

Detection of CRB1 mutations in families with retinal dystrophy through phenotype-oriented mutational screening

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Abstract. Mutations in the crumbs homolog (CRB)1 gene are among the common causes of severe early onset retinal dystrophy. Some characteristic clinical phenotypes are frequently associated with mutations in CRB1. The aim of this study was to examine whether characteristic phenotype-directed mutational screening facilitated the detection of CRB1 mutations. The study included 22 probands with at least one of the potential CRB1-associated phenotypes for retinal dystrophy. Variants were detected using Sanger sequencing. The complete sequences of the coding and adjacent intronic regions of CRB1 were analyzed, revealing homozygous or compound heterozygous mutations in CRB1 in seven of 22 probands, involving six novel (c.136delA, c.1841G>T, c.3017C>A, c.3488G>T, c.3991C>T and c.4089dupTGTTGCTT) and four known (c.2222T>C, c.2671T>G, c.3676G>T and c.4005+2T>G) mutations. The mutations were present in three of four probands with macular nummular pigmentation and in four of seven probands with early onset retinitis pigmentosa with macular involvement. The results suggested that macular nummular pigmentation is a gene-specific indication for CRB1-associated retinal dystrophy and confirm that CRB1 mutations are also common causes of early onset retinitis pigmentosa. Identification of gene-specific phenotypes is useful in identifying genetic defects underlying heterogeneous retinal dystrophy.

Introduction

Hereditary retinal dystrophy is a broad group of clinically and genetically heterogeneous diseases that usually cause

irreversible blindness. Retinitis pigmentosa (RP) is the most common form, with Leber congenital amaurosis (LCA) being the most severe form. The clinical manifestations as well as the causative genes frequently overlap in the different forms of these diseases (1). Mutations in almost 200 genes have been described in association with hereditary retinal diseases (RetNet: <http://www.sph.uth.tmc.edu/Retnet/sum-dis.htm>). However, mutations in each of these genes usually contribute to only a small fraction of the diseases (2-4). Thus, detection of disease-causing mutations among these genes is a great challenge in individual patients, and involves great effort and expense. Identification of a gene-specific genotype-phenotype correlation would facilitate mutation detection, similar to CYP4V2 mutations and Bietti crystalline corneoretinal dystrophy (5,6).

Mutations in crumbs homolog (CRB)1 are among the common causes of severe early onset retinal dystrophy, including LCA and early onset RP (4,7-31). Although a firm genotype-phenotype correlation between CRB1 mutations and specific phenotypes has yet to be determined, some characteristic clinical findings have been suggested to associate with CRB1 mutations.

The aim of the present study was to investigate whether characteristic phenotype-directed mutational screening facilitated the detection of CRB1 mutations. Probands with some of the CRB1-associated phenotypes were selected for this study. The Sanger sequencing of CRB1 coding and adjacent intronic regions revealed homozygous and compound heterozygous mutations in CRB1 in seven of 22 Chinese probands with at least one of the CRB1-associated phenotypes.

Materials and methods

Clinical characteristics of the probands. Probands from 22 unrelated families with at least one of the CRB1-associated phenotypes participated in this study (Table I). Clinical phenotypes in this study were mainly based on the fundus photos and the age of onset (1-10 years of age). CRB1-associated characteristic clinical findings included: i) severe early onset retinal dystrophy (including LCA and early onset RP) (4,7-31); ii) nummular pigmentary changes at the posterior fundus; iii) preservation of the para-arteriolar retinal pigment epithelium (PPRPE) (14); iv) Coats-like vasculopathy (CLV) (12); and v) pigmented paravenous chorioretinal atrophy (PPCRA) (20).

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Table I. Classification of the probands in this study.

Clinical characteristics	No. of cases	
	Screened	CRB1 mutations
A. Macular nummular pigmentation	4	3
B. Pigmented paravenous chorioretinal atrophy	4	0
C. Early onset retinitis pigmentosa with macular involvement (<10 years)	7	4
D. Other retinal dystrophy with macular dot pigmentation	7	0
Total	22	7

The LCA, PPRPE and CLV phenotypes were not studied since LCA has been previously analyzed, whereas PPRPE and CLV were not identified in our patients. Macular dot pigmentation in seven probands was also selected to examine the specificity of macular nummular pigmentation.

Informed consent was obtained from each participating individual or their guardians prior to the collection of clinical data and venous blood. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center and followed the tenets of the Declaration of Helsinki.

Genomic DNA. Genomic DNA was prepared from venous leukocytes using a method described previously (32). The procedure for Sanger sequencing of CRB1 was the same as that described in our previous study and the same sets of primers were used (4). The informatics analysis for the detected variants was also as described previously (4). Only homozygous or compound heterozygous variants that were predicted to be pathogenic were considered to be disease-causing because: i) CRB1 mutations were only reported to associate with recessive retinal dystrophy (8); and ii) single heterozygous null mutations for recessive genes were common in the general population (33). Controls were healthy university students with normal visual acuity without any known hereditary retinopathy and family history of such diseases as those controls used in the genetic study of myopia (34).

Results

Homozygous or compound heterozygous mutations in CRB1. Complete sequencing analysis of CRB1 revealed homozygous and compound heterozygous variants in seven of the 22 probands (Fig. 1), involving six novel and four known variations (Table II). Of the 10 variants, four were predicted to be truncated and the remaining six variants were missense. The residues with the six missense changes were relatively conserved in different species (Table II and Fig. 2) and involved in the functional domain of the CRB1 protein (Table II). Five of the six missense variants were predicted to affect the encoded protein by PolyPhen-2, while two variants were predicted to be damaging by sorting intolerant from tolerant (SIFT) (Table II). Only the missense change p.M741T, was predicted to be benign or tolerated by PolyPhen-2 or SIFT, but it was reported to be causative in a previous study (31). Therefore, all 10 variants detected in this study were considered to be pathogenic.

Clinical data of the seven probands with CRB1 mutations. Each proband had either poor vision or nystagmus at <8 years of age. Visual acuity ranged from finger counting to 0.6 (Table III). Homozygous or compound heterozygous mutations were identified in three of four probands with macular nummular pigmentation and in four of seven probands with early onset RP with macular involvement. However, no mutations were identified in the 11 probands with either pigmented paravenous chorioretinal atrophy or other retinal dystrophy with macular dot pigmentation (Tables I and III). All seven probands with CRB1 mutations were isolated cases (Fig. 1). Fig. 3 shows the representative fundus changes of three probands with CRB1 mutations (Fig. 3A-C) and one proband without CRB1 mutation (Fig. 3D) but with nummular pigmentation.

Discussion

A recent review on CRB1 mutations suggested an average prevalence of homozygous or compound heterozygous CRB1 mutations in patients with different forms of retinal dystrophy as follows: 6.6% (109/1,645) for LCA or early onset retinal dystrophy, 1.2% (4/335) for RP, 66.7% (18/27) for RP with PPRPE and 26.7% (8/30) for RP with CLV (8). Those findings suggested that the sequencing analysis of patients with CRB1-specific phenotypes may facilitate mutation detection of CRB1-associated retinopathy. The frequency of CRB1 mutations in patients with macular nummular pigmentation was not available due to the unavailability of the frequency of this fundus change in patients analyzed in previous studies. However, macular nummular pigmentation is rarely observed in patients with mutations in other genes, although it has been described in different studies in a number of patients with CRB1 mutations (9,16,24,25,35-37). Therefore, it may be considered a characteristic indication for CRB1-associated retinal dystrophy.

In the present study, the entire coding region and adjacent intronic sequences of CRB1 were analyzed in 22 probands with one of the possible CRB1-associated phenotypes, using Sanger sequencing. Homozygous and compound heterozygous mutations in the CRB1 gene, involving six novel and four known variants, were identified in 31.8% (7/22) of the probands, including 75% (3/4) with macular nummular pigmentation and 57.2% (4/7) with early onset RP with macular involvement. No novel mutations were detected in 192 alleles of 96 healthy individuals. The frequent association of CRB1 mutations with early onset of RP and LCA

Table II. Potential pathogenic mutations detected in 7 of the 22 probands.

Nucleotide change	Effects	Protein domain	Bioinformatics analysis			Status in probands		Novel or reported
			Conservation	PolyPhen or splice site	SIFT	Homozygous	Heterozygous	
c.136delA	Frameshift	-	-	-	-	-	QT495	Novel
c.1841G>T	p.G614V	LamG	Yes	Missense, PBD (1.00)	Tolerated (0.18)	RP114	RP200	Novel
c.2222T>C	p.M741T	LamG	Yes	Missense, benign (0.395)	Tolerated (0.41)	-	QT659	Hanein <i>et al</i> (31)
c.2671T>G	p.C891G	EGF	Yes	Missense, PD (0.475)	Damaging (0)	-	RP344	Bernal <i>et al</i> (28)
c.3017C>A	p.S1006Y	LamG	Yes	Missense, PBD (0.964)	Tolerated (0.14)	-	QT495, RP82	Novel
c.3488G>T	p.C1163F	EGF	Yes	Missense, PBD (0.999)	Damaging (0)	-	QT432	Novel
c.3676G>T	p.G1226X	-	-	-	-	-	QT432, RP82	Li <i>et al</i> (4)
c.3991C>T	p.R1331C	EGF	Yes	Missense, PBD (0.941)	Tolerated (0.18)	-	RP200	Novel
c.4005+2T>G	Splicing site	-	-	Splicing site eliminated	-	-	RP344	Li <i>et al</i> (4)
c.4089dupTGTGCTT	Frameshift	-	-	-	-	-	QT659	Novel

-, Not applicable; PD, possibly damaging; PBD, probably damaging. No novel variants were detected in 192 alleles in 96 controls. SIFT, sorting intolerant from tolerant.

Table III. Clinical data of the probands with CRB1 homozygous or compound heterozygous mutations.

Proband ID	CRB1 mutations	Gender	Age (years) at		First symptom	Visual acuity (right; left)	Fundus changes	ERG responses		Classification in Table I
			Exam	Onset				Rod	Cone	
QT432	c.[3488G>T];[3676G>T]	M	4.5	1	NYS	FC	GRD, MNP, YCLS, BSP	Not detectable	Not detectable	A
QT495	c.[136delA];[3017C>A]	M	22	EC	PV; NYS	0.02; 0.04	GRD, BSP	NA	NA	C
QT659	c.[2222T>C];[4089dupTGTGCTT]	M	4.5	EC	NYS; HH	NA	GRD	Not detectable	Not detectable	C
RP82	c.[3017C>A];[3676G>T]	F	5	3	PV	0.6; 0.6	GRD, BSP	Not detectable	Reduced	C
RP114	c.[1841G>T];[1841G>T]	M	6	2	PV; NYS	0.3; 0.3	GRD, MNP, YCLS, BSP	Not detectable	Not detectable	A
RP200	c.[1841G>T];[3991C>T]	M	20	8	PV	0.1; FC	GRD	Not detectable	Not detectable	C
RP344	c.[2671T>G];[4005+2T>G]	F	7	6	PV	0.2; 0.2	GRD, MNP, BSP	Not detectable	Reduced	A

All probands were isolated cases without a family history or a consanguinity marriage between their parents. CRB1, crumbs homolog 1; EC, early childhood; PV, poor vision; NYS, nystagmus; HH, high hyperopia; NA, not available; FC, finger counting; GRD, generalized retinal dystrophy; MNP, macular nummular pigmentation; YCLS, yellowish crystalline-like spots in mid-peripheral retina; BSP, bone-spicule pigmentation at mid-peripheral retina.

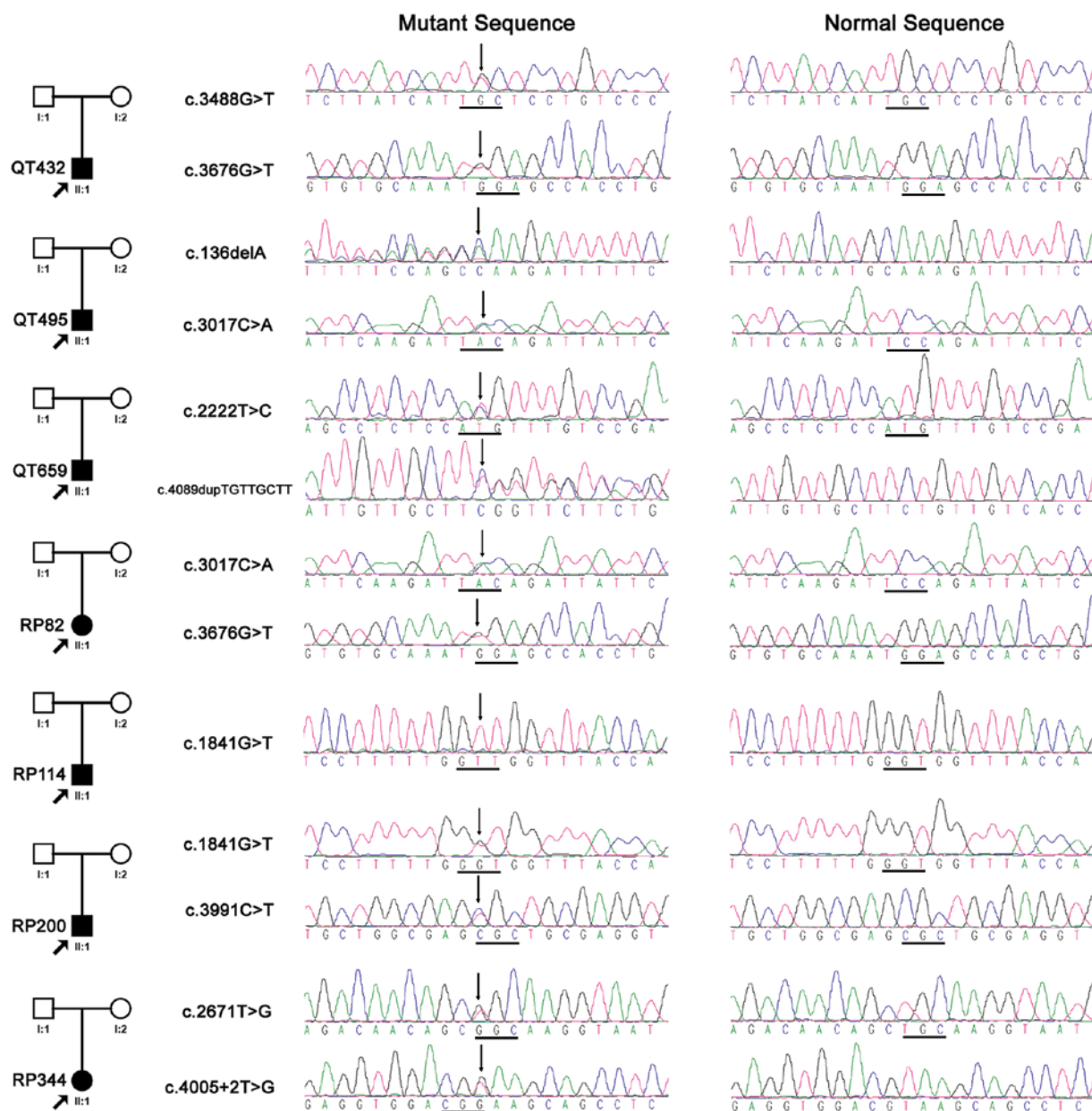


Figure 1. Sequence chromatography. Homozygous or compound heterozygous mutations in crumbs homolog (CRB)1 of each of the seven probands are shown on the left; the corresponding normal sequences are shown on the right.

suggests that early onset RP is a spectrum from LCA to RP with early onset RP being detected within this spectrum. Thus, genes associated with early onset RP are potentially good candidates for LCA and vice versa.

Results of the bioinformatics analysis suggest that these mutations are likely to exert pathogenic effects on the encoded proteins (Fig. 2). Our study provides additional evidence that macular nummular pigmentation may be considered a gene-specific indication for CRB1-associated retinal dystrophy. The analysis also suggests that CRB1 mutations are common causes for early onset RP, as observed by their contribution to LCA. Findings of a recent study also showed that CRB1 mutations are a relatively common cause of autosomal recessive early onset retinal degeneration (38), suggesting that certain CRB1-associated specific phenotypes, including PPRPE and CLV, may not be common in the studied

populations. This may also be due to the relatively small sample size of this study.

Identification of gene-specific phenotypes may facilitate mutation identification and subsequent genetic counseling as well as provide useful evidence to determine causative variants underlying the extremely heterogeneous hereditary retinal diseases. This is particularly true for isolated patients with mutations in multiple genes, which are likely to become increasingly common in the era of next-generation sequencing. Systematic and careful records of clinical data, particularly of fundus photos of different regions and at different time-points, may provide additional gene-specific phenotypes for CRB1 and for other genes associated with retinal dystrophy. This record keeping should be encouraged in future investigations of mutations related to retinal dystrophy.

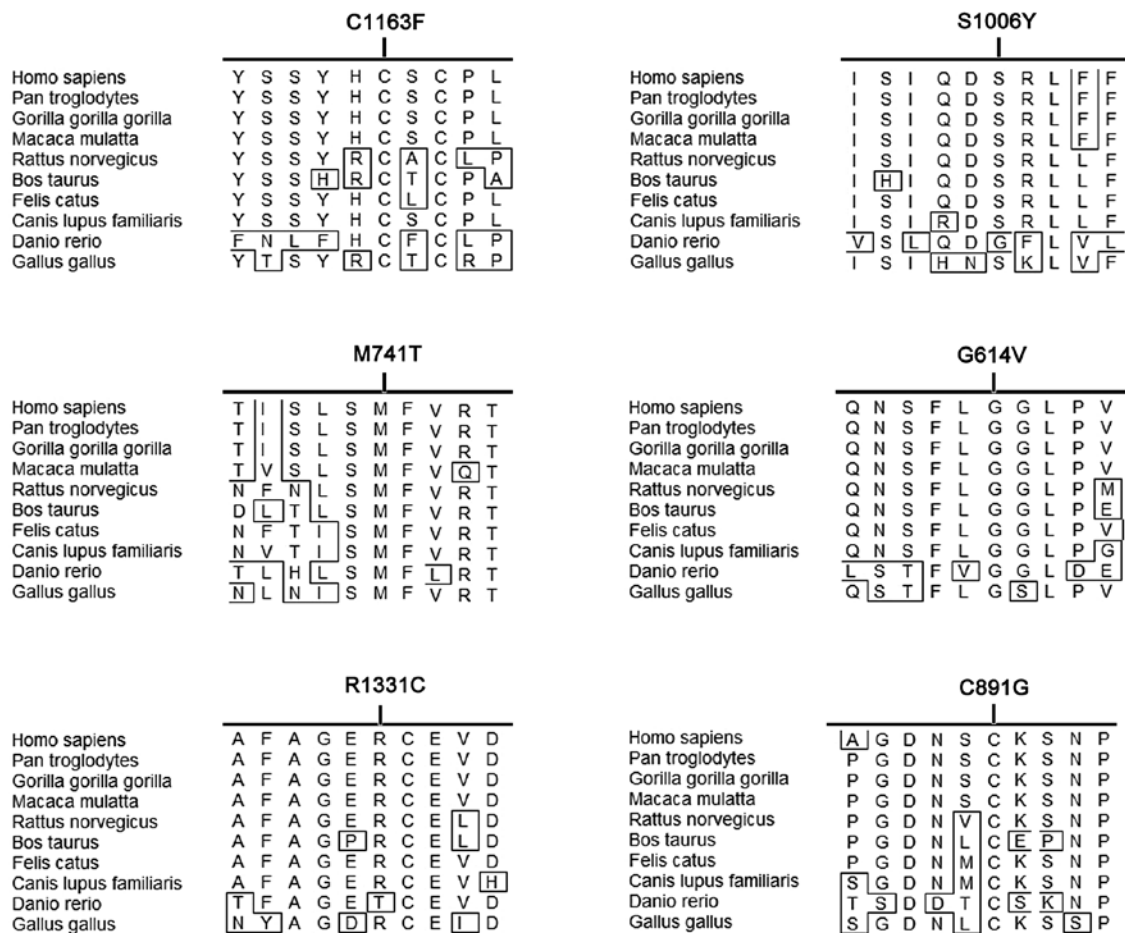


Figure 2. Protein sequence alignment for 10 crumbs homolog (CRB)1 orthologs. Of the six residues affected by the six missense mutations, four are well conserved through all 10 species while the other two are relatively conserved in nine of the ten species. The squares indicate residues that were not conserved in specific species.

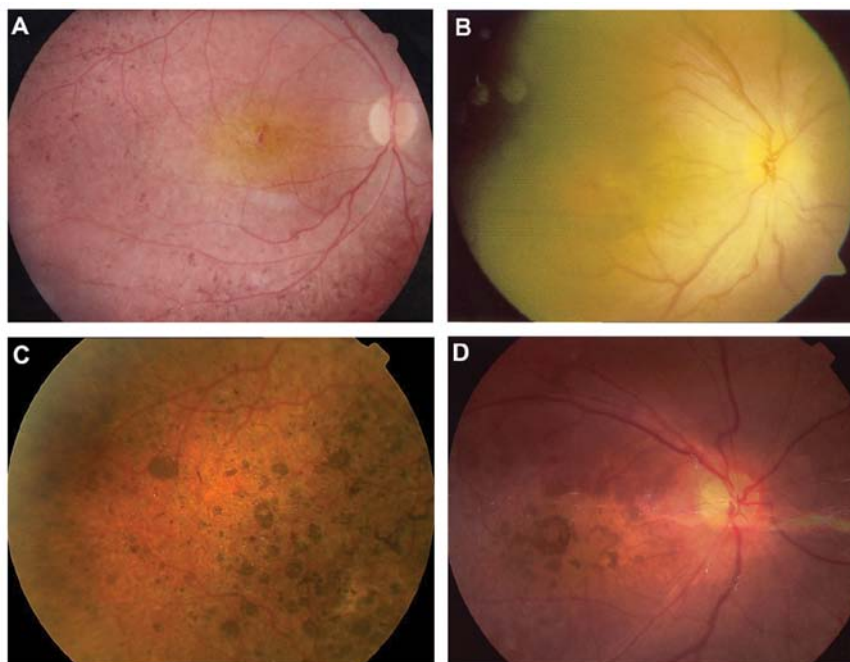


Figure 3. Fundus photos: (A) Generalized retinal dystrophy with early macular involvement and bone-spicule pigmentation in mid-peripheral retina, which was obtained from proband QT495 with crumbs homolog (CRB)1 compound heterozygous mutations (c.[136delA]; [3017C>A]). (B) Generalized retinal dystrophy with early macular involvement, which was obtained from proband QT659 with CRB1 compound heterozygous mutations (c.[2222T>C]; [4089dupTGTGCTT]). (C) The sample was obtained from proband RP114 with homozygous CRB1 mutations (c.[1841G>T]; [1841G>T]), and indicates generalized retinal dystrophy, nummular pigmentation, and yellowish crystalline-like spots. (D) Only proband with nummular pigmentation but without CRB1 mutations was identified.

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