1.50(−1.50)10° −2(−0.75)180°	Clear	Moderate RPE changes in the periphery CME	CME with relatively spared parafoveal structure	Patchy loss of AF outside the vascular arcade, normal AF within posterior pole except AF modification due to CME in the fovea

BCVA: best corrected visual acuity; CME: cystoid macular edema; ND: not detectable; FAF: Fundus Autofluorescence; OD: Oculis dextra (right eye); OS: Oculis Sinistra (left eye); IOL: intra ocular lens; CF: counting fingers; HM: hand motion; LP: light perception; RPE: retinal pigment epithelium; RP: retinitis pigmentosa; OHT: ocular hypertension; ONL: Outer Nuclear Layer

Table 3

Function data

Patient	Colour vision (15 saturated Hue)	Binocular Goldman visual field, III4 isopter	Full field ERG	Multifocal ERG
53	NP	Inf to 5°	ND	ND
54	Dyschromatopsia without axis	Inf to 5°	ND	ND
229	Normal	40 central degree with 2 peripheral island of perception	ND	ND
368	Normal	120° horizontally, 60° vertically with relative central annular scotoma	Residual responses consistent with severe rod-cone dysfunction	Residual responses to central hexagones
409	Dyschromatopsia without axis	100° horizontally, 60° vertically with annular scotoma	Residual cone responses	ND
547	Bilateral tritaonopia	20 central degrees both horizontally and vertically	ND	ND
1183	NP	Inf to 5°	ND	ND
1731	OD NP, OS tritaonopia	5 central degrees	ND	ND
03144	Normal	20 central degrees both horizontally and vertically	ND	ND
3969	Dyschromatopsia without axis	20 central degree with 2 peripheral island of perception	ND	ND
4240	Normal	130° vertically and 110° horizontally	30% decreased scotopic responses with photopic responses at the lower limit of normal	Decreased responses to central hexagones

NP: not performed; ND: not detectable

Table 4

Average prevalence of *CRB1* mutations in retinal dystrophy patients in published reports

Dystrophy	Prevalence*	Patients with two CRB1 alleles	Patients with one CRB1 allele	Added cohort size	References
LCA/EORD	10.1%	109	57	1645	(Bernal, et al., 2003; Coppieters, et al., 2010; den Hollander, et al., 2004; den Hollander, et al., 2001; den Hollander, et al., 2007; den Hollander, et al., 1999; Hanein, et al., 2004; Henderson, et al., 2010; Henderson, et al., 2007; Li, et al., 2011; Lotery, et al., 2001; Seong, et al., 2008; Simonelli, et al., 2007; Vallespin, et al., 2007; Walia, et al.)
RP	2.7%	4	5	335	(Bernal, et al., 2003; den Hollander, et al., 2004; Vallespin, et al., 2007)
RP+PPRPE	74.1%	18	2	27	(den Hollander, et al., 2004; den Hollander, et al., 1999)
RP+ret telangiectasia	53.3%	8	8	30	(den Hollander, et al., 2004; den Hollander, et al., 2001; Henderson, et al., 2010)
Classic Coats disease	0.0%	0	0	18	(den Hollander, et al., 2004)

* The average prevalence was calculated on the basis of all the published reports indicating phenotypes of patients with *CRB1* mutations and the size of screened cohorts.