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Autofluorescence Imaging and Phenotypic variance in a Sibling pair with Early Onset Retinal Dystrophy due to defective CRB1 function

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Abstract

Purpose—To phenotype two siblings with autosomal recessive early onset retinal dystrophy due to CRB1 mutations.

Methods—Autofluorescence (AF) imaging, high resolution optical coherence tomography (OCT), and full-field electroretinography (ERG) were performed. The results of DNA sequencing from polymerase chain reaction (PCR) products of the CRB1 gene were obtained from hospital records.

Results—Two siblings, 14-years-old and 17-years-old, were compound heterozygote for 749 del Ser and C948Y mutations in the gene encoding CRB1. AF imaging documented the preservation of retinal pigment epithelium (RPE) along the arterioles. High resolution OCT showed abnormally thick retinae with increased lamination.

Conclusion—Leber congenital amaurosis caused by CRB1 is a unique form of early onset retinal dystrophy because it spares the para-arteriolar RPE and causes abnormal retinal lamination with thickening. These findings, detectable with AF imaging and high resolution OCT, can be combined with electrophysiology and genetic testing to molecularly classify retinal degenerations efficiently.

Introduction

The Crumbs gene (CRB1) encodes a large transmembrane protein required for maintenance of apical-basal epithelial polarity and is involved in stalk membrane formation during photoreceptor morphogenesis (Pellicka et al., 2002). Mutations in CRB1, the human homolog of *Drosophila* Crumbs, cause autosomal recessive retinitis pigmentosa (Richard et al., 2006); (den Hollander et al., 2001). Clinically, it is known to manifest with sparing of para-arteriolar retinal pigment epithelium (Vallespin et al., 2007), Coats-like exudative vasculopathy, and as an early onset retinal dystrophy (also known as Leber congenital amaurosis) (Massey-Harroche et al., 2007).

Herein, we describe genotype-phenotype correlations of two patients with CRB1 RP using recently available imaging techniques.

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Case Report

Genetic Analyses

Patients' and their parents' DNA were screened on the arRP array (<http://www.asperophthalmics.com/ARRPgenetest.htm>) containing mutant alleles of 8 known arRP genes: AIPL1, CRB1, CRX, GUCY2D, RPE65, RPGRIP1, MERTK and LRAT (Asper Ophthalmics). The slides were imaged with the Genorama QuattroImager and the sequence variants were identified by Genorama genotyping software.

Segregation analysis showed that all family members are heterozygous carriers. Father is a heterozygote carrier for 749 del Ser, Mother is a heterozygote carrier for C948Y. Both Cases 1 and 2 are compound heterozygous for 749 del Ser and C948Y.

Case 1

A 14-year-old boy was referred for the evaluation of Crumbs associated early onset retinal dystrophy. He was genetically tested elsewhere and known to be a compound heterozygote for 749 del Ser and C948Y mutations. On exam, the patient had horizontal sensory nystagmus. His best corrected visual acuity was count fingers at 2 feet in the right eye and 20/200 in the left eye. Axial length was 21.37 mm in the right and 21.53mm in the left. The anterior segment examination was normal.

Dilated fundus examination showed slightly pale optic nerves, macular atrophy, and nummular pigment clumping in both eyes. There were also diffuse drusen like deposits in the posterior pole. There was no cystoid macular edema or Coats-like exudation. There was para-arteriolar RPE preservation observed funduscopically and demonstrated with AF (Figure 1). Cirrus-OCT (Carl Zeiss, Dublin, CA, USA) showed abnormal inner retinal lamination and loss of photoreceptors (Figure 1). There is an increased retinal thickness in the nasal retina compared to controls (Figure 3).

Full field ERG was performed using Dawson-Trick-Litzkow (DTL) recording electrodes and Ganzfeld stimulation according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards (Figure 4). Scotopic responses were extinguished and the photopic cone responses were difficult to evaluate secondary to photomyoclonic reflex.

Case 2

The 17-year-old sister of the above patient was also a compound heterozygote for 749 del Ser and C948Y mutations, as confirmed with old records from another institution. She had horizontal sensory nystagmus and best-corrected acuity vision was 20/200 in the right eye and 20/400 in the left eye. Axial length was 19.98 mm in the right and 19.76 in the left. Anterior segment exam was unremarkable.

Dilated fundus examination showed optic nerve pallor, macular atrophy, and peripheral intraretinal pigment migration less severe than her brother (Figure 2). There were a few drusenoid deposits in the posterior pole. Fundus AF imaging showed RPE preservation around the arterioles. There is an increased retinal thickness in the nasal retina compared to controls (Figure 3).

Full field ERGs showed generalized retinal dysfunction affecting rods more than cones (Figure 4). Scotopic rod specific ERG appeared to be extinguished. Maximal ERG a- and b- wave amplitudes were 70 microvolts and 86 microvolts in the right; 51 microvolts and 78 microvolts in the left. Transient photopic ERG b-wave amplitudes were 28 microvolts in the right and 37 microvolts in the left. Burian-Allen contact lens electrodes were used for 30 Hz flicker ERG

and obtained the values of 25 microvolts 30 Hz flicker response. Transient photopic ERG obtained with Burian-allen contact electrodes showed 40 microvolts in the right eye.

Discussion

Gene specific therapeutics for early onset retinal dystrophy patients are currently undergoing clinical trials (Jacobson et al., 2003); (Jacobson et al., 2006); (Downs et al., 2007); (Koenekoop et al., 2007); (Bainbridge et al., 2008); (Maguire et al., 2008). The key to gene-based treatments is efficient and accurate genotyping (Stone, 2007a); (Stone, 2007b). Therefore, it is essential to phenotype and genotype individuals with retinal dystrophies for genetic counseling and enrollment into treatment trials. The CRB1 gene is known to function in eye development and mutations result in the loss of photoreceptor signaling. CRB1 mutant retinæ are thicker and have a coarse lamination pattern similar to patterns observed during stages of normal human retinal development (Stone, 2007a), as opposed to other inherited retinal degenerations. CRB1 gene product interacts with members of the peripheral membrane-associated guanylate kinase (MAGUK) family membrane protein known as palmitoylated 5 protein (MPP5), MPP3 and MPP4 to form a scaffold at the retinal outer limiting membrane. (Kantardzheva et al., 2006; Kantardzheva et al., 2005; Stöhr et al., 2003; van Rossum et al., 2006).

Additionally, in this sibling pair, the younger brother manifested more severe retinal dysfunction than the older sister. This demonstrates variance in expressivity of compound heterozygotes for 749 del Ser and C948Y mutations in the gene encoding CRB1 gene.

AF is most commonly used to study peripapillary and macular RPE. To our knowledge, this is the first application of AF imaging that shows para-arteriolar RPE preservation in Crumbs associated RP. Another distinct clinical feature of CRB1 mutant retinæ is the abnormally thicker retina with a coarse lamination pattern that appears on OCT.

In summary, this case ~~small~~ series uses fundus AF and high resolution OCT to demonstrate para-arteriolar sparing of RPE and abnormal retinal lamination. These clinical features, distinguishing Crumbs RP from other retinal dystrophies, should prompt screening for the CRB1 gene. Molecular diagnosis of RP is an important tool in the era of emerging therapeutic options.

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Fig. 1.

Fundus photograph of a 14-year-old boy with early onset retinal dystrophy due to mutations in the *CRB1* gene. Posterior pole shows prominent xanthophyll as well as macular pigment mottling. The disc looks fairly normal and blood vessels are slightly attenuated. Note the nummular pattern throughout the fundus (white arrowheads) and intraretinal pigment migration. Preservation of the RPE adjacent to retinal arterioles is observed in the autofluorescence imaging (*right eye, insert*). Fourier domain OCT macular images of both eyes shows thickening of retina with less distinct lamination (*right column*).

Fig. 2.

Right eye fundus photograph of a 17-year-old girl with early onset retinal dystrophy due to mutations in the *CRB1* gene. Posterior pole exhibits macular atrophy and pigment mottling. The disc shows some pallor. Peripheral intraretinal pigmentation, RPE migration and a few diffuse fine drusenoid deposits throughout the entire fundus is observed. Fundus autofluorescent examination shows the preservation of the RPE along the para-arterioles (*insert*). *Bottom*, Fourier domain OCT macular images from case 2. Note the coarsely-laminated thick retinae. Cystoid degeneration is seen in the left macula.

	Normal		Patient		<i>P</i> value
	Mean (um)	SD(um)	Mean (um)	SD(um)	
Nasal 800 um	326.1626	9.00033	350.6783	6.95936	*0.002
Nasal 600 um	313.2474	8.94466	335.7460	10.25605	*0.005
Nasal 400 um	295.6979	10.89002	319.1348	15.93660	*0.011
Nasal 200 um	273.9819	6.84170	293.0058	6.99478	*0.003
Temporal 200 um	259.3752	9.37190	257.9268	13.41161	0.850
Temporal 400 um	270.1304	6.73750	276.6085	15.18937	0.571
Temporal 600 um	326.1626	10.33032	293.9275	22.55679	0.850
Temporal 800 um	308.9624	8.15014	315.4548	15.00339	0.450

Fig. 3.

Increased nasal retinal thickness in patients with mutations in the *CRB1* gene. The thickness of the total retina and the distance between RPE and NFL in the nasal and temporal boundary of the ring were measured using Photoshop caliper. The same parameters were measured up to 800 μm nasally and temporally from the center of the fovea. The mean total retinal thickness +/- standard deviation (SD) in the nasal and temporal boundary were illustrated. Normal values were collected from 14 age-matched controls.

Fig. 4.

Full-field electroretinogram (ERG) findings were consistent with severe generalized rodcone dysfunction affecting the rod system more than the cone system due to compound heterozygote 749 del Ser and C948Y mutations in the gene encoding CRB1. ISCEV standardized ERG traces from a normal individual are shown for comparison. Traces from the left eye of Case 1 and right eye of Case 2 were shown.