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Biallelic *RP1*-associated retinal dystrophies: Expanding the mutational and clinical spectrum

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Purpose: To evaluate the phenotypic spectrum of autosomal recessive *RP1*-associated retinal dystrophies and assess genotypic associations.
Methods: A retrospective multicenter study was performed of patients with biallelic *RP1*-associated retinal dystrophies. Data including presenting symptoms and age, visual acuity, kinetic perimetry, full field electroretinogram, fundus examination, multimodal retinal imaging, and *RP1* genotype were evaluated.
Results: Nineteen eligible patients from 17 families were identified and ranged in age from 10 to 56 years at the most recent evaluation. Ten of the 21 unique *RP1* variants identified were novel, and mutations within exon 2 accounted for nearly half of alleles across the cohort. Patients had clinical diagnoses of retinitis pigmentosa (13), cone-rod dystrophy (3), Leber congenital amaurosis (1), early-onset severe retinal dystrophy (1), and macular dystrophy (1). Macular atrophy was a common feature across the cohort. Symptom onset occurred between 4 and 30 years of age (mean 14.9 years, median 13 years), but there were clusters of onset age that correlated with the effects of *RP1* mutations at a protein level. Patients with later-onset disease, including retinitis pigmentosa, had at least one missense variant in an exon 2 DCX domain.
Conclusions: Biallelic *RP1* mutations cause a broad spectrum of retinal disease. Exon 2 missense mutations are a significant contributor to disease and can be associated with a considerably later onset of retinitis pigmentosa than that typically associated with biallelic *RP1* mutations.

Inherited retinal disorders are a significant cause of vision loss worldwide, and they have a complex genetic causality. Retinitis pigmentosa (RP), the most common of these diseases, can be caused by mutations in more than 80 genes (RetNet). The phenotypic spectrum associated with individual genes is also broad: mutations in individual genes can cause syndromic as well as nonsyndromic RP (e.g., *USH2A* [1]), impact protein function such that both recessive and dominant retinal dystrophies are possible (e.g., *RPE65* [2]), and lead to different clinical presentations and diagnoses (e.g., *RPGR* [3], *PRPH2* [4]) even among family members with a shared mutation [5]. Thus, although inherited retinal disorders such as macular dystrophy (MD) and RP are distinct in their clinical manifestations, they can have a shared genetic causality. Understanding this complexity is increasingly relevant to clinical care with a growing number of gene-specific therapies being evaluated in preclinical models and clinical trials.

RP1-associated retinal dystrophies illustrate this phenotypic variability. *RP1* encodes a photoreceptor-specific microtubule-associated protein found within the outer segment axoneme of both rods and cones that is needed for the stability and organization of the outer segment membranous discs [6-8]. Mutations in *RP1* were initially identified as a cause of autosomal dominant RP (adRP) [9,10] and subsequently autosomal recessive RP (arRP) [11,12], and they have been estimated to cause up to 11% of each adRP and arRP in some populations [13-15]. The onset and severity of *RP1*-associated RP are related to the mode of inheritance. Individuals with *RP1*-associated adRP typically begin to experience nyctalopia and decreased peripheral vision in their 20s and 30s with the potential to retain near-normal visual acuity (VA) into their 50s and 60s [13,16,17]. In contrast, arRP due to mutations in *RP1* typically manifests before 10 years of age and is characterized by secondary macular involvement that can result in legal blindness by age 20 [11,17-24]. The spectrum of disease was recently broadened to include MD and cone-rod dystrophy (CRD) secondary to biallelic *RP1* mutations with age of onset typically later than *RP1*-associated arRP [25-27].

Mutations leading to adRP versus arRP are spatially segregated within *RP1*. The mutations associated with adRP fall within a mutational hot spot in exon 4, the largest of the three coding exons, that extends from codon 500 to

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approximately codon 984, although only a few variants fall before codon 658 [22,25,28]. Mutations associated with arRP are found elsewhere in exon 4, including within a region overlapping the proximal extent of the broader adRP cluster, as well as in exons 2 and 3 [13,22,25]. Nonsense and frameshift mutations throughout exon 4 are predicted to result in truncated rather than absent protein secondary to transcript escape from nonsense-mediated decay [29]. Indeed, mRNA corresponding to an *RP1* truncation mutation has been identified in patient lymphoblasts, and truncated protein correctly localized to the axoneme has been found in a mouse model [7,30]. A dominant-negative mechanism for *RP1*-associated adRP has been suggested by animal studies [30], although the mechanisms by which dominant versus recessive exon 4 mutations impose their differential effects are not understood. Various categorizations of mutations have been proposed in addition to the recent consideration of the impact of hypomorphic variants as determinants of the severity of biallelic disease [18,20,25].

Limited phenotypic characterization is available, however, for individuals with biallelic disease in which one or both mutations fall outside of exon 4. Exons 2 and 3 contain tandem doublecortin (DCX) domains, with homology to the brain-specific microtubule-associated protein DCX that are necessary for *RP1* association with microtubules and axoneme organization [8]. The recent identification of a homozygous missense change in the exon 2 DCX domain in 12 unrelated Kuwaiti families with MD indicates the importance of these domains [27], but the spectrum of disease associated with DCX mutations remains to be defined. The function and clinical importance of the BIF domain in exon 4, which is homologous to the Drosophila protein bifocal (Bif) needed for photoreceptor morphogenesis [31], also remain undefined, although they overlap with the reported range of the adRP hotspot. The purpose of this study is to assess more broadly the phenotypic range associated with biallelic *RP1* mutations with a particular focus on the clinical correlates of DCX domain mutations.

METHODS

This retrospective multicenter study was conducted at Massachusetts Eye and Ear (MEE) and the Casey Eye Institute (CEI). It was approved by the institutional review boards of each institution and met the tenets of the Declaration of Helsinki. Individuals with two pathogenic or likely pathogenic *RP1* variants causing recessive dystrophy were eligible.

Genetic testing: Diagnostic and research-based genetic testing was primarily performed with next-generation panel-based approaches. MEE patients had genetic testing performed locally with the genetic eye diseases inherited retinal diseases panel (GEDi-R) [32] with the exception of patient MEE4, who had Sanger sequencing performed to verify familial mutations identified by an outside laboratory. CEI patients had testing performed through a variety of experienced laboratories. Variant interpretation during patient screening for inclusion was performed in accordance with American College of Medical Genetics and Genomics guidelines for sequence variant interpretation [33]. Variants with an allele frequency greater than 1% in the Genome Aggregation Database (gnomAD) [34] were considered benign polymorphisms and filtered out. Alleles with a high frequency in the MEE internal database were also excluded. In addition to frequency data, variant pathogenicity was determined by predictions from in silico modeling, including SIFT [35], PolyPhen-2 [36], MutationTaster [37], and scientific evidence available in the literature. Segregation analysis was available for a subset of patients, and analysis of relatedness among patients MEE1–3 was assessed with previously described methods [38].

Clinical assessments: Individuals were evaluated in ophthalmic genetics or retina clinics. VA was measured using Snellen and Early Treatment of Diabetic Retinopathy Study (ETDRS) charts. Kinetic perimetry was performed using either the Goldmann perimeter or the Octopus 900 perimeter (Haag-Streit, Bern, Switzerland). Full-field electroretinograms (ERG) were performed using Burian Allen electrodes and custom ERG systems at both institutions with previously described parameters at MEE [39,40] and International Society for Clinical Electrophysiology of Vision (ISCEV) standards at CEI [41,42]. Retinal imaging included fundus photography (Topcon Medical Systems, Oakland, NJ; Optos, Marlborough, Massachusetts, USA), spectral-domain optical coherence tomography (SD-OCT: Spectralis, Heidelberg Engineering, Heidelberg, Germany; Cirrus, Carl Zeiss, Oberkochen, Germany), and fundus autofluorescence (FAF; Spectralis; Optos).

RESULTS

Genetic analysis: Twenty-one individuals from 17 families, including two sibling pairs, had two variants in *RP1*. Two patients, MEE6 and CEI22989, were excluded from further analysis (Appendix 1). MEE6 had a mutation associated with arRP [c.1625C>G, p.(Ser542*)] [22] as well as a novel frameshift mutation [c.2041dupA, p.(Ile681Asnfs*17)] located within the adRP hotspot in exon 4. Although MEE6 had a negative family history and these variants were found to be in trans by cloning and long-range PCR, no family members were available for genetic testing or clinical evaluation to assess the mode of inheritance. CEI22989 had a pathogenic

RP1 variant that is a common cause of adRP [c.2028C>T, p.(Arg677*)] [9] as well as a variant of unknown significance (VUS) well represented in gnomAD [c.4250T>C, p.(Leu1417Pro); gnomAD 141/102,562].

The resulting 19 patients comprised a cohort with presumed autosomal recessive inheritance. Individuals were ethnically diverse with countries of origin including Brazil, Kuwait, Saudi Arabia, Malaysia, Vietnam, and the United States. Segregation testing confirmed biparental inheritance when performed (MEE4, 5, 11–13; CEI26396, CEI26528, CEI26529, CEI29023). Ten of the 21 unique identified *RP1* variants were novel and predicted to be pathogenic (Table 1; Appendix 2; Figure 1). Eight patients from seven families had homozygous variants with only one of these individuals (MEE7) reporting parental consanguinity. Four patients from Kuwait (MEE1–4) were homozygous for the mutation p.(Asp202Glu); two were siblings (MEE1, MEE2) and were not near relatives of a third Kuwaiti individual (MEE3) based on an analysis of relatedness. No relationships were known between MEE1–3 and MEE4.

Several variants merit additional comment. The c.3155delT mutation [p.(Tyr1053Thrfs*4)] in CEI23745 was first proposed to cause adRP but later reported in a compound heterozygous state in arRP with segregation analysis confirming biparental inheritance and with the carrier parent having a normal ophthalmic evaluation [15,16]. CEI29023 was a compound heterozygote for a truncating mutation near the adRP hotspot that was initially described in isolation and without segregation data [c.1598_1601del, p.(Arg533Lysfs*12)] [26]. Multiple recessively inherited mutations cluster in this region [22,25], and in the present report, segregation analysis confirms biparental inheritance of the two *RP1* variants. Neither set of parents was available for clinical evaluation to verify the presumed autosomal recessive action of these variants, but they did not have any known retinal problems. In addition, the clinical features of the two patients were consistent with earlier reports of *RP1*-associated arRP. Finally, CEI24459 had a third *RP1* variant [c.4526T>G; p.(Ile1509Ser)] interpreted as a VUS based on pathogenicity predictions (SIFT – tolerated; MutationTaster – polymorphism), as well as prior reports [13].

The total number of mutations in exons 2 and 4 was equal across the cohort (exon 2: 18; exon 4: 18) with seven and 13 unique variants present respectively in each (Table 1; Figure 1). All exon 2 mutations, as well as the single exon 3 mutation, fell within the DCX domains (Figure 1). The most common variant in exon 2 was the previously reported missense mutation c.606C>A, p.(Asp202Glu) [43] that was homozygous in four individuals from three families. The second most frequent variant was c.515G>T; p.(Leu172Arg) [44], which was identified with a second allele in five individuals from four families. Two novel missense mutations, as well as two variants resulting in early transcript termination and thus nonsense-mediated decay, were also identified in exon 2. The single exon 3 mutation identified was present in two individuals and was predicted to result in nonsense-mediated decay. All exon 4 variants were predicted to result in the premature termination of translation, and three of these mutations fell within the BIF domain. An evaluation of the *RP1* sequence data, which were available for MEE1–3 and MEE5–13, verified the absence of three exon 4 mutations recently reported to be present at a high frequency in patients with biallelic *RP1* dystrophies [25,45].

Clinical data: Clinical assessments were performed in 19 individuals (9 females, 10 males) ranging in age from 10 to 56 years at the most recent evaluation. Eleven patients had more than one visit over intervals between two and 43 years (Appendix 3). RP was the most common clinical diagnosis (n = 13), but CRD (n = 3), Leber congenital amaurosis (LCA; n = 1), early-onset severe retinal dystrophy (EOSRD; n = 1), and MD (n = 1) were also represented (Table 1). There was overlap in the age of onset and features of patients with LCA, EOSRD, and RP reflecting variation in how clinicians define these diagnostic categories. Of the 18 patients for whom presenting symptoms were available, 16 included nyctalopia or impaired dark adaptation as primary complaints, and two described poor central vision. Symptom onset was between ages 4 and 30 with a mean of 14.9 years and median of 13 years. The sub-group of patients biallelic for predicted protein-truncating or protein null mutations had an earlier age of onset (n = 8, mean 6.3 years, median 5 years) than those with either a protein-truncating or protein null mutation plus a missense mutation (n = 6, mean and median 20.0 years) or two missense mutations (n = 5, mean 22.6 years, median 22 years; Table 2). Individuals with diagnoses of MD and CRD were only found within the two genotype groups characterized by at least one missense variant.

Cross-sectional VA across the group ranged from 20/20 to light perception (Appendix 3). Two individuals diagnosed with RP by age 5 had data spanning approximately 40 years showing a profound reduction in VA (MEE9, MEE10). No individuals were reported to have nystagmus. Kinetic perimetry showed midperipheral and peripheral visual field loss in most of the patients for whom it was available with significant central depression in a smaller number (Appendix 4). ERGs showed nondetectable scotopic signals and either nondetectable or severely depressed photopic function in 12 patients (Appendix 3). Fundus exams and retinal imaging

demonstrated peripheral retinal findings indicative of generalized photoreceptor degeneration in all but two patients (Figure 2; Appendix 5, Appendix 6). MEE1 and MEE4, who had been diagnosed with MD and CRD, had normal peripheral retinal exams but large regions of fovea-sparing macular atrophy. Similar areas of macular atrophy, however, were also seen in individuals with RP (e.g., MEE5, 11; CEI23745, CEI24459; CEI29345). FAF and SD-OCT demonstrated correlates of the clinical fundus exam (Figure 2; Appendix 5, Appendix 6).

Four individual cases emphasize the phenotypic variability found within these patients (Figure 2, Appendix 3, Appendix 5). MEE1, who had a homozygous exon 2 missense mutation, was asymptomatic until age 30 and had a clinical diagnosis of MD with near-normal ERG responses and extensive macular atrophy. CEI26529, who had a missense mutation and a protein-truncating mutation, presented as an adolescent with subjectively decreased VA and central scotomas with subnormal ERG responses resulting in a diagnosis of CRD. MEE5, who had RP associated with a missense mutation and

Table 1. Genotypes and clinical diagnoses.

Patient	Allele 1	Allele 2	Diagnosis	Symptom onset (y)
MEE1[a]	c.606C>A; p.(Asp202Glu) [43]	c.606C>A; p.(Asp202Glu)	MD	30
MEE2[a]	c.606C>A; p.(Asp202Glu) [43]	c.606C>A; p.(Asp202Glu)	RP	25
MEE3	c.606C>A; p.(Asp202Glu) [43]	c.606C>A; p.(Asp202Glu)	RP	22
MEE4[b]	c.606C>A; p.(Asp202Glu) [43]	c.606C>A; p.(Asp202Glu)	CRD	20
MEE5[b]	c.126G>A; p.(Lys42Asn)[c]	c.312_315delCCTA; p.(Leu105Valfs*10)[c]	RP	30
MEE7	c.1462delG; p.(Glu488Lysfs*44) [26]	c.1462delG; p.(Glu488Lysfs*44)	RP	4
MEE8	c.3428delA; p.(Asn1143Ilefs*25) [19]	c.3428delA; p.(Asn1143Ilefs*25)	RP	7
MEE9	c.4788delT; p.(Asp1597Thrfs*29)[c]	c.4788delT; p.(Asp1597Thrfs*29)	RP	4
MEE10	c.668del; p.(Gly223Glufs*41)[c]	c.1126C>T; p.(Arg376*) [49]	RP	5
MEE11[b]	c.668del; p.(Gly223Glufs*41)[c]	c.1468G>T; p.(Glu490*)[c]	RP	5
MEE12[b]	c.1234dupA; p.(Met412Asnfs*7) [24]	c.4171delC; p.(Gln1391Lysfs*7)[c]	RP	4
MEE13[b]	c.491C>G; p.(Pro164Arg)[c]	c.1199_1200del; p.(Gln400Argfs*18)[c]	RP	28
CEI23745	c.515T>G; p.(Leu172Arg) [44]	c.3155delT; p.(Tyr1053Thrfs*4) [16]	RP	27
CEI26396[b]	c.5017delC; p.(Tyr1673Metfs*37)[c]	c.5017delC; p.(Tyr1673Metfs*37)	LCA	6
CEI26528[a,b]	c.515T>G; p.(Leu172Arg) [44]	c.4582_4585delATCA; p.(Ile1528Valfs*10) [50]	CRD	12
CEI26529[a,b]	c.515T>G; p.(Leu172Arg) [44]	c.4582_4585delATCA; p.(Ile1528Valfs*10) [50]	CRD	13
CEI29023[b]	c.515T>G; p.(Leu172Arg) [44]	c.1598_1601del; p.(Arg533Lysfs*12) [26]	EOSRD	10
CEI24459	c.139dup; p.(Gln47Profs*15)[c]	c.5248G>T; p.(Glu1750*) [15]	RP	15
CEI29345	c.121T>C, p.(Tyr41His) [26]	c.515T>G; p.(Leu172Arg) [44]	RP	16

Abbreviations: CRD – cone-rod dystrophy; EOSRD – early-onset severe retinal dystrophy; LCA – Leber congenital amaurosis; MD – macular dystrophy; RP – retinitis pigmentosa; y – years [a] Siblings; [b] Biparental inheritance confirmed by segregation analysis; [c] Novel mutation

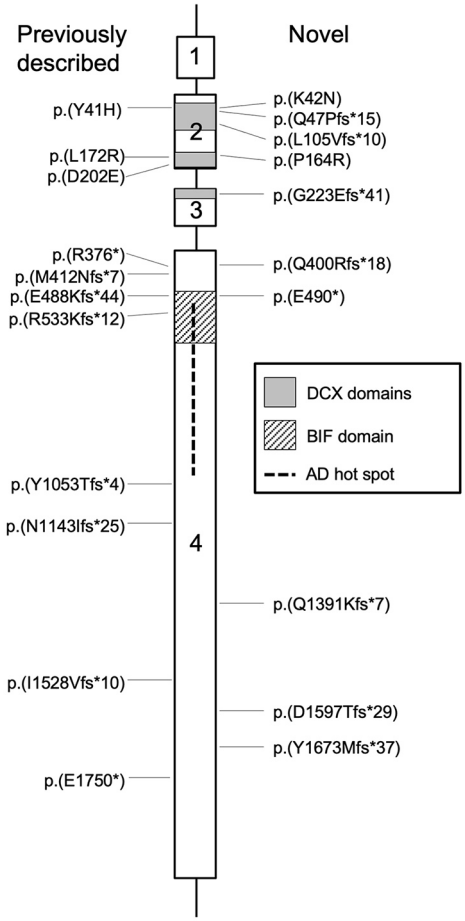

Figure 1. Schematic of *RP1* gene organization showing mutations associated with biallelic disease. The mutations shown were identified in the present study as novel or previously reported. Mutations previously reported in the literature but absent in this cohort are not shown.

a protein null mutation, was asymptomatic until age 30 and had sharply demarcated macular atrophy in her 40s similar to that of MEE1. Finally, MEE9, who had a homozygous protein-truncating exon 4 mutation and a diagnosis of RP, had nyctalopia at age 4, subnormal VA of 20/50 with nondetectable ERG responses at age 9, and visual field constriction to less than 10° by age 13.

DISCUSSION

Data from this cohort, which to our knowledge represents the largest reported group of unrelated patients with biallelic *RP1*-associated disease, further broaden the phenotypic spectrum of biallelic disease and demonstrate the clinical importance of the DCX homology domains. A subset of this cohort (MEE7–12, CEI26396) exhibited the severe early-onset disease previously described in association with biallelic *RP1* mutations, with a median onset of age 5. The clinical diagnosis of LCA found within this group indicates the importance of assessing patients with early-onset disease for mutations in *RP1*. The genotypes in this early-onset subset were comprised almost exclusively of exon 4 protein-truncating mutations with the exception of two individuals heterozygous for an exon 3 protein null mutation. Only one patient with a similar genotype (CEI24459) had a later age of symptom onset, with atypical preservation of VA at age 20 for this genotype. The exon 4 mutations identified in these patients were excluded from the adRP hotspot but were otherwise found throughout exon 4, including within the BIF domain.

The clinical presentations of the remaining patients differed from this pattern with an apparent relationship to the genotype. Six patients with a missense mutation in an exon 2 DCX domain combined with either a protein null mutation in exon 2 or a protein-truncating mutation in exon 4 had symptom onset at a median age of 20 years with two apparent age clusters. Three of these individuals (CEI26528, CEI26529, CEI29023), including two siblings with CRD, had symptom onset between ages 10 and 13 and shared a missense mutation within an exon 2 DCX domain [p.(Leu172Arg)]. The other three (MEE5, 13; CEI23745) began to experience RP

symptoms between ages 27 and 30. A final group of five patients had two missense mutations in the exon 2 DCX domains and a median onset age of 22 years. Four of these individuals, including two siblings, were homozygous for the exon 2 missense mutation [p.(Asp202Glu)]. Despite the identical genotype, diagnoses included MD, CRD, and RP. This variability differs from a recent report by Riera et al. [27] of multiple Kuwaiti families in which homozygosity for p.(Asp202Glu) was associated with MD, but individuals homozygous for this variant with diagnoses of RP have also been reported although with limited phenotypic detail [43,46].

Several clinically relevant points are emphasized by the latter two groups in this cohort in particular. First, they provide additional examples of biallelic *RP1* mutations causing cone-predominant disease while also extending the mutational spectrum. The cases reported here agree with recent observations that *RP1*-associated CRD and MD are associated with the presence of at least one mutation with a lesser impact on protein function [25,27]. While Verbakel et al. [25] reported predominantly mild variants in the distal end of exon 4, the mutations associated with the diagnoses in the present study were exclusively missense changes in the exon 2 DCX domains. Indeed, although two recent studies on recessive *RP1*-associated dystrophies described high proportions of patients with hypomorphic exon 4 variants [25,45], we did not encounter these alleles in our cohort, which was instead notable for the high representation of missense mutations in the exon 2 DCX domains.

Second, a clear genotype–phenotype relationship with regard to the extent of retinal involvement was not apparent in the presence of exon 2 missense mutations. Mutation severity and the anticipated impact on protein function were previously observed to correlate with the severity of biallelic *RP1*-associated retinal dystrophies, such that hypomorphic mutations, including exon 2 missense mutations, were identified in individuals with MD and CRD but not RP [25]. In the present cohort, which included a substantial number of individuals with exon 2-involving biallelic disease, a broader disease spectrum was identified. In particular, although exon 2 missense mutations were identified in individuals

Table 2. *RP1* genotypes grouped by mutation effect on protein.

Genotype category	Exons involved	Patient	Clinical diagnosis	Symptom onset (y)	Median onset age (y; range)
Two missense mutations	2, 2	MEE1[a]	MD	30	22.0 (16–30)
	2, 2	MEE2[a]	RP	25	
	2, 2	MEE3	RP	22	
	2, 2	MEE4	CRD	20	
	2, 2	CEI29345	RP	16	
Missense variant plus protein-truncating or protein null mutation	2, 2	MEE5	RP	30	20.0 (10–30)
	2, 4	MEE13	RP	28	
	2, 4	CEI23745	RP	27	
	2, 4	CEI26528[a]	CRD	12	
	2, 4	CEI26529[a]	CRD	13	
	2, 4	CEI29023	EOSRD	10	
Two protein-truncating or protein null mutations	2, 4	CEI24459	RP	15	5.0 (4–15)
	3, 4	MEE10	RP	5	
	3, 4	MEE11	RP	5	
	4, 4	MEE7	RP	4	
	4, 4	MEE8	RP	7	
	4, 4	MEE9	RP	4	
	4, 4	MEE12	RP	4	
	4, 4	CEI26396	LCA	6	

Abbreviations: CRD – cone-rod dystrophy; EOSRD – early-onset severe retinal dystrophy; LCA – Leber congenital amaurosis; MD – macular dystrophy; RP – retinitis pigmentosa; y – years; [a] Siblings

with MD and CRD consistent with previous reports [25-27], the present cohort included individuals with RP and EOSRD who possessed at least one exon 2 missense mutation (MEE2, 3, 5, 13; CEI23745, CEI29345; CEI29023). Two of the missense variants reported here have also previously been reported in association with biallelic RP, although limited clinical data are available on individuals with these or other exon 2-involving genotypes beyond homozygosity for p.(Asp202Glu) [15,26,27,43,44,46,47]. A common although not exclusive feature of individuals in this cohort with one or more exon 2 missense mutations was prominent macular atrophy, which was present in individuals with MD as well as RP (e.g., Figure 2A, C).

Finally, biallelic *RP1*-associated RP can have a later onset than previously appreciated, and this feature appears influenced by genotype. With the exception of CEI29023, the median age of symptom onset for individuals with RP associated with one or more missense mutations in the exon 2 DCX domains was in the early 20s and thus notably later than that of individuals with biallelic protein null or protein-truncating mutations (Table 2). Consistent with this finding, a later age of symptom onset than typically associated with biallelic *RP1*-associated RP was reported for an individual with a homozygous DCX-affecting missense variant (p.F227V) [47]. Similarly, slowly progressive retinal degeneration was described in a mouse line homozygous for a DCX missense mutation [48].

Further evaluation is needed to understand the mechanisms of *RP1*-associated retinal dystrophies. The macular involvement in biallelic RP demonstrated in this cohort both in early- and later-onset cases is consistent with the localization of RP1 to both cones and rods [6], but its earlier onset in contrast to the late macular involvement in *RP1*-associated adRP is striking and not understood. The axoneme abnormalities and disrupted RP1 microtubule binding that result from the deletion of the DCX domains but not C-terminal truncation in cell culture and mouse models hint at the intracellular perturbations that may contribute to clinical diversity [8]. The p.(Asp202Glu) DCX mutation did not disrupt RP1 localization in HEK293T cells overexpressing this protein [27], but it is possible that a cellular phenotype may be more apparent in photoreceptors. It also remains unclear why frameshift mutations in exon 4, which result in presumed protein production due to transcript escape from nonsense-mediated decay, albeit with the unknown preservation of localization and function for the full mutational spectrum, can have a loss-of-function versus dominant negative effect based on their location within the gene. Finally, the phenotypic variability seen in the four patients homozygous for the same *RP1* variant (MEE1–4) suggests the existence of unidentified modifying

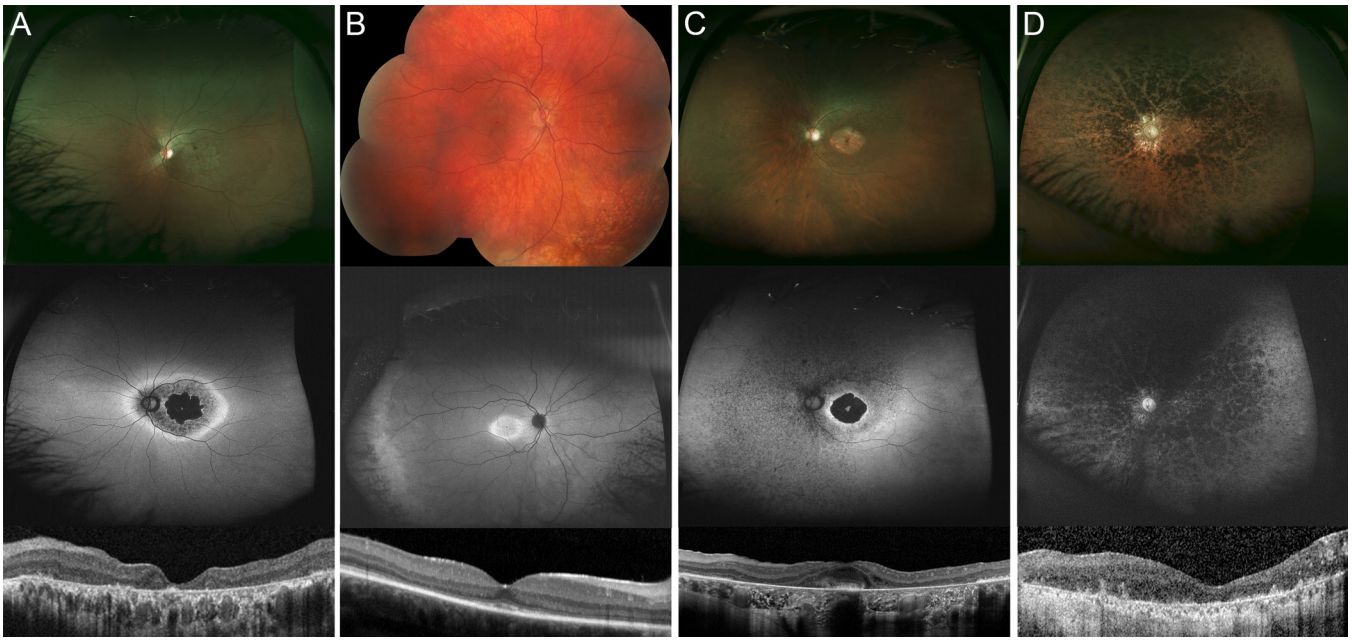

Figure 2. Retinal imaging from patients with a representative spectrum of biallelic *RP1*-associated disease. Imaging for each patient includes fundus photography (top image), fundus autofluorescence (middle image), and OCT (bottom image). The patients shown had macular dystrophy (**A**: MEE1, images acquired at age 38), cone-rod dystrophy (**B**: CEI26529, images acquired at age 15), adult-onset retinitis pigmentosa (**C**: MEE5, images acquired at age 46), and early-onset RP (D: MEE9, images acquired between ages 45–47).

factors. Similarly, a genotype associated with RP in this study [CEI29345: p.(Y41H), p.(L172R)] was previously observed in a patient reported to have MD [26]. Patient-derived induced pluripotent stem cells (iPSCs) and retinal organoids could be used to evaluate the consequences of the mutational spectrum as well as potential differences in the impact on rod versus cone function. These systems can also be used to assess potential therapies that introduce normal RP1 protein to individuals with biallelic disease. The later ages of onset reported here suggest a longer window of therapeutic opportunity for gene therapy in some individuals with biallelic *RP1* retinal dystrophies than anticipated.

In summary, this cohort of patients broadens the phenotypic spectrum of biallelic *RP1*-associated retinal dystrophies to include later-onset RP, and it demonstrates the clinical significance of missense mutations in the exon 2 DCX domains. A prospective natural history study of *RP1*-associated dystrophies would be of value in assessing and understanding this clinical heterogeneity in a larger group of patients. This information, in conjunction with mechanistic insights that could be obtained from patient-derived iPSCs, has increasing significance given the frequency of *RP1*-associated dystrophies and the expanding array of therapeutic strategies.

APPENDIX 1: RP1 ALLELES FOUND IN PATIENTS WITH EITHER DOMINANT RP1-ASSOCIATED DISEASE OR WITH AN UNCERTAIN MODE OF INHERITANCE.

To access the data, click or select the words "Appendix 1."

APPENDIX 2: RP1 ALLELE FREQUENCIES AND PREDICTED PATHOGENICITY.

To access the data, click or select the words "Appendix 2."

APPENDIX 3: SUMMARY OF CLINICAL DATA.

To access the data, click or select the words "Appendix 3."

APPENDIX 4: VISUAL FIELDS OF PATIENTS WITH BIALLELIC RP1-ASSOCIATED RETINAL DYSTROPHIES.

To access the data, click or select the words "Appendix 4."

APPENDIX 5: SUMMARY OF FUNDUS APPEARANCE AND RETINAL IMAGING.

To access the data, click or select the words "Appendix 5."

APPENDIX 6: RETINAL IMAGING FROM PATIENTS WITH BIALLELIC RP1-ASSOCIATED RETINAL DYSTROPHIES.

To access the data, click or select the words "Appendix 6."

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