Butterfly-Shaped Pattern Dystrophy

A Genetic, Clinical, and Histopathological Report

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Objectives: To identify the disease-causing mutation in a large family segregating dominantly inherited butterfly-shaped pattern dystrophy (BPD) and to describe the microscopic pathological changes observed in a member of this family.

Methods: Seventeen individuals at risk for dominantly inherited BPD in a family were examined and blood samples obtained. Linkage analysis and mutation screening of the human retinal degeneration slow (RDS)/peripherin locus were performed. Light and electron microscopic examinations were performed on 1 postmortem eye of 1 affected individual.

Results: Four individuals demonstrated macular degenerative changes with diminished visual acuity, and 3 others exhibited early signs of atrophy without visual deficits. Microscopic examination of the left eye of 1 patient revealed an area of total loss of the retinal pigment epithelium (RPE) and photoreceptor cell layer with intact choriocapillaris and lipofuscin-containing cells in the subretinal space. Outside the area of RPE atrophy, the RPE was greatly distended by lipofuscin. The disease locus in this family was mapped to 6p21.2, the region of the RDS/peripherin gene. Further analysis identified a G→A change at nucleotide position 637 of RDS/peripherin, predicting a novel Cys213Tyr substitution in all affected members of the family.

Conclusions: This study describes a new RDS/peripherin mutation for BPD and provides the first combined genetic-pathological study of this condition, to our knowledge.

Clinical Relevance: Accumulation of lipofuscin in RPE is a prominent feature of several retinal disorders, including age-related macular degeneration. Further elucidation of the cellular and molecular mechanism of BPD may provide insight into pathogenesis and lead to novel treatment approaches for this and other macular degenerations.

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SUBJECTS AND METHODS

Approval from the Johns Hopkins Hospital Joint Committee on Clinical Investigation was obtained for this study, and informed consent was obtained from all patients. Seventeen individuals at risk for a dominantly inherited BPD in a large white family, initially described by Singerman et al21 in 1977, participated in this study (Figure 1). A history of the patient’s vision was obtained from all patients, and best-corrected visual acuities were assessed. Slitlamp biomicroscopy, ophthalmoscopic examination, and fluorescein angiography were performed. Light and electron microscopic examinations were performed on 1 postmortem eye of 1 affected individual using previously described methods.22

Blood samples were obtained by venipuncture, and genomic DNA was extracted. Genetic linkage to the RDS/peripherin locus was assessed using the short tandem repeat polymorphism DNA markers D6S1549 and D6S1557.21,13 Mutation screening was performed by direct sequencing of polymerase chain reaction–amplified DNA fragments corresponding to the 3 exons of the gene using published primer pairs.12,13 The primer pairs used for polymerase chain reaction amplification were as follows: for exon 1, 1a 5′-GCTGTCTGAGGAACAC-3′, 1b 5′-TCTGACCCAGGATGAAAG-3′; for exon 2, 2a5′-AGCCCATCTCCAGCTGTCTG-3′, 2b5′-CTTACACCTTACCCAGCG-3′; and for exon 3, 3a5′-GATTGCCTCTAAATCTCCTCC-3′, 3b5′-ATGGTGCCTCTTCCTGGAG-3′. The nucleotide change at position 637 that was identified by DNA sequencing was independently confirmed by demonstration of elimination of a predicted PvuII restriction enzyme site.

RESULTS

CLINICAL CHARACTERIZATION

Seventeen individuals who were at risk for a dominantly inherited BPD in a large family were examined. Of these, 6 individuals had diminished visual acuity and funduscopic abnormalities, and 3 additional individuals demonstrated pigmented mottling without accompanying deficits in visual acuity, suggestive of the early stages of this disorder (Table). Although the extent of pigmentary abnormalities varied, we noted a degree of RPE atrophy in every affected individual. Several family members had been previously evaluated,21 and reevaluation during this study demonstrated progressive geographic atrophy of the RPE.

Ophthalmoscopic examination of individual IV:7, a 52-year-old woman who had an initial visual acuity of 20/30 OD and 20/70 OS, demonstrated butterfly-shaped lesions in the central macula associated with yellow pigment deposits at the level of RPE and numerous flecks at the RPE level in the posterior pole for both eyes (Figure 2A). These lesions were strikingly similar to those observed in the individual’s father (III:14) and his cousin (III:2) in a previous study.21 Patient III:1, then 53 years old, with a visual acuity of 20/40 OD and 20/20 OS, also demonstrated bilateral butterfly-shaped accumulations of yellowish deposits at the level of the RPE with yellow flecks in the posterior pole (Figure 2B). Fluorescein angiography revealed a central, dark, butterfly-shaped lesion surrounded by a region of hyperfluorescence, with dark spots in the posterior pole corresponding to the observed yellow flecks (Figure 2C).

In patients of the oldest surviving generation, including the patient for whom we have obtained pathological studies, we observed more extensive retinal atrophy. In an 80-year-old man (III:6) with a visual acuity of 20/800 OU, we observed bilateral geographic atrophy of the central retina and RPE, which was clearly demarcated from healthy-appearing tissue in the periphery (Figure 2D). This was confirmed by fluorescein angiography, which demonstrated marked RPE atrophy centrally and healthy tissue in the periphery (not shown).

CLINICOPATHOLOGICAL CASE REPORT

In 1961, a 45-year-old white woman (III:20) had bilateral blurred vision and a doughnut-shaped, depigmented area inferior to the right macula and a faint discoloration in the left macula. In 1964, she was noted to have an additional depigmented area superior to her left fovea and, in the following year, 2 new areas of depigmentation were observed inferior and superior to the fovea of this eye. Although her vision was still 20/20 OU at the time, a central scotoma was discovered in her left eye later that same year, at which point her visual acuity was still measured at 20/20 OS.

In 1974, when she was initially examined by Singerman et al,21 her visual acuity was 20/30 OU, and funduscopic examination of the right eye revealed an ovoid area of RPE disturbance surrounding a central grayish-yellow round area with a dark, slightly refractile mate-
rial that was deep to the retina at its center. This ovoid area was adjacent to a region of dark yellow subretinal material around the right fovea. Examination of the left macula revealed similar changes, although the RPE abnormalities and the central dark refractile material were less well defined. Fluorescein angiography of the right eye demonstrated an early, vertically oriented, ovoid area of hyperfluorescence, corresponding to the RPE disturbances observed funduscopically. An angiogram of the left eye revealed similar abnormalities, and neither showed hyperfluorescent spots peripheral to the central lesion.

In August 1997, the patient was reexamined as part of a cohort used to map the family’s genetic defect to the RDS/peripherin locus. At that time, her visual acuity was 20/64 OD and 20/800 OS. Ophthalmoscopic examination revealed atrophy of the RPE, left eye more than the right eye. She died of complications from chronic obstructive pulmonary disease on October 31, 1997, at which time her left globe was harvested within 12 hours for histopathological examination.

Internal examination disclosed a 3 × 2.5-mm depigmented area with sharp margins located at the center of the macula (Figure 3). Microscopic examination of 545 serial sections through the macula disclosed a 3.5-mm area of loss of RPE and photoreceptor cell layer (Figure 4A, left margin, 4B, right margin). Peripheral to this region, the RPE cells were distorted and greatly distended by lipofuscin, whereas the photoreceptors were partially atrophic (Figure 4C and D). A few large, round pigmented cells containing lipofuscin were present in the subretinal space and in the outer aspect of retina (Figure 4E). Immunological staining for factor 8 disclosed normal staining of the choriocapillaris endothelium outside the area of atrophy, while about 75% of the capillaries in the area of atrophy of the RPE and photoreceptor cell layer demonstrated endothelial staining (Figure 4F).

Ultrastructural examination revealed a 11-µm-thick section of Bruch’s membrane composed of vesicular materials distended from its normal thickness of 2 µm (Figure 5). Small mound-shaped deposits of granular material with wide-spaced collagen and basal laminar deposits were present along the internal aspect of Bruch’s membrane. In some areas of the atrophic zone, residual RPE basement membrane was present. The choriocapillaris was examined extensively and was intact, except for 1 vessel that had no endothelium (Figure 5). Outside the area of atrophy, the RPE was greatly distended by melanin, melanolipofuscin, and especially by lipofuscin granules (Figure 5).

**LINKAGE ANALYSIS AND MUTATION DETECTION**

Genotype analysis using short tandem repeat markers D6S1549 and D6S1557 revealed a positive linkage between the disease phenotype and the RDS/peripherin locus (data not shown). Sequence analysis of the RDS/peripherin coding region in 6 affected patients revealed
a G→A change at the nucleotide position 637, predicting a novel Cys213Tyr substitution. This change also eliminated a PvuII restriction enzyme site and therefore was used as a rapid screening assay for the presence of the mutation. The PvuII site change segregated with the disease phenotype in all affected members of the cohort, giving a positive logarithm of the odds (lod) score of 3.37 (Figure 1B). The lack of a PvuII site was not found in 102 control chromosomes examined.

This report provides, to our knowledge, the first clinico-pathological correlate of BPD caused by a defined mutation in the RDS/peripherin gene. The disease is characterized clinically by the ophthalmoscopic observation of macular atrophy associated with deposits at the level of the RPE. Our light microscopic and transmission electron microscopic studies demonstrated that this atrophy was limited to the outer retina, with loss of the RPE and photoreceptor cell layer, engorgement of surrounding RPE cells with lipofuscin, and sparing of the choriocapillaris. These histopathological findings stand in contrast to the clinical observations of other groups such as Prensky and Bresnick who described peripapillary abnormalities in 3 older members of a large cohort with BPD, which they compared with the peripapillary variant of choroidal dystrophy, in which the choriocapillaris atrophies focally.

Previous studies have demonstrated that mutations in the RDS/peripherin gene can yield retinal disease phenotypes and, specifically, that mutations in the cysteine residue at position 213 can lead to pattern dystrophy. We have identified a novel Cys213Tyr mutation in the RDS/peripherin gene responsible for BPD in this family. The cysteine residue at codon 213 is highly conserved be-

Figure 2. A, Fundus examination of the right eye of individual IV:7 showing a typical butterfly-shaped pigmentary lesion in the macula with yellow flecks in the posterior pole. B, Fundus examination of the right eye of individual III:1. Butterfly-shaped accumulation of yellowish retinal pigment epithelium (RPE) deposits with flecks in the posterior pole. C, Fluorescein angiogram of the right eye of individual III:1 showing a large, hypofluorescent, butterfly-shaped macular lesion. The yellow flecks observed funduscopically in the posterior pole are noted to block fluorescence. D, Fundus examination of the right eye of individual III:6, showing widespread retinal atrophy with pigment clumping.

Figure 3. Gross examination of the left eye of individual III:20, demonstrating a 3×2.5-mm atrophic lesion with depigmentation and sharp margins located at the center of the macula.

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between human, bovine, mouse, and rat. It has been suggested that this cysteine residue may play an important role in intrachain and/or interchain disulfide bond formation.\(^{20,23}\) One can postulate that the Cys213Tyr mutation could interrupt disulfide bond formation and thereby disrupt the integrity of the photoreceptor disc membrane, leading initially to degeneration of photoreceptor cells and ultimately resulting in an accumulation of lipofuscin in the RPE. In turn, this could result in the degeneration of the RPE cell layer, with sparing of the choriocapillaris, as we observed on histopathological examination.

A mutation in the amino acid residue next to Cys213, Cys214Ser, has been reported in a family with autosomal dominant retinitis pigmentosa rather than BPD.\(^{16}\) It is not clear why these adjacent mutations produce distinct clinical pictures, although it is well known that RDS/peripherin mutations can cause numerous distinct phenotypes.\(^{9,17}\) Future studies will hopefully help elucidate the molecular mechanisms that determine clinical phenotype, including the relative importance of primary structural mutations and modifier gene effects.

Figure 4. Light microscopic analysis of left retina from individual III:20. The nasal (A) and temporal (B) margins of the lesion show an abrupt transition between areas of photoreceptor and RPE atrophy and areas of relatively intact retina (hematoxylin-eosin, original magnification \(\times 40\)). C, A higher-power view of the margin of the lesion discloses RPE cells distended with lipofuscin and partial atrophy of the photoreceptor layer (hematoxylin-eosin, original magnification \(\times 100\)). D, An area outside the atrophic lesion demonstrating RPE cells that are distended with lipofuscin (hematoxylin-eosin, original magnification \(\times 400\)). E, Area of outer retina showing melanophages containing lipofuscin (hematoxylin-eosin, original magnification \(\times 400\)). F, Immunological staining using Factor VIII antibodies demonstrating relatively intact choriocapillaris endothelium (original magnification \(\times 100\)).
Figure 5. Electron micrograph demonstrating a retinal pigment epithelium cell containing lipofuscin (large arrowhead) and melanolipofuscin (small arrowhead), Bruch’s membrane with vesicular material (arrow), and intact endothelium (star).

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REFERENCES