Compound dominant-null heterozygosity in a family with RP1-related retinal dystrophy

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Purpose: To report on the presence of autosomal dominant and compound dominant-null RP1-related retinitis pigmentosa in the same non-consanguineous family.

Observation: The father was minimally symptomatic and referred by his optometrist aged 38. He was diagnosed with rod-cone dystrophy, confirmed to be caused by the previously reported RP1 c.2613dupA mutation. He was reassured that his 11-year-old daughter had a 50% chance of inheriting the same mutation and that the condition, if she had it, would most likely be similar. Clinical phenotyping of his daughter however revealed an early onset cone-rod dystrophy. The mother was entirely asymptomatic and clinically normal. Sanger sequencing of the RP1 gene in the daughter confirmed the presence of biallelic mutations – the dominant c.2613dupA variant from her father and a c.3843dupT truncating variant inherited from her mother, both located in exon 4 of the RP1 gene. The maternal c.3843dupT has previously been reported.

Conclusions and importance: Pathogenic variants in exon 4 of RP1 are known to cause differential dominant and recessive disease. The presence of both phenotypes in a single family has not yet been reported. The father, being minimally symptomatic, is affected by a known dominant variant which truncates the RP1 protein more proximally. However, inheritance of both variants in a compound heterozygous state in the daughter resulted in a much more severe, early onset cone-rod phenotype in a pattern akin to recessive disease. This raises challenges for genetic counselling and development of gene-based therapies for RP1 mutations.

1. Introduction

Mutations in the RP1 gene are an important cause of both autosomal dominant and recessive forms of retinal dystrophy.1-4 The RP1 gene itself comprises 4 exons - 3 of which are coding - in which the terminal exon is the largest and encodes over 80% of the RP1 protein, a microtubule associated protein. As might be expected, most reported mutations lie within this exon. Due to its location in the terminal exon, nonsense mutations are expected to escape nonsense mediated decay and result in the production of truncated RP1 protein. This is supported by detection of both wild-type and mutant RP1 mRNA transcript in patient peripheral blood cells.5 Premature truncations in exon 4 produce a variably inherited retinal dystrophy with differing phenotypes. The majority of truncating variants give rise to later onset, dominantly inherited rod-cone dystrophy.6 Biallelic inheritance may be associated with an early onset retinitis pigmentosa (RP), early onset severe retinal dystrophy (EOSRD), cone-rod or macular dystrophy, all typically arising at a younger age than the dominant disease.7,8 There appears to be a dominant cluster or ‘mutational hot spot’ between amino acids 500 to 1053. The exact molecular mechanisms that would account for this are not yet elucidated. The N-terminus of the RP1 protein contains two doublecoritin domains and a bifocal (BIF) homologous domain, which are thought to be important for localization in the outer segment and photoreceptor morphogenesis respectively.9,10 The function of the C-terminus however - encoded by exon 4 - is not yet understood.

Here, we report on a family comprising of a father, minimally affected by a known dominant pathogenic variant; an unaffected mother who is a carrier of a recessive premature truncating variant; and their daughter with an early-onset recessive phenotype due to biallelic inheritance. To our knowledge, this is the first report to present both phenotypes of RP1-related retinal dystrophy within a single family.
2. Methods

A retrospective review was undertaken of the medical records of a non-consanguineous family comprising three individuals - the daughter (age 11), father (age 38) and mother (age 44). The family were seen in a specialist retinal genetics clinic in a single centre at the Oxford Eye Hospital, Oxford University Hospitals NHS Foundation Trust. Written consent was obtained for genetic testing and participation in the study in adherence with the Declaration of Helsinki. Detailed medical, ocular and family history was obtained from the participants. Full ophthalmic examination was undertaken followed by retinal imaging including short-wave autofluorescence imaging, optical coherence tomography (OCT) (Heidelberg Spectralis, Heidelberg Engineering GmbH, Germany) and fundus photography (Optos, Nikon).

Peripheral blood samples were taken from the father and daughter for genetic testing by next generation sequencing (Illumina MiSeq, Illumina, San Diego, CA). Molecular analysis of 111 retinal genes associated with retinitis pigmentosa and retinitis pigmentosa-like phenotypes were undertaken probing the \textit{RP1} gene with customised HaloPlex target enrichment system kit (Agilent Technologies, Santa Clara, California, USA) capturing the coding exons and 10 base pairs of the flanking introns of the 111 genes. Putative pathogenic variants identified by next generation sequencing were confirmed by Sanger sequencing. Segregation analysis of the \textit{RP1} c.3843dupT variant was undertaken in the unaffected mother as part of confirming pathogenicity.

3. Case 1

The father (case 1) was newly diagnosed with retinitis pigmentosa at the age of 38, almost incidentally. He admitted to difficulties with night vision but aside from this he was largely unaffected and had excellent visual acuity of 6/4 in both eyes. As he was adopted, there was no family history available, although his biological father was known to be of Nigerian lineage and his mother was Caucasian and from the UK. Funduscopic examination revealed mid-peripheral pigmentary changes superonasal to the disc (Fig. 1, bottom row). Fundus autofluorescence revealed more extensive retinopathy than evident on fundus examination and OCT showed a well-preserved perifoveal ellipsoid zone, but beyond which outer retinal architecture was less well defined. All features were consistent with a late-onset, dominant retinitis pigmentosa phenotype. Genetic testing revealed heterozygosity for the c.2613dupA (Arg872Thrfs*2) pathogenic variant (Table 1). The variant is predicted to result in a frameshift and lead to premature termination of translation. This variant has previously been reported in the literature in individuals and families with autosomal dominant retinitis pigmentosa, associated with dominant inheritance.\textsuperscript{11}

4. Case 2

Case 2 is the 11-year-old daughter in the family (Table 1). She presented at this age with difficulties in discriminating fine detail and reading, and as such sat at the front of her class at school. She was otherwise healthy and on systems examination there were no signs of syndromic retinitis pigmentosa. Anterior segment examination was unremarkable. The retina demonstrated peripheral pigmentary changes and loss of the foveal reflex, consistent with probable early involvement of foveal cones. The visual acuity in both eyes was reduced and measured 6/18 (0.48 logMAR). Fundus photographs demonstrated more
Table 1

Summary of patient demographics, phenotype and genotype data.

| Case | Sex | Age | BCVA | RE/LE | DNA mutation (exon) | Predicted protein change (length of expressed protein, amino acids) | Predicted mutation effect | Phenotype | Reference |
|------|-----|-----|------|-------|---------------------|---------------------------------------------------------------|--------------------------|-----------|-----------|
| 1 (father) | M | 38 | 6/4 | c.2613dupA (4) | (p.Arg872Thrfs*2) | Dominant | Dominant | Rod-cone dystrophy | Tiwari et al., 2016 |
| 2 (daughter) | F | 11 | 6/18 | c.2613dupA (4) | (p.Arg872Thrfs*2) | Dominant | Recessive | Cone-rod dystrophy | This study |
| 3 (mother) | F | 44 | 6/6 | c.3843dupT (4) | (p.Pro1282*) | Recessive | Not affected | | Mizobuchi 2021 |

BCVA = best corrected visual acuity.

6. Discussion

To the best of our knowledge, ours is the first report of differential inheritance and phenotype of RP1-retinal dystrophy within a single non-consanguineous family. We present full segregation data and clinical imaging to verify the distinct phenotypes of the father and daughter. The father, heterozygous for the c.2613dupA variant, displays a clinical course and phenotype typical of dominant disease. The daughter, however, is a compound heterozygote demonstrating a more severe, early-onset phenotype with early foveal cone involvement. The c.3843dupT allele inherited from her mother has previously been reported in a biallelic state. A similar c.3843delT pathogenic variant at the same nucleotide has also been reported to cause disease in the homozygous state. According to American College of Medical Genetics and Genomics criteria, the c.3843dupT variant fulfils the requirements for being determined as pathogenic (PV1, PM2, PM3, PM4).

While not common, the co-existence of dominant and recessive phenotypes within a single family has been documented for other genes. Consanguinity may be associated with such a situation - for example, homozygosity of dominant p.Arg220Gln PRPH2 pathogenic variants in a young child with severe early onset retinal dystrophy, with their parents suffering adult-onset maculopathy. However, the event is also observed in the absence of known consanguinity. A Jewish-American family affected by NR2E3-related retinal disease contains family members affected by dominant retinitis pigmentosa due to the p.Gly56Arg variant, as well as others who manifest enhanced S-cone syndrome. The clinical spectrum of bestrophinopathies presents another example of varying phenotypes, with pathogenic variants associated with Best vitelliform macular dystrophy (BVMD) giving rise to a autosomal recessive bestrophinopathy (ARB) a compound heterozygous state. In IMPG1, subclinical findings of foveal irregularity have been demonstrated in asymptomatic paternal carriers of the recessively inherited IMPG1 c.807+1G>T splice site mutation. Our family report, in addition to the highlighted cases in the literature, highlights the increasingly complex picture of many inherited retinal dystrophies. Full clinical and genetic evaluation of family members, including multimodal imaging, is essential.

The inheritance of RP1-related retinal dystrophy due to pathogenic variants in exon 4 is complex, as exemplified by this family report. Chen et al. first described a mutational hotspot between amino acids p.500 to p.1053, distal to - and including a portion of - the BIF-homologous protein region (p.486 to p.635) (Fig. 2). The bifocal protein in extensive circumferential pigmentary retinopathy with waxy pallor of the optic disc (Fig. 1, top row). Additionally, short-wavelength fundus autofluorescence showed loss of foveal hypofluorescence, typically associated with healthy macular pigment. Optical coherence tomography (OCT) demonstrated a marked disturbance of normal outer retinal architecture, with no visible ellipsoid zone. The phenotype was consistent with early onset cone-rod dystrophy. Genetic testing of the daughter revealed compound heterozygosity at the RP1 locus with c.2613dupA (p.Arg872Thrfs*2) and c.3843dupT (p.Pro1282*) mutations. RP1 c.3843dupT (p.Pro1282*) is predicted to result in a frameshift and a premature truncation of the RP1 protein. No additional pathogenic variants in the 110 other genes were detected in the gene panel.

5. Case 3

The mother (case 3) had no visual complaints to date (aged 44). There was no known prior history of visual impairment in her ancestors; and the couple had no other children together. Her ophthalmic examination was entirely normal with visual acuity of 6/6 in both eyes. The fundus photography and autofluorescence imaging confirmed normal retina (Fig. 1, middle row) and optical coherence tomography demonstrated a healthy macular architecture. The mother was confirmed to be a carrier of the c.3843dupT (p.Pro1282LeufsTer12) variant (Table 1).
Drosophila is thought to be important for photoreceptor morphogenesis.\textsuperscript{10} Frameshift-causing mutations within exon 4, the terminal exon, are insensitive to nonsense-mediated decay and result in viable transcripts.\textsuperscript{7} It is not well understood in terms of protein domains how premature truncating variants within this area of exon 4 (described as class II by Chen) produce disease in a heterozygous state - i.e. are dominantly inherited - whilst the remainder act in a recessive fashion, even though in both the C-terminus of the protein is affected. Further case reports have largely validated the general principles of this classification, however, there are several examples of pathogenic variants that fall within the class II cluster that appear to be recessively inherited.\textsuperscript{12} A notable example is the p.Ser542Ter founder variant which underlies approximately 5% of autosomal recessive retinitis pigmentosa in a Spanish population, with heterozygous members of these families appearing to be clinically normal.\textsuperscript{20} Further examples include the p.Ser574Asnfs*8 pathogenic variant which has been found to cause disease in a homozygous state only - including in a case of uniparental disomy of chromosome 8 \textsuperscript{21} - and p.Asp799+19. Whilst the pathogenic variants within our family do align with the model (Fig. 2), it cannot fully predict the mode of inheritance. The unusual situation within this family also suggests that so-called dominant pathogenic variants share the same loss of function as recessive exon 4 variants, which is only apparent in cases of biallelic inheritance. The molecular mechanism for this is not yet understood, but presumably is due to loss of function of the C-terminus in the RPI protein.

Patients with biallelic pathogenic variants in RPI have been reported to present with a similar phenotype to the daughter in the family, with either rod-cone dystrophy with early macular involvement or early onset severe retinal dystrophy (EOSRD). There exists some phenotypic variability, with presentations of cone-rod and macular dystrophy in late teens to early adult life.\textsuperscript{7} An analysis by Huckfeldt et al. suggests correlation between age of onset of symptoms and mutation type, with inheritance of at least one missense mutation being associated with later onset disease in comparison to biallelic premature truncating variants.\textsuperscript{8} Both alleles in the daughter (p.Arg872Thrfs*2 and p.Pro1282*) are premature-truncating exon 4 variants and her phenotype is consistent with other individuals reported. It remains to be seen if the clinical course of the daughter will differ from other individuals with biallelic inheritance due to the presence of the dominant c.2613dupA pathogenic variant, which in heterozygous individuals is presumed to exert latent or slow-acting pathogenicity by a gain-of-function mechanism.

Despite animal models, the exact mechanism by which premature-truncating variants cause retinal dystrophy remains unclear. Liu et al. previously demonstrated in the p.Glu662Ter mouse model - mimicking the common dominant human p. Arg677Ter variant - that mutant Rpi protein continues to localize within photoreceptor outer segments in a similar fashion to wildtype and possesses the same ability to stimulate microtubule polymerization.\textsuperscript{8} It should be noted, however, that this mouse model is generated on a C57B/L background and does not appear to undergo retinal degeneration; whereas this can be observed on an albino A/J background.\textsuperscript{22} The lack of retinal degeneration in the heterozygous state limits further study in this animal model, as the albino model potentially introduces other retinal confounders. It may be that the p.Glu662Ter allele is not in fact dominant in the mouse, as we have already observed several exon 4 mutations in humans act recessively, despite being within the predicted dominant cluster.

We previously observed in dominant RPI patients a phenotypic variability that appears to correlate with the predicted length of the truncated protein.\textsuperscript{2} This could be in keeping with a toxic gain-of-function effect. Known protein interactants with RPI include its paralog, RP1L1, with whom it shares a common N-terminus\textsuperscript{23} and missense mutations in which are associated with occult macular dystrophy.\textsuperscript{24} Double heterozygote knock-out mice (i.e. Rpi1 ± Rpi1l1+/−) demonstrate greater ERG abnormalities than the sum of single heterozygotes, implying functional interaction.\textsuperscript{23} Further characterization of the function of the C-terminus, perhaps through generation of alternative animal models, is required to understand the differential inheritance of exon 4 pathogenic variants.

The mutational spectrum of RPI-related retinal dystrophy presents challenges to engineering gene-based therapies. Adenovirus-associated vector (AAV) approaches have resulted in approved therapy for biallelic RPE-65 related retinal dystrophy,\textsuperscript{3} and there are a number of ongoing clinical trials for other inherited retinal dystrophies. However, the size of the RPI coding sequence is approximately 7 kbp and cannot be packaged in a single AAV vector. A dual vector approach\textsuperscript{25} may be able to augment levels of wild-type RPI protein within photoreceptors.

Expression of wild type Rpi protein in the p.Glu662Ter homozygous knock-in mouse prevented retinal degeneration up to the 12-month time point. In some lines, however, there was deemed to be relative overexpression of the wild-type protein which led to an accelerated retinal degeneration. It is not clear if this is due to abnormal stoichiometry and interactions between wild-type Rpi protein and/or other protein interactants with the prematurely-truncated p.Glu662Ter mutant Rpi, or a non-specific cellular response to protein overexpression. In any case, with such an approach the potential latent dominant-negative effects of a dominant pathogenic variant would not be addressed. Precise restoration of a single wild-type allele by base-editing\textsuperscript{27} or prime-editing\textsuperscript{28} may be able to address both recessive and dominantly inherited forms of
RP1-related retinal dystrophy.

In conclusion, this case report highlights the clinical heterogeneity of exon 4 pathogenic variants. The presentation of a compound heterozygous case involving a recessive and pathogenic variant highlights that genetic counselling should address the small possibility of biallelic inheritance, which would result in a more severe phenotype. Further molecular characterization is required to elucidate disease mechanisms across the spectrum of RP1 mutations and inform future development of novel therapies.

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Declaration of authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

Consent

Written consent to publish this case has not been obtained. This report does not contain any personal identifying information.

Declaration of competing interest

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References

1. Sullivan LS, Bowne SJ, Reeves MJ, et al. Prevalence of mutations in eyeGene probes with a diagnosis of autosomal dominant retinitis pigmentosa. Invest Ophthalmol Vis Sci. 2013;54(9):6255–6261.
2. Nanda A, McClements ME, Clouston P, Shankis ME, MacLaren RE. The location of exon 4 mutations in RP1 raises challenges for genetic counseling and gene therapy. Am J Ophthalmol. 2019;202:22–29.
3. 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.
4. Riazuddin S, Zulfiquar 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.
5. Liu Q, Lyubarsky A, Skalet JH, Pugh EN, Pierce EA. RP1 is required for the correct stacking of outer segment discs. Invest. Ophthalmol. Visual Sci. 2003;44(10):4171–4183.
6. Chen LJ, Lai TTY, Tam POS, et al. Compound heterozygosity of two novel truncation mutations in RP1 causing autosomal recessive retinitis pigmentosa. Invest. Ophthalmol. Visual Sci. 2010;51(4):2236–2242.
7. Verbalk S, Van Huet RAC, Den Hollander AI, et al. Macular dystrophy and cone-rod dystrophy caused by mutations in the RP1 gene: extending the RP1 disease spectrum. Invest. Ophthalmol. Visual Sci. 2019;60(4):1192–1203.
8. Hucklefield RM, Grigorian F, Place E, et al. Biallelic RP1-associated retinal dystrophies: expanding the mutational and clinical spectrum. Mol Vis. 2020;26:423–433.
9. Liu Q, Zuo J, Pierce EA. The retinitis pigmentosa 1 protein is a photoreceptor microtubule-associated protein. J Neurosci. 2004;24(29):6427–6436.
10. Bahri SM, Yang X, Chia W. The Drosophila bifocal gene encodes a novel protein which colocalizes with actin and is necessary for photoreceptor morphogenesis. Mol Cell Biol. 1997;17(9):5521–5529.
11. Tiwari A, Bahr A, Bahr L, et al. Next generation sequencing based identification of disease-associated mutations in Swiss patients with retinal dystrophies. Sci Rep. 2016;6, 28755.
12. Mizoshiki K, Hayashi T, Oishi N, et al. Genotype-phenotype correlations in RP1-associated retinal dystrophies: a multi-center cohort study in Japan. J Clin Med. 2021;10(11):2265.
13. Eiserberger T, Neuhaus C, Khan AO, et al. Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. In: Li T, ed. PLoS ONE. 8, 2013, e78496, 11.
14. Silva RS, Salles MV, Motta FL, Sallum JMF. Retinitis pigmentosa due to RP1 biallelic variants. Sci Rep. 2020;10(1):1–4.
15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and Genomics and the association for molecular pathology. Genet Med. 2015;17(5):405–424.
16. Khan AO. Recessive pediatric-onset cone-rod dysfunction or dominant maculopathy in a consanguineous family harboring the peripheral mutation p.Arg220Gln. Ophthalmic Genet. 2019;40(1):60–63.
17. Fischer P, Gouras P, Roduit R, et al. Mutations in NR2E3 can cause dominant or recessive retinal degenerations in a same family. Hum Mutat. 2009;30(3):342–351.
18. Xuan Y, Zhang Y, Zong Y, et al. The clinical features and genetic spectrum of a large cohort of Chinese patients with vitelliform macular dystrophies. Am J Ophthalmol. 2020;216:69–79.
19. El Shamieh S, Boulanger-Scemama E, Lancelot ME, 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. Avila-Fernandez A, Corton M, Nishiguchi KM, et al. Identification of an RP1 prevalent founder mutation and related phenotype in Spanish patients with early-onset autosomal recessive retinitis. Ophthalmology. 2012;119(12):2616–2621.
21. Bedoukian E, Aleman T, O’Neill E. ePOs2: associated recessive retinitis pigmentosa caused by paternal uniparental disomy. Genet Med. 2022;24(3):S54.
22. The severity of retinal degeneration in Rph1 gene-targeted mice is dependent on genetic background | IOVS | ARVO Journals.
23. Yoshihisa T, Liu J, Gao J, et al. Essential and synergistic roles of RP1 and RP1L1 in rod photoreceptor axoneme and retinitis pigmentosa. J Neurosci. 2009;29(31):9748–9760.
24. RP1L1 and Inherited Photoreceptor Disease: A Review - Survey of Ophthalmology.
25. Russell B, Bennett J, Wellman JA, et al. Efficacy and safety of verteporfin photodynamic therapy (AVV2-68PE65v2) in patients with RP665-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet. 2017;390(10097):849–860.
26. McClements ME, MacLaren RE. Adeno-associated virus (AAV) dual vector strategies for gene therapy encoding large transgenes. Yale J Biol Med. 2017;90(4):611–625.
27. Levy JM, Yeh WH, Pendse N, et al. Cytosine and adenine base editing of the brain, liver, retina, and skeletal muscle of mice via adeno-associated viruses. Nat Biomed. Eng. 2020;4(1):97–110.
28. Kantor A, McClements ME, MacLaren RE. Crispr-cas9 dna base-editing and prime-editing. Int J Mol Sci. 2020;21(17):1–22.