The Location of Exon 4 Mutations in RP1 Raises Challenges for Genetic Counseling and Gene Therapy

ANIKA NANDA, MICHELLE E. MCCLEMENTS, PENNY CLOUSTON, MORAG E. SHANKS, AND ROBERT E. MACLAREN

• PURPOSE: **Mutations in the photoreceptor gene *RP1* lead to recessive or dominantly inherited retinitis pigmentosa (RP). Since the dominantly inherited phenotype is generally milder than recessive cases, it raises the possibility that it could arise by haploinsufficiency; however, most mutations are in the terminal exon 4, which would be predicted to generate truncated proteins. We therefore assessed a cohort of RP patients with confirmed mutations in *RP1* to examine the genetic basis of the exon 4 mutations.**

• DESIGN: **Observational case series.**

• METHODS: **A retrospective review of 15 patients, aged between 36 and 84, with *RP1* mutations in exon 4 confirmed by Sanger sequencing. All patients underwent full ophthalmic examination.**

• RESULTS: **Two patients had homozygous mutations in *RP1*, p.(Glu1526*) and p.(Ser486fs), and presented with severe early-onset retinal degeneration. Their first-degree relatives were unaffected. Thirteen patients had dominantly inherited RP presenting in adult life with a rod-cone dystrophy phenotype. Four novel mutations were identified. All mutations were predicted to produce truncated RP1 protein of variable lengths, as follows: p.(Arg677*), p.(Gln679*), p.(Leu722*), p.(Ile725Argfs*6), p.(Ser734*)x2, p.(Leu762Tyrfs*17)x2, p.(Leu866Lysfs*7)x2, p.(Arg872Thrfs*2)x2, and p.(Gln917*).**

• CONCLUSION: **The RP1 protein with a predicted length between 677 and 917 amino acids seems to have a dominant negative effect, whereas proteins shorter (486 amino acids) or longer than this (1526 amino acids) lead to a more severe phenotype, but only in homozygous individuals. Since mutations at various points along exon 4 have divergent consequences, genetic testing alone may be insufficient for counseling, but recessive inheritance should be considered likely in severe early-onset cases. (Am J Ophthalmol 2019;202:23–29. © 2019 Elsevier Inc. All rights reserved.)**

MUTATIONS IN RETINITIS PIGMENTOSA 1 (*RP1*) ACcount for a significant proportion (3%-5%) of autosomal dominant retinitis pigmentosa (RP) cases, and are also a rare cause of recessive RP.[1,2] The *RP1* gene, located on chromosome 8, comprises 4 exons (3 coding) and is expressed exclusively in photoreceptors.[3] It is thought to participate in the regulation of microtubule polymerization that is necessary for the precise stacking of outer segment discs. The misalignment of discs caused by mutations in *RP1* results in a destabilized structure and leads to photoreceptor cell death.[4] A number of disease-causing mutations have been identified in *RP1*, with the majority clustered in exon 4, the largest and terminal exon of the gene. Initially *RP1* mutations causing RP were identified to be of autosomal dominant inheritance, with multiple case reports outlining its late onset and mild degeneration.[5] Since the original description, there have been several other cases demonstrating that *RP1* mutations inherited recessively lead to more severe disease,[6,7] indicating that complete absence of the RP1 protein owing to predicted null mutations leads to accelerated photoreceptor death.

Since null mutations are tolerated in the carrier (heterozygous) state, the mechanism by which truncating *RP1* mutations cause autosomal dominant disease is widely debated. Furthermore, with exon 4 accounting for more than 50% of the length of the final protein, it is not known at which point the truncated RP1 protein becomes pathogenic. The purpose of this study was therefore to study the phenotype in a cohort of 15 patients with truncating exon 4 mutations in *RP1* in more detail, to better understand the molecular mechanism of the disease.

METHODS

ALL PATIENTS WERE IDENTIFIED AT SPECIALIST RETINAL GEnetics clinics at Oxford Eye Hospital. Consent for genetic testing was obtained in adherence with the Declaration

AJO.com Supplemental Material available at AJO.com.
Accepted for publication Jan 24, 2019.

From the Oxford University Hospitals NHS Foundation Trust and NIHR Oxford Biomedical Research Centre (A.N., R.E.M.) and Nuffield Department of Clinical Neurosciences (M.E.M., R.E.M.), John Radcliffe Hospital, Oxford, United Kingdom; and Oxford University Hospitals NHS Foundation Trust, Oxford Medical Genetics Laboratories, The Churchill Hospital, Oxford, United Kingdom (P.C., M.E.S.).

Inquiries to Anika Nanda, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, UK; e-mail: anika.nanda@ouh.nhs.uk

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2019.01.027

TABLE. Summary Demographic, Visual Acuity, and Genetic Data for Retinitis Pigmentosa 1 Case Series Patients

Case Number	Sex	Current Age	Last Recorded Visual Acuity (BCVA/PH)		DNA Mutation	Exon	Protein Mutation	Protein Length	Approximate Decade of Presentation (Years)	Reference
			OD	OS						
1	M	38	PL	PL	c.1458_1461dup homozygous	4	p.(Ser486fs)	487	10	(Khaliq et al,[10] 2005)
2	M	49	HM	PL	c.4576G>T homozygous	4	p.(Glu1526*)	1525	10	New mutation
3	M	62	6/24	PL	c.2285_2289del	4	p.(Leu762Tyrfs*17)	777	20	(Payne et al,[11] 2000)
4	F	66	6/12-1	6/7.5-3	c.2596_2597del	4	p.(Leu866Lysfs*7)	871	20	(Carss et al,[12] 2017)
5	M	63	5/60	HM	c.2172_2185del	4	p.(Ile725Argfs*6)	729	40	(Bowne et al,[13] 1999)
6	F	73	6/6	6/6-2	c.2613dup	4	p.(Arg872Thrfs*2)	872	60	(Payne et al,[11] 2000)
7	F	52	6/4.8	6/4.8	C.2029C>T	4	p. (Arg677*)	677	30	(Bowne et al,[13] 1999)
8	M	36	6/5	6/5	c.2613dup	4	p.(Arg872fs)	872	30	(Payne et al,[11] 2000)
9	M	76	6/7.5	6/7.5	c.2035C>T	4	p.(gln679*)	679	60	(Sullivan et al,[14] 1999)
10	F	85	6/7.5	6/9	c.2199_2200del	4	p.(Ser734*)	733	70	New mutation
11	M	73	5/60	NPL	c.2749C>T	4	p.(Gln917*)	917	50	New mutation
12	M	66	6/7.5	6/6	c.2285_2289del	4	p.(Leu762Tyrfs*17)	777	50	(Payne et al,[11] 2000)
13	M	86	NPL	6/18	c.2199_2200del	4	p.(Ser734*)	733	40	New mutation
14	M	47	6/7.5	6/7.5	c.2596_2597del	4	p.(Leu866Lysfs*7)	871	20	(Carss et al,[12] 2017)
15	F	41	6/6	6/7.5	c.2161_2162ins	4	p.(Leu722*)	722	30	New mutation

Case 1 and Case 2 are patients diagnosed with autosomal recessive *RP1* and Cases 3-15 are patients diagnosed with autosomal dominant *RP1*.
HM = hand movements; LP = light perception; NLP = no light perception.

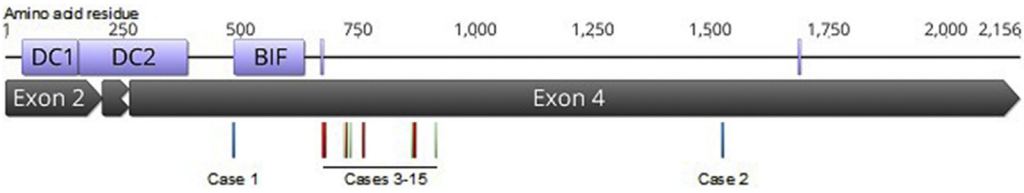

FIGURE 1. Schematic diagram of *RP1*. All known mutations and those identified in this case series have been plotted onto the *RP1* gene, showing their location in exon 4. The locations of homozygous mutations are shown in blue (previously unreported), and dominant mutations are shown in red (known mutations) and green (previously unreported mutations identified in this cohort). BIF = bifocal domain; DC = doublecortin domain.

of Helsinki. A comprehensive medical and ocular history was taken from the patient prior to examination, together with a family history. All patients had a full anterior and posterior ophthalmic examination with slit-lamp biomicroscopy, autofluorescence imaging, optical coherence tomography (spectral-domain optical coherence tomography and BAF; Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), and Snellen visual acuity. In a few cases where diagnosis was more difficult, some patients underwent electroretinography.

For all patients except Patient 2, enrichment for *RP1* was achieved as part of a customized HaloPlex enrichment system kit (Agilent Technologies, Santa Clara, California, USA) designed to capture the coding exons and 10 base pairs of the flanking introns of 111 retinal genes. The number of genes captured on the panels increased over time and ranged from 45 genes in the first iteration to 118 genes in the current version. HaloPlex reactions were prepared as per manufacturer's instructions. Libraries were pooled into batches of 14 and sequenced on an Illumina MiSeq instrument (Illumina, USA) using a MiSeq v3 kit, as per manufacturer's instructions. Reads were aligned using BWA[8] and variants were called using Platypus.[9] Patient 2 was analyzed using an Agilent SureSelect Custom Design 105 gene panel, sequenced on an ABI SOLiD 4 system. All variants identified by next-generation sequencing were confirmed by Sanger sequencing (Supplemental Table 1; Supplemental Material available at AJO.com). Panel gene content and coverage data for tested genes is described in Supplemental Table 2 (Supplemental Material available at AJO.com).

RESULTS

• **AUTOSOMAL RECESSIVE CASES:** Two unrelated autosomal recessive cases, Case 1 and Case 2 (Table and Figure 1), suffered from severe early-onset disease, with blindness reported in the first decade of life. Both patients are of Pakistani origin and are from multiply consanguineous families, increasing the probability of inheritance of rare recessive mutations.[15] The homozygous mutations identified in these patients are located in exon 4, c.1458_1461dup p.(Ser486fs) and c.4576G>T p.(Glu1526*). The predicted protein lengths of 487 and 1525 amino acids are significantly shorter and longer, respectively, than the proteins predicted from dominant mutations in this cohort. The phenotypes of homozygous Case 1 and Case 2 are described below, showing the similarities in early onset and severity.

Case 1 (Figure 2A and Figure 3A) involved a 38-year-old man who first noticed visual problems at the age of 9 years while at school in Pakistan. Within a short time, his peripheral and central vision declined significantly. He has a highly consanguineous family with 2 cousins also affected by early-onset retinal dystrophy. He continues to work full time with best-corrected visual acuity of light perception in both eyes. On examination, he has a fine nystagmus with extensive pigmentary retinopathy encroaching on the central retina. Autofluorescence revealed large atrophic patches in the periphery. His parents have been examined and are reported to be unaffected; his mother is a confirmed heterozygous carrier of the familial mutation.

Case 2 (Figure 2B and Figure 3B) involved a 49-year-old man who suffered from poor night vision since the age of 7; at 15 he was registered blind and he attended a specialist school. He has a complicated family history with multiple consanguineous marriages and 2 genes segregating with retinal dystrophy. His mother and father are asymptomatic and are first cousins. His nephew and maternal uncle's children are also affected with RP due to recessive mutations in *SPATA7*. On examination, he has significant pigmentary retinopathy, atrophy in the macular region, and best-corrected visual acuity of hand movements and light perception in the right and left eye, respectively.

• **AUTOSOMAL DOMINANT CASES:** In the 13 dominantly inherited cases studied (Cases 3-15; Table and Figure 4), the average age of presentation was 33 years. All had frameshift mutations or point mutations leading to premature stop codons clustered between bases c.2029-2749 in exon 4, with protein lengths ranging from 677 to 917 amino acids (Figure 1). Three additional variants of unknown significance were found in 2 cases (Case 3 and Case 4). The majority of patients identified with autosomal dominant mutations had reasonable visual acuity, with autofluorescence imaging

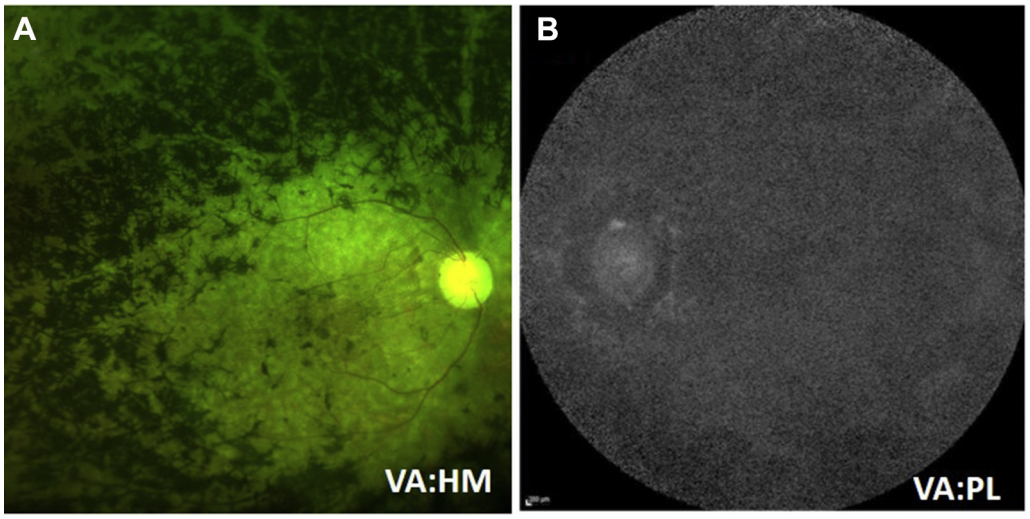

FIGURE 2. OPTOS (A, right eye) and autofluorescence (B, left eye) imaging of Case 2, a severely affected retinitis pigmentosa patient with homozygous RP1 c.4576G > T mutation in exon 4.

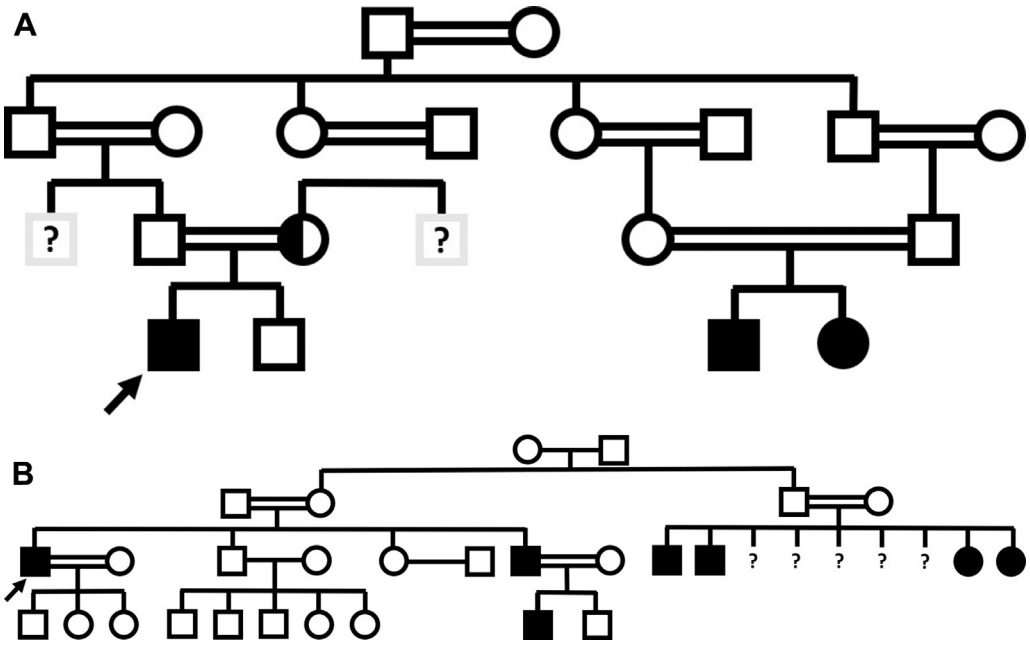

FIGURE 3. A. Pedigree for Case 1: homozygous c.1458_1461dup p.(Ser486fs). B. Pedigree for Case 2: homozygous c.4576G > T p.(Glu1526*).

showing mild peripheral pigmentary retinopathy only (Table). Additional information for selected cases is reported below.

Case 3 (male, 62 years old), c.2285_2289del p.(Leu762Tyrfs*17): The mutation identified in *RP1* in Case 3 is the same as that of Case 12; however, results of genetic sequencing also identified a novel variant of uncertain clinical significance in *PRPH2*, c.464C>T, in Case 3. The subject in Case 12 is of similar age at 66 years old but has vision of 6.7.5 and 6/6 in his right and left eyes, respectively, much better than in Case 3, with vision of 6/24 and light perception. *PRPH2* is also known to cause autosomal dominant RP, and this novel mutation could have an additional effect worsening the phenotype in Case 3. An additional variant was found in RPGRILP.

Case 8 (male, 36 years old), c.2613dup: The patient in Case 8 has a daughter aged 10 years, who presented with difficulties at school and fine detail work that has resulted in her sitting nearer to the front of the class. She otherwise copes well with her visual difficulties with spectacles alone. On

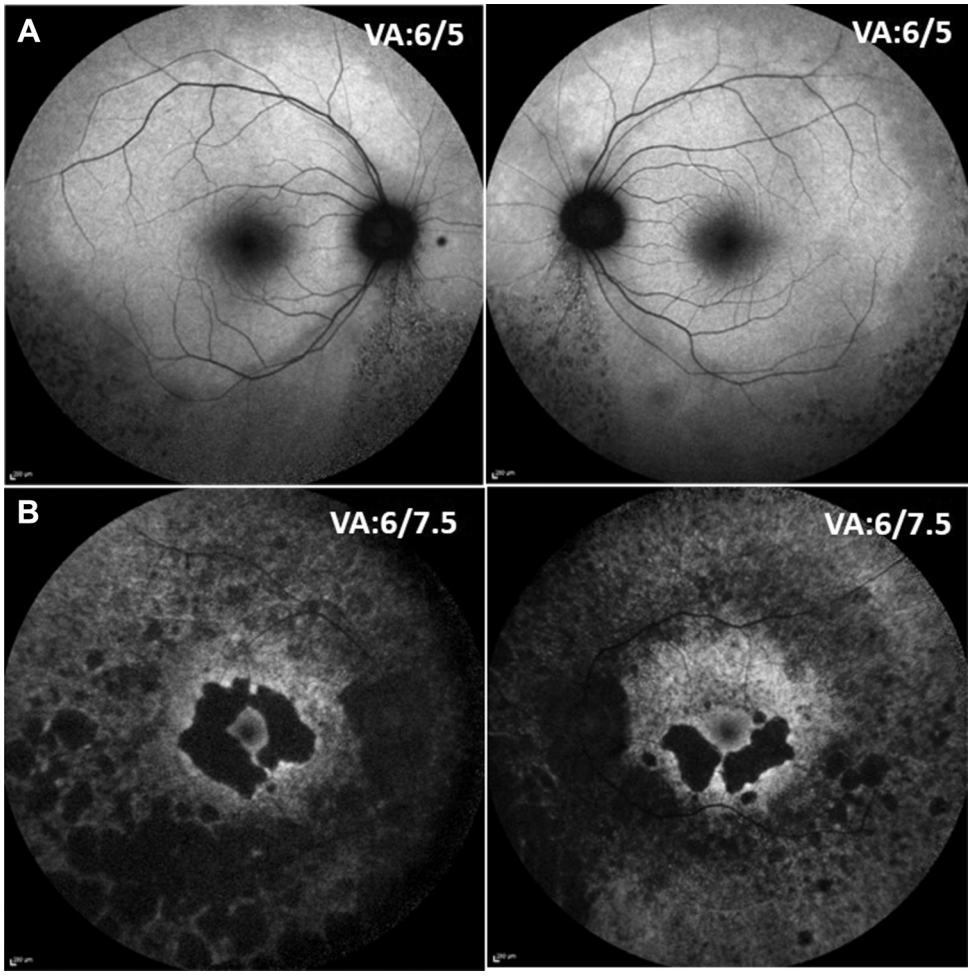

FIGURE 4. A. Case 8: Autofluorescence imaging with visual acuities, of patient at age 37 years, showing early-stage focal pigmentary retinopathy with heterozygous RP1 mutation c.2613dup in exon 4. B. Case 9: Autofluorescence imaging with visual acuities, of patient at age 76 years, showing features of late-stage retinitis pigmentosa with heterozygous RP1 mutation c.2035C > T in exon 4.

examination, she has typical RP signs but seems to have a considerably more severe phenotype (OD: 6/12, OS: 6/9) than her father, presented here, who still has good vision binocularly.

Case 10 (female, 85 years old), with a novel *RP1* mutation, c.2199_2200del, p.(Ser734*): The patient in this case was incidentally diagnosed at the age of 70 after suffering an episode of temporal arteritis. Her mutation is located in the region of the gene where other dominant mutations of this cohort cluster, but she claims to have never suffered from symptoms of RP. Her mild pigmentary retinopathy follows the inferior arcade with borderline rod responses on electroretinogram, helping to confirm her RP.

DISCUSSION

THIS COHORT DEMONSTRATES A CLEAR PHENOTYPIC distinction between disease-causing heterozygous mutations and homozygous mutations in *RP1*. All 15 mutations were confirmed by Sanger sequencing and are located in exon 4, the largest exon of the gene. Dominant mutations seem to cluster in the upstream region of exon 4 (Figure 1), presenting generally with mild and late-onset disease. On either side of this cluster, but still within exon 4, are the 2 homozygous mutations, which present with a severe phenotype of RP leading to blindness within the first decade of life. However, since heterozygous carriers of these mutations are asymptomatic, this excludes haploinsufficiency as the disease-causing mechanism.

All dominant exon 4 mutations in this series are predicted to avoid nonsense-mediated decay, instead creating truncated proteins of varied lengths.[16] Chen and associates[17] describe them as "Class 2" dominant negative mutations. From examination of autofluorescence and optical coherence tomography imaging of this case series, there is variation in phenotype that fits a waveform pattern of severity. Case 7 has the most upstream dominant mutation at c.2029, with 6/6 vision bilaterally and pigmentary

changes confined to the inferior-nasal region, but 52 amino acids downstream at c.2172 the patient in Case 5 has vision of 5/60 and hand movements. A further 12 base pairs downstream, to c.2199 (Case 10), an improvement in visual function is seen until c.2749, amino acid 917, where the patient (Case 11) has less than 10% of visual field remaining and a severely atrophic retina. The length of the truncated RP1 protein may have varying detrimental effects; however, all phenotypes are milder than the recessive variant. Mutations found upstream to exon 4 are likely to be subject to nonsense-mediated decay.

Haploinsufficiency has been suggested as a disease mechanism for autosomal dominant RP1 disease. Mouse models described by Gao and associates report reduced levels of RP1 protein in $RP1^{+/-}$ mice, which also had subnormal electroretinogram findings compared to $RP1^{+/+}$; it was predicted that a similar mechanism may occur in humans.[18] The theory of haploinsufficiency in RP1 disease can be disputed, as a family member of the patient in Case 1 is a confirmed heterozygous carrier and yet does not exhibit signs or symptoms of RP. In addition, parents and siblings of the patient in Case 2 do not suffer from typical RP symptoms either. Similar findings were reported in a study by Chen and associates, in which heterozygous family members did not have the RP phenotype.[17] These examples show that a single functional copy of *RP1* can prevent the development of RP, which supports the likelihood that truncated proteins exert a dominant negative effect in autosomal dominant cases.

The *RP1* mutations in this case series are typically clustered in the first third of exon 4 after the bifocal (BIF) domain located at p.486 to p.635. The BIF domain and doublecortin complexes, located at the 5′ end of exon 4, are thought to be crucial for the maintenance of photoreceptor morphogenesis.[19] Mutations downstream of the BIF domain lead to a loss of approximately a third to two thirds of the C-terminal end of the protein.[20] The homozygous mutation in Case 1, c.1458_1461dup, is located within the BIF domain of exon 4. This frameshift mutation is likely to cause loss of function owing to the absence of this vital functional domain required for photoreceptor development. Truncated proteins from mutations within or prior to the BIF domain are considered loss of function[17]; hence, heterozygous carriers of mutations in this location have protein product only from their unaffected allele. This seems to be sufficient for normal function.

It is also possible that the variation in RP1 disease severity is a result of factors other than the primary gene defect, such as differences in genetic background or environment that could account for the phenotype variations observed in the patients in Cases 10 and 13, who share the same genetic mutation. These patients also share the same autosomal dominant *RP1* mutation, yet they present with different phenotypes. The more severe phenotype of the patient in Case 3 may be related to the coexistence of a novel *PRPH2* mutation in this patient.

With the advent of gene therapy, disease inheritance patterns must be assessed to configure the best therapeutic approach. For disease caused by a dominant negative mechanism, a gene therapy strategy would require silencing of the expression of the mutated allele.[21] As it is apparent in RP1 disease that a single allele is adequate for normal functionality, further gene supplementation would not be necessary. CRISPR gene editing could also be considered. For autosomal recessive cases of RP1 disease, gene supplementation would be required. Adeno-associated virus vector packaging currently limits the size of the gene that can be inserted. *RP1* consists of 6468 base pairs, making it too large for current adeno-associated virus vectors. However, dual-vector therapy is proving successful, where the transduction of the cell by 2 vectors allows for recombination of genetic material and transcription of a large gene to take place.[22]

The data presented here demonstrate the differences in the phenotype of RP1 disease that seem to be caused primarily by the mutation location. This genetic study had enabled us to further narrow down the region of the RP1 protein that causes dominant negative effects, distinguishing it from that which has lack of function and can only cause RP when in the fully recessive disease state. Since all these mutations lie in exon 4, careful genetic counseling and gene therapy planning will be required in patients with *RP1* mutations.

FUNDING/SUPPORT: NIHR OXFORD BIOMEDICAL RESEARCH CENTRE AND THE WELLCOME TRUST. FINANCIAL DISCLOSURES: Robert E MacLaren and Michelle E McClements are employed by the University of Oxford and are consultants to Nightstar Therapeutics, however, neither company has declared any interest in the topic of the paper. The following authors have no financial disclosures: Anika Nanda, Penny Clouston, and Morag E. Shanks. All authors attest that they meet the current ICMJE criteria for authorship.

REFERENCES

1. Baum L, Chan WM, Yeung KY, Lam DS, Kwok AK, Pang CP. RP1 in Chinese: eight novel variants and evidence that truncation of the extreme C-terminal does not cause retinitis pigmentosa. *Hum Mutat* 2001;17(5):436.
2. Ferrari S, Di Iorio E, Barbaro V, Ponzin D, Sorrentino FS, Parmeggiani F. Retinitis pigmentosa: genes and disease mechanisms. *Curr Genomics* 2011;12(4):238–249.
3. Liu Q, Zhou J, Daiger SP, et al. Identification and subcellular localization of the RP1 protein in human and mouse photoreceptors. *Invest Ophthalmol Vis Sci* 2002;43(1):22–32.

4. Liu Q, Lyubarsky A, Skalet JH, Pugh EN Jr, Pierce EA. RP1 is required for the correct stacking of outer segment discs. *Invest Ophthalmol Vis Sci* 2003;44(10):4171–4183.
5. Berson EL, Grimsby JL, Adams SM, et al. Clinical features and mutations in patients with dominant retinitis pigmentosa-1 (RP1). *Invest Ophthalmol Vis Sci* 2001;42(10):2217–2224.
6. Pierce EA, Quinn T, Meehan T, McGee TL, Berson EL, Dryja TP. Mutations in a gene encoding a new oxygen-regulated photoreceptor protein cause dominant retinitis pigmentosa. *Nat Genet* 1999;22(3):248–254.
7. Lafont E, Manes G, Sénéchal A, et al. Patients with retinitis pigmentosa due to RP1 mutations show greater severity in recessive than in dominant cases. *J Clin Exp Ophthalmol* 2011;2(12).
8. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25(14):1754–1760.
9. Rimmer A, Phan H, Mathieson I, et al. Integrating mapping-, assembly- and haplotype-based approaches for calling variants in clinical sequencing applications. *Nat Genet* 2014;46(8):912–918.
10. Khaliq S, Abid A, Ismail M, et al. Novel association of RP1 gene mutations with autosomal recessive retinitis pigmentosa. *J Med Genet* 2005;42(5):436–438.
11. Payne A, Vithana E, Khaliq S, et al. RP1 protein truncating mutations predominate at the RP1 adRP locus. *Invest Ophthalmol Vis Sci* 2000;41(13):4069–4073.
12. Carss KJ, Arno G, Erwood M, et al. Comprehensive rare variant analysis via whole-genome sequencing to determine the molecular pathology of inherited retinal disease. *Am J Hum Genet* 2017;100(1):75–90.
13. Bowne SJ, Daiger SP, Hims MM, et al. Mutations in the RP1 gene causing autosomal dominant retinitis pigmentosa. *Hum Mol Genet* 1999;8(11):2121–2128.
14. Sullivan LS, Heckenlively JR, Bowne SJ, et al. Mutations in a novel retina-specific gene cause autosomal dominant retinitis pigmentosa. *Nat Genet* 1999;22(3):255–259.
15. Woods CG, Cox J, Springell K, et al. Quantification of homozygosity in consanguineous individuals with autosomal recessive disease. *Am J Hum Genet* 2006;78(5):889–896.
16. Hentz MW, Kulozik AE. A perfect message: RNA surveillance and nonsense-mediated decay. *Cell* 1999;96:307–310.
17. Chen LJ, Lai TY, Tam PO, et al. Compound heterozygosity of two novel truncation mutations in RP1 causing autosomal recessive retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2010;51(4):2236–2242.
18. Gao J, Cheon K, Nusinowitz S, et al. Progressive photoreceptor degeneration, outer segment dysplasia, and rhodopsin mislocalization in mice with targeted disruption of the retinitis pigmentosa-1 (Rp1) gene. *Proc Natl Acad Sci U S A* 2002;99(8):5698–5703.
19. El Shamieh S, Boulanger-Scemama E, Lancelot M, et al. Targeted next generation sequencing identifies novel mutations in RP1 as a relatively common cause of autosomal recessive rod-cone dystrophy. *Biomed Res Int* 2015;2015:485624.
20. Riazuddin SA, Zulfiqar F, Zhang Q, et al. Autosomal recessive retinitis pigmentosa is associated with mutations in RP1 in three consanguineous Pakistani families. *Invest Ophthalmol Vis Sci* 2005;46(7):2264–2270.
21. McClements ME, MacLaren RE. Gene therapy for retinal disease. *Transl Res* 2013;161(4):241–254.
22. McClements ME, MacLaren RE. Adeno-associated virus (AAV) dual vector strategies for gene therapy encoding large transgenes. *Yale J Biol Med* 2017;90(4):611–623.