Mpdz Null Allele in an Avian Model of Retinal Degeneration and Mutations in Human Leber Congenital Amaurosis and Retinitis Pigmentosa

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PURPOSE. To identify the defective gene in the sex-linked, recessively inherited retinal dysplasia and degeneration (rdd) chicken and to search for the human equivalent disease.

METHODS. Microsatellites from chicken chromosome Z were genotyped in 77 progeny of a carrier male (rdd/+) and an affected female (rdd/W), and candidate genes were sequenced. Retinal cross-sections from rdd and wild-type birds were analyzed by immunohistochemistry. The human orthologous gene was screened in a panel of archival DNAs from 276 patients with retinitis pigmentosa (RP) or Leber congenital amaurosis (LCA) using melting curve analysis and DNA sequencing.

RESULTS. The rdd locus was refined to an approximately 3-Mb region on chromosome Z. Sequence analysis identified a C→T change in the mpdz gene that created a premature stop codon (c.1372C→T, p.R458X), which segregated with the disease phenotype. As expected, the full-length mpdz protein was absent in rdd retinas, but in wild-type birds, it localized to the retinal outer limiting membrane, where it may have a role in the interactions between photoreceptors and Müller glia cells. The screen to identify the human equivalent disease found 10 heterozygous variants in the orthologous gene in patients with RP (three missense and two null alleles) and LCA (four missense and one null allele).

CONCLUSIONS. These findings reveal that MPDZ is essential for normal development of the retina and may have a role in maintaining photoreceptor integrity. The identification of human mutations suggests that MPDZ plays a role in human retinal disease, but the precise nature of this role remains to be determined. (Invest Ophthalmol Vis Sci. 2011;52:7432–7440) DOI:10.1167/iovs.11-7872

The retinal dysplasia and degeneration (rdd) phenotype arose spontaneously in commercial chicken stocks over 30 years ago,1,2 and the associated eye defect was shown to be inherited in a sex-linked recessive manner.3 Rdd chicks are sighted at hatch, but vision gradually deteriorates until by 10 weeks all the birds are blind. The earliest pathologic signs are holes in the retinal pigment epithelial (RPE) layer and undulations in the outer nuclear, outer plexiform, and inner nuclear layers in prehatched embryos.1,2,4 As the disease develops, there is widespread pigmentary disturbance, progressive thinning of all the retinal layers, and degeneration of the photoreceptor and inner nuclear layers, causing the retina to become buckled.5 It has been suggested that the rdd chicken has features that resemble a severe early-onset pigmentary retinopathy in humans.5,6

Retinal dystrophies are a common cause of human morbidity and many have a genetic basis. Thus far, 214 unique Mendelian human retinal diseases have been mapped to specific chromosomal loci, and the defective genes for 172 of these have been identified (RetNet; http://www.sph.uth.tmc.edu/retnet/). However, identification of the genes underlying recessively inherited diseases has tended to lag behind their dominant and X-linked equivalents because of a lack of large pedigrees for mapping studies. The prospect of identifying the disease-causing genes in chickens, with large “family” sizes of 50 or more progeny, has been simplified with the publication of the chicken genome and the revelation that its physical size is only one-third that of the human.7 This is further supported by the higher density of recombination in the chicken genome and the remarkable conservation of synteny between the genomes. Furthermore, the development of specific treatments for these conditions based on the genetic information generated has been limited in part by the lack of suitable animal models to test therapies.

Here we report the molecular defect that causes the rdd phenotype in chickens and attempt to identify the analogous...
human condition so that the value of this animal model for targeted therapy can be assessed.

**MATERIALS AND METHODS**

**Animals**

The *rdd* flock and sighted White Leghorn birds were maintained at the Roslin Institute, as described elsewhere. All animal husbandry and experimental procedures were conducted under the appropriate Home Office project and personal licenses in a manner consistent with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and in compliance with the UK Animals (Scientific Procedures) Act 1986.

**Genotyping**

Genomic DNA was extracted from chicken blood using the DNAzol method (Invitrogen, Paisley, UK). High-resolution genotyping was performed with previously uncharacterized microsatellite markers downloaded as simple repeat sequences from the chicken genome database at the University of California at Santa Cruz (UCSC; http://www.genome.ucsc.edu/). Primer sequences were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/), and DNA oligonucleotides were labeled with fluorochrome FAM, HEX, or TET (Invitrogen). PCR products, generated with fluorescence-labeled polymorphic microsatellite markers on chicken DNA, were size fractionated on a 3130xl Genetic Analyzer (Applied Biosystems, Warrington, UK) and viewed using GeneMapper version 4.0 (Applied Biosystems).

**Bioinformatic Analysis of the Candidate Genes**


**Reverse Transcription–Polymerase Chain Reaction**

Total chicken retina RNA was extracted (Trizol; Invitrogen) and reverse transcribed with an oligonucleotide (dT)$_3$ primer and reverse transcriptase (Superscript II; Invitrogen). For each candidate gene to be analyzed, the full-length chicken cDNA was directly downloaded from the Ensembl Web site (http://www.ensembl.org/index.html). Where the full-length cDNA was not available, the corresponding human ortholog was identified by synteny at the UCSC Web site, the human cDNA was downloaded, and a BLASTN search was performed against the chicken cDNA database at the Ensembl Web site. A series of overlapping fragments was aligned to construct a putative full-length chicken cDNA. Specific primer pairs were designed using Primer3, as a series of approximately 500-bp overlapping fragments to span the entire coding region. An aliquot of total cDNA was used in a standard PCR to amplify the cDNA fragment of interest.

To test the levels of chicken transcripts in various tissues, specific primer pairs for *mpdz* (dAGT CAT CGT CCA GCC AAG TC and dCAA TCT GTA ACC GGC CAT CT) and *gapdh* (dGGA AAG TCA TCC CTG AGC TG and dCAT CAA AGG TGG AGG AAT GG) were used in the PCR on total cDNA for 25 cycles with an annealing temperature of 57°C. The reaction products of 243 bp and 237 bp, respectively, were resolved by electrophoresis through a 2% agarose gel.

**Immunohistochemistry**

Eyes were dissected from 17-day-old embryos, fixed in buffered 4% paraformaldehyde (Sigma-Aldrich Ltd, Gillingham, UK), and embedded in paraffin wax. Four-micrometer chicken retina cross-sections were incubated with polyclonal rabbit anti-chicken mpdz, followed by rabbit immunoglobulin detection system (EnVision; Dako, Cambridge, UK). The rabbit anti-chicken mpdz serum had been raised using the synthetic peptide KDSSETEQGSPSQPC-CONH$_2$ corresponding to amino acid residues 497 to 511 in chicken mpdz as immunogen and affinity-purified before use (Eurogentech S.A., Liege, Belgium). The rationale for designing a peptide immunogen C-terminal to the premature stop codon mutation (residue 458) was to use *rdd* tissue as a negative control. The sections were analyzed using a fluorescence microscope (Eclipse E1000; Nikon, Tokyo, Japan).

**Western Blot Analysis**

Eyes from 1-day-old White Leghorn or *rdd* chicks were harvested for retinal protein extraction according to standard methods. Ten micrograms of total protein extract was resolved under reducing conditions by polyacrylamide gel electrophoresis (XCell SureLock System; Invitrogen) and Tris-acetate SDS running buffer. The gel proteins were transferred onto a 0.45-µm polyvinylidene difluoride filter membrane (XCell II Blot Module; Invitrogen) and analyzed using the chicken *mpdz* antibody, anti-rabbit immunoglobulin conjugated to horseradish peroxidase as a secondary antibody, followed by chemiluminescence detection (Fischer Scientific UK Ltd., Loughborough, UK) according to standard methods.

**Immunofluorescence and Confocal Microscopy**

Adult rat eyes were harvested and cryopreserved according to standard procedures. Twenty-micrometer retinal cross-sections were incubated with a polyclonal rabbit antibody against *Mpdz* (Invitrogen) or Crb1 (a gift from Jan Wijnholds, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands), followed by an Alexa Fluor 488–conjugated secondary anti-rabbit immunoglobulin (Invitrogen). Nuclei were counterstained with propidium iodide (Invitrogen). Slides were analyzed using an inverted confocal microscope (Eclipse TE2000-4; Nikon) with Nikon software (EC-1).

**Patients**

Patients with retinitis pigmentosa (RP) or Leber congenital amaurosis (LCA) were recruited by ophthalmologists or clinical geneticists in charge of their treatment. Blood samples were taken for DNA extraction with the patients’ informed consent according to the principles of the Declaration of Helsinki using a process approved by the Leeds Teaching Hospitals Trust Research Ethics Committee.

**Mutation Screening in Archival DNA Samples**

Patient DNA samples were PCR amplified with master mix (HotShot; Clent Life Science, Stourbridge, UK) in the presence of an intercalating dye (LCGreen-Plus; Clent Life Science) and *MPDZ* oligonucleotide primers (Supplementary Table S1, http://www.iovs.orglookup/suppl/doi:10.1167/iovs.11-7872/-/DSupplemental) according to the supplier’s...
instructions. PCR products were subjected to high-resolution melting-curve analysis (Lightscanner; Clent Life Science), and the results were inspected using the manufacturer’s software. Melting curves that deviated from the normal pattern were identified, and a PCR aliquot of the sample that gave the variant band was sequenced.

Comparative Genomics
Protein sequence conservation across mammalian and nonmammalian vertebrates was investigated using the UCSC Genome Browser. This study used the Vertebrate Multiz Alignment and Conservation package across 15 species.

RESULTS
Identifying the rdd Mutation
The rdd phenotype has been shown to be inherited as a Z-linked recessive trait. The avian sex chromosomes differ from those in mammals in that the male is homogametic, denoted ZZ, and the female is heterogametic, denoted ZW. We have previously bred 77 progeny from a cross of a carrier male (rdd/H11001) with an unaffected female (rdd/W) and used haplotype analysis to locate the rdd locus to within a 12.5-Mb region on chromosome Z between the markers MCW0055 and FIGURE 1. Diagram of the refined interval for the rdd locus after genotyping analysis. Genotyping was performed using standard techniques. The image shown was downloaded from the UCSC Genome Browser and depicts the refined interval on chromosome Z. The known chicken protein-coding genes (RefSeq Genes) and the predicted genes based on alignment with the human genome (Human Proteins mapped by chained tBLASTn) are shown.

FIGURE 2. Molecular analysis of m�� in the rdd chicken. (A) Sequence analysis of the m�� cDNA in White Leghorn (WL) and rdd birds. Note the C→T substitution (c.1372C→T) changes the arginine residue to a stop codon mutation. (B) The C→T mutation segregates with the disease phenotype, as expected, for a sex-linked recessive condition. In both gels, lane 1 represents the 100-bp standard ladder.
ALD0250. Using the same progeny DNAs generated for the initial linkage screen, we further refined the locus with previously uncharacterized microsatellite markers (data not shown) to an approximately 3-Mb region between 28,186,873 and 31,157,461 bp on the chicken May 2006 assembly (galGal3) of the UCSC Genome Browser (Fig. 1). This region contained 14 predicted genes. Bioinformatics analysis revealed that the mouse homolog of one of these, Mpdz (multiple PDZ domain protein), also known as Mupp1 (multi-PDZ domain protein-1), had been shown to interact with Crb1 (crumbs homolog 1). Mutations in CRB1 (MIM *604360) cause recessively inherited RP and LCA in human patients.9,10 Mpdz was therefore considered a strong candidate gene.

Given that retinal RNA was readily available from the birds, we used direct cDNA sequencing to search for the pathogenic mutation in rdd. As opposed to analyzing genomic DNA, to cover the possibility of identifying noncoding changes that affect expression or splicing. The coding sequence of the White Leghorn chicken mpdz gene was identified in retinal RNA by a combination of reverse-transcription PCR and DNA sequencing (Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7872/-/DCSupplemental). Sequence analysis of mpdz cDNA in rdd birds identified a C→T substitution that creates a premature stop codon mutation (c.1372C→T, p.R458X) (Fig. 2A). This mutation does not appear to affect the level of mpdz transcripts in rdd retina significantly, suggesting a lack of nonsense-mediated decay of the mutated transcript (Supplementary Fig. S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7872/-/DCSupplemental). Using a restriction enzyme digest assay on genomic DNA, the c.1372C→T mutation was absent from the DNA of other breeds of chickens and was shown to be unique to the White Leghorn and rdd-affected and carrier birds, segregating with the disease phenotype in a sex-linked recessive manner (Fig. 2B).

A Defect in the Outer Limiting Membrane Causes the rdd Phenotype

To investigate the precise consequence of the mpdz premature stop codon mutation, we performed immunohistologic analysis on rdd and White Leghorn retinas with a polyclonal rabbit anti-chicken mpdz (Fig. 3A). We analyzed histologic sections from 17-day-old embryos because there was minimal retinal disorganization in rdd leading up to this developmental stage. We confirmed that the lack of full-length mpdz protein in rdd caused the observed phenotype. However, in wild-type birds, mpdz localized to the outer limiting membrane (OLM) between the photoreceptor inner segment and the outer nuclear layer. To confirm the specificity of our antibody, we performed Western blot analysis of retinal protein extracts from 1-day-old birds using the anti-chicken mpdz (Supplementary Fig. S3, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7872/-/DCSupplemental). We observed that only the wild-type retinas contained an immunoreactive species of approximately 210 kDa of the expected size for mpdz. To confirm whether localization of mpdz at the OLM was specific, we purchased a commercially available polyclonal rabbit anti-mouse Mpdz for analysis by immunofluorescence and confocal microscopy on 12-week-old rat retinal cross-sections (Fig. 3B). We observed intense immunostaining at the OLM that overlapped with the localization of the Mpdz interactant, Crb1. This expression coincided with the site of the earliest observed pathology in the rdd chicken and implied that the primary defect was a poorly formed OLM.

Screening for MPDZ Mutations in Human Disease

Because rdd pathology resembles human RP9,10 and mutations in the known MPDZ interactant CRB1 cause RP9 and LCA,10 we investigated the possibility that mutations in the 46-exon human MPDZ (MIM *603785) gene cause RP or LCA. A panel of archival DNA from 276 patients with retinal dystrophies (149

![Figure 3. Localization of mpdz in the chicken retina. (A) 4% paraformaldehyde-fixed paraffin-embedded sections (± µm) of (i) White Leghorn and (ii) rdd retinas from 17-day-old embryos immunostained with polyclonal rabbit anti-chicken mpdz using standard methods. Note mpdz immunoreactivity was observed as intense brown staining at the OLM. (B) Cryosections (20 µm) of adult rat retinas stained with polyclonal rabbit antibody against (i) Mpdz, (ii) Crb1, or (iii) rabbit IgG negative control are shown. Bound primary antibody was visualized with Alexa Fluor 488–conjugated secondary antibody (green), and the nuclei were counterstained with propidium iodide (red). The rat retinas detached from the nucleated retinal pigment epithelial layer during tissue preparation. Intense green fluorescence corresponding to Mpdz (i) and Crb1 (ii) immunoreactivity was observed at the OLM, whereas the IgG control (iii) was immunonegative. Scale bars, 50 µm. RPE (retinal pigment epithelium), OLM, ONL (outer nuclear layer), OPL (outer plexiform layer), INL (inner nuclear layer), IPL (inner plexiform layer), and GCL (ganglion cell layer).]
RP and 127 LCA) was screened using high-resolution melting curve analysis. Primer sequences used are depicted in Supplementary Table S1 (http://www iovs org/lookup/suppl/doi:10.1167/ iovs.11-7872-/DCSupplemental). This approach identified 10 heterozygous MPDZ sequence changes consisting of five variants in patients with RP (c.132–133delCT, p.F45QfsX27; c.322C→G, p.P108A; c.658A→C, p.I220L; c.3685C→T, p.R1229W; c.5255C→G, p.S1752X) and five variants in patients with LCA (c.1788–1790AGA→GG, p.D597AfsX5; c.2344G→A, p.G782R; c.4220A→G, p.Y1407C; c.4793C→T, p.P1598L; c.5072A→G, p.D1691G) (Fig. 4). These variants, comprising three null alleles and seven missense changes, were not present in the SNP database (http://www.ncbi.nlm.nih.gov/snp) and were absent from 190 ethnically matched control DNAs (380 chromosomes). Assuming recessive inheritance, we sequenced the entire coding region, and splice recognition signals, of MPDZ in the 10 patients but did not identify a second mutation. To investigate the significance of the seven missense substitutions, we compared the evolutionary conservation of the corresponding wild-type amino acids (Supplementary Fig. S4, http://www iovs org/lookup/suppl/doi:10.1167/ iovs.11-7872-/DCSupplemental). Residues p.G782, p.R1229, p.Y1407, and p.D1691, were found to be totally conserved, suggesting their functional importance. However, p.P108, p.I220, and p.P1598 were not fully conserved, suggesting that their variants may represent rare polymorphisms and should be interpreted with caution. The clinical history of the patients carrying a heterozygous MPDZ null allele or a missense substitution in an evolutionarily conserved residue are depicted in Supplementary Table S2 (http://www iovs org/lookup/suppl/doi:10.1167/ iovs.11-7872-/DCSupplemental). Even though the numbers were limited, there appeared to be no obvious correlation between the mutation type and the age of disease onset or severity.

The lack of a second mutation in human patients carrying MPDZ variants argues against recessive inheritance. However, the size of the MPDZ gene (46 exons spread over 145 kb) means that deletions encompassing whole exons, which would give rise to alleles that fail to amplify, cannot be excluded and may account for the second allele in some cases. Mutations in the promoter region or in introns would also have been missed. To investigate these possibilities, an RNA-based strategy focusing on expression levels and cDNA sequencing was considered. We initially performed reverse-transcription PCR on blood lymphocyte total RNA, extracted from healthy persons, to assess MPDZ expression. However, the absence of an amplification product (data not shown) suggested that MPDZ was not expressed in blood lymphocytes and that an alternative source of RNA, from cells that express MPDZ, would be needed from patients before this explanation could be explored.

Alternatively, heterozygous MPDZ variants may cause dominant disease in humans, so we analyzed any families from whom DNA was available for segregation of the variant with the disease phenotype (Supplementary Fig. S5, http://www iovs.org/lookup/suppl/doi:10.1167/ iovs.11-7872-/DCSupplemental). Using this approach, we showed that p.F45QfsX27 was inherited by an affected sibling and by an unaffected child who was younger than the age of onset of RP in this family (see Supplementary Table S2, http://www iovs.org/lookup/suppl/doi:10.1167/ iovs.11-7872-/DCSupplemental) and that p.I220L was...

![FIGURE 4] MPDZ mutation screen in human RP and LCA. (A) Schematic representation of the human MPDZ protein showing the distribution of the novel human sequence changes that were identified in RP and LCA. (B) Sequence chromatograms showing the 10 heterozygous MPDZ variants (I–X) in the DNA of human RP and LCA patients.
transmitted from an affected mother. We also showed that p.P1598L did not fully segregate with LCA in a pedigree with multiple affected persons, confirming that this variant is not pathogenic.

A third possibility to consider was that heterozygous MPDZ variants could act as modifiers of disease severity caused by mutations in other retinal dysgenehrosis genes, or act in concert with mutations in other genes to cause digenic or trigenic disease. The MPDZ variant, p.P1598L, that did not segregate with LCA (Supplementary Fig. S5, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7872/-/DCSupplemental) could act as a modifier, leading to a more severe phenotype. However, detailed clinical data were not available for this family, which was recruited in rural Pakistan; hence, no useful comparison could be made between family members with and without the MPDZ allele. Given the known interaction between MPDZ and CRB1, this would appear the most likely locus/modifier pairing. However, because RPE65 (MIM +180,069) is similar to CRB1 in that mutations can cause either RP or LCA, this gene was also considered a potential partner. The possibility of digenic or trigenic inheritance with CRB1 or RPE65 mutations was investigated by sequencing the entire coding region and splice recognition signals of these genes in the 10 patients with MPDZ sequence changes. Primer sequences used are depicted in (Supplementary Table S3, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7872/-/DCSupplemental). We identified homozygous CRB1 gene mutations in two LCA patients and RPE65 mutations in another two. GUCY2D (MIM *600179) mutations had been found in the fifth LCA case (SGJ, unpublished data, 2010). The results are summarized in Table 1. Notably, the RP patients with MPDZ variants did not have CRB1 or RPE65 mutations. This could imply that patients with certain CRB1, RPE65, or GUCY2D mutations have more severe disease (LCA) when a heterozygous MPDZ variant is also present, whereas the MPDZ variants seen in RP patients could be dominant alleles or could modify the severity of mutations in retinal genes other than CRB1 and RPE65 leading to RP.

**Discussion**

Here we report that a premature stop codon mutation in the mpdز gene causes sex-linked, recessively inherited retinal degeneration and dysplasia (rdd) in chickens, and we describe heterozygous sequence changes in the corresponding human ortholog in five patients with RP and five patients with LCA. The lack of a second mutation in human patients carrying MPDZ variants argues against recessive inheritance, though the deletion of whole exons, intronic or promoter mutations, and gene rearrangements would have been missed. Alternatively, our findings in human patients with LCA suggest that heterozygous MPDZ variants may act as modifiers of mutations in CRB1, RPE65, or GUCY2D. In RP, such changes could either cause dominant disease or act in concert with alleles at other loci. In case of recessive inheritance in humans, the contrast with sex-linked recessive inheritance in rdd chickens (where the male is the homogametic sex, denoted ZZ, and the female is heterogametic, denoted ZW) could be due to inactivation of the mutated Z chromosome in half of all cells in male chickens. Thus, in a heterozygous human patient, regardless of sex, each retinal cell expresses both normal and affected MPDZ alleles from chromosome 9p23; in contrast, in the heterozygous male chicken retina, half of all cells express only a single normal allele of mpdز from the Z chromosome, having inactivated the other Z chromosome carrying the mutated allele. These cells may then derive a clonal advantage over cells that have inactivated the normal mpdز allele and form a healthy OLM.

MPDZ (MIM *603785) contains one L27 domain at the amino terminus and 13 PDZ domains. The L27 domain is a protein interaction module usually found in scaffold proteins, consisting of three alpha helices that form a heteromeric complex with other L27 domain-containing proteins. PDZ domains, named after the first three PDZ domain-containing proteins identified (Postsynaptic density protein 95/ Drosophila Discs large tumor suppressor/tight junction protein Zona occludins-1), are composed of approximately 90 amino acids that form six beta strands and two alpha helices. PDZ domain-containing proteins are usually targeted to submembranous structures, where they bind to the carboxyl termini or internal peptide sequences of receptors and channels. Here they form a platform for mediating the assembly of signaling and cell polarity complexes. MPDZ interacts directly with many different proteins (see Genecards version 3, http://www.genecards.org/), including claudins and associated tight junction proteins. Different PDZ domains within MPDZ are involved in these reactions, suggesting that there is scope for multiple simultaneous interactions, the nature of which depends entirely on the tissue in which the protein is expressed.

MPDZ is found in the central nervous system, and many other tissues, where it has been shown to localize to tight, gap, neuromuscular, synaptic, and adherens junctions. There MPDZ is involved in a diverse range of pathways that maintain cell integrity and function. These include regulating the conductive permeability of tight junctions, preventing spontaneous acrosomal exocytosis, regulating AMPA receptor clustering in hippocampal neuronal synapses, maintaining GABAergic and K+ conductance, and regulating intracellular signaling in response to osmotic stress in kidney cells, melatonin receptor signaling, and olfactory receptor signaling.

In the eye, MPDZ localizes to the OLM between the photoreceptor inner segment and outer nuclear layer. The OLM is a specialized site of adhesion that links the photoreceptors with Müller glial cells, maintaining the correct photoreceptor shape and orientation, and is thought to contain both adherent and tight junctions. However, although the RP cells are also enriched with tight junctions, we did not observe any apparent localization of MPDZ to this layer (SF and WJC, personal communication, 2010). Expression of MPDZ at the OLM coincides with the site of the earliest observed pathology in the rdd chicken and implies that the primary defect is a poorly formed OLM. The Mpdز interactant Crb1 also localizes to the OLM, as do the interactants PaI1 and Mpp4. Crb1 knockout mice develop lesions in the OLM that become progressively worse during exposure to light. No other animal models with loss of Mpdز function have been reported before this study.

To conclude, our findings highlight the importance of MPDZ function in normal eye development. Because chicken eyes are relatively large, rdd birds could be used to test therapies for human blindness based on small molecules that promote stop-codon read-through. Furthermore, it has been suggested that disruption of the OLM may improve the chance of successful integration of transplanted photoreceptor precursor cells. Given that disruption of the OLM is one of the earliest pathologic observations in the rdd chicken, rdd would be a strong model in which to test this theory.

**Acknowledgments**

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### Table 1. Summary of the MPDZ Sequences Variants Identified in RP and LCA Patients

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<th>Effect</th>
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<th>Ethnicity</th>
<th>Clinical History Available</th>
<th>DNA from Other Family Members</th>
<th>CRB1 or RPE65 Mutations</th>
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The exon in which the variant was identified, the change in the human cDNA sequence (NM_003829), the effect on the 2041 amino acid protein (NP_003820), whether a missense change is an evolutionary conserved residue (Supplementary Fig. S4, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7872/-/DCSupplemental), the ethnicity of the patient, whether a clinical history is available (Supplementary Table S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7872/-/DCSupplemental), whether DNA is available from other family members (Supplementary Fig. S5, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7872/-/DCSupplemental), and the identity of CRB1 or RPE65 mutations are shown. NA, not applicable.

*The CRB1 mutation segregates with the disease phenotype and has been previously reported.18
†The patient has biallelic GUCY2D mutations, c.2302C→T, p.R768W and c.1214–129delCTGCTT, p.L442L43del (SGJ, personal communication, 2010).
ics Department, IIS-Fundación Jiménez Díaz, Madrid, Spain) for providing DNA samples for MPDZ mutation screening.

References


