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Clinical Phenotype and Course of *PDE6A*-Associated Retinitis Pigmentosa Disease, Characterized in Preparation for a Gene Supplementation Trial

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IMPORTANCE Treatment trials require sound knowledge on the natural course of disease.

OBJECTIVE To assess clinical features, genetic findings, and genotype-phenotype correlations in patients with retinitis pigmentosa (RP) associated with biallelic sequence variations in the *PDE6A* gene in preparation for a gene supplementation trial.

DESIGN, SETTING, AND PARTICIPANTS This prospective, longitudinal, observational cohort study was conducted from January 2001 to December 2019 in a single center (Centre for Ophthalmology of the University of Tübingen, Germany) with patients recruited multinationally from 12 collaborating European tertiary referral centers. Patients with retinitis pigmentosa, sequence variants in *PDE6A*, and the ability to provide informed consent were included.

EXPOSURES Comprehensive ophthalmological examinations; validation of compound heterozygosity and biallelism by familial segregation analysis, allelic cloning, or assessment of next-generation sequencing–read data, where possible.

MAIN OUTCOMES AND MEASURES Genetic findings and clinical features describing the entire cohort and comparing patients harboring the 2 most common disease-causing variants in a homozygous state (c.304C>A;p.(R102S) and c.998 + 1G>A;p.?).

RESULTS Fifty-seven patients (32 female patients [56%]; mean [SD], 40 [14] years) from 44 families were included. All patients completed the study. Thirty patients were homozygous for disease-causing alleles. Twenty-seven patients were heterozygous for 2 different *PDE6A* variants each. The most frequently observed alleles were c.304C>A;p.(R102S), c.998 + 1G>A;p.?, and c.2053G>A;p.(V685M). The mean (SD) best-corrected visual acuity was 0.43 (0.48) logMAR (Snellen equivalent, 20/50). The median visual field area with object III4e was 660 square degrees (5th and 95th percentiles, 76 and 11 019 square degrees; 25th and 75th percentiles, 255 and 3923 square degrees). Dark-adapted and light-adapted full-field electroretinography showed no responses in 88 of 108 eyes (81.5%). Sixty-nine of 108 eyes (62.9%) showed additional findings on optical coherence tomography imaging (eg, cystoid macular edema or macular atrophy). The variant c.998 + 1G>A;p.? led to a more severe phenotype when compared with the variant c.304C>A;p.(R102S).

CONCLUSIONS AND RELEVANCE Seventeen of the *PDE6A* variants found in these patients appeared to be novel. Regarding the clinical findings, disease was highly symmetrical between the right and left eyes and visual impairment was mild or moderate in 90% of patients, providing a window of opportunity for gene therapy.

Invited Commentary page 1251

Supplemental content

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Retinitis pigmentosa (RP) is a hereditary, degenerative retinal disease that causes severe visual impairment and visual acuity loss because of progressive degeneration of primarily the rod and secondarily the cone photoreceptors. The disease manifests with early-onset nyctalopia, followed by daytime visual field defects progressing from the midperiphery to the periphery and the center. Best-corrected visual acuity typically remains relatively well preserved until macular involvement by macular edema and/or photoreceptor atrophy causes central visual acuity loss.

To date, sequence variations in 89 genes are known to be associated with RP.[1] In 1995, the gene encoding for the α subunit of the rod photoreceptor cyclic guanosine monophosphate (cGMP) phosphodiesterase (*PDE6A*) was identified as the seventh RP locus, on chromosome 5q31.2-q34 (OMIM, 180071 and 613810).[2,3] It includes 22 coding exons and encodes 859 amino acid residues. Rod photoreceptor cGMP phosphodiesterase is made of 4 subunits, the catalytic α subunit (PDE6A), the catalytic β subunit (PDE6B), and 2 inhibitory γ subunits (PDE6G). The enzyme functions to hydrolize the intracellular cytoplasmic cGMP level, which causes closure of cyclic nucleotide-gated channels, an essential step in vertebrate phototransduction.[4] The *PDE6A* gene appears to account for disease in less than 4% of families with autosomal recessive RP in North America, approximately 2% of the cases in cohorts of French and Pakistani patients, and approximately 1% of families with inherited retinal diseases in Israel.[5-8]

Sequence variations in *PDE6A* do not cause retinal dystrophy in only humans. Biallelic sequence variations in the *PDE6A* gene have been identified to cause autosomal recessive progressive retinal atrophy in the Cardigan Welsh Corgi dog.[9,10] Additionally, induced and natural mouse models have been studied for variations of the disease phenotype (eg, pace of photoreceptor degeneration) associated with different PDE6A sequence variations.[10,11] In preclinical gene supplementation trials, mice showed more effective photoreceptor cell rescue when the rod-specific transgene AAV2/8(Y733F)-Rho-Pde6α was delivered before the onset of disease. It was thus concluded that the success of therapeutic clinical trials will depend on identifying patients as early as possible to maximize the number of rods still viable and treatable with gene therapy.[12-14]

In 2012, the RD-CURE Consortium started working on a collaborative project for the clinical translation of gene therapy for patients with inherited retinal dystrophies; *PDE6A*-associated RP is one of the diseases for which a therapeutic transgene is currently evaluated in a human gene therapy trial, using recombinant adeno-associated viral vectors. The aim of the present study was to assess clinical features and genetic findings of patients with RP associated with biallelic sequence variations in the *PDE6A* gene in preparation for this gene supplementation trial.

Key Points

Question What are the clinical features and course of retinitis pigmentosa associated with biallelic sequence variations in the *PDE6A* gene?

Findings In this longitudinal cohort study of 57 adults, 17 of the *PDE6A* variants appeared to be novel. Disease was highly symmetrical between right and left eyes, and visual impairment was mild or moderate in 90% of patients.

Meaning These data suggest that *PDE6A*–retinitis pigmentosa may be amenable to gene therapy.

Methods

The study (ClinicalTrials.gov Identifier NCT02759952) was conducted in accordance with the Declaration of Helsinki, with approval from the ethics committee of the University of Tübingen. Written informed consent was obtained from all participants. Patients were recruited from the clinics for hereditary retinal degenerations at the Centre of Ophthalmology of the University of Tübingen and 12 collaborating European tertiary referral centers. Patient travel and accommodation costs were compensated.

Ophthalmological Testing

All patients were examined at the Centre for Ophthalmology of the University of Tübingen, Germany, between January 2001 and December 2019. A comprehensive ophthalmological examination was performed, including best-corrected visual acuity (BCVA) with Early Treatment Diabetic Retinopathy Study charts, a semiautomated 90° kinetic visual field (VF) exam with objects lll4e and I4e (Octopus 900 [Haag-Streit]), full-field electroretinography (ff-ERG) and multifocal electroretinography testing according to International Society for Clinical Electrophysiology of Vision standards (Espion [Diagnosys]), spectral-domain optical coherence tomography imaging (Spectralis HRA+OCT [Heidelberg Engineering]), and slitlamp and dilated fundus examinations and photography. Results of BCVA testing were converted to logarithm of the minimum angle of resolution (logMAR) visual acuity values.[15] Visual field parameters were assessed as total VF area in square degrees for both objects III4e and I4e, using the built-in software of the testing device.

Genetic Testing and Variant Classification

The patients enrolled in this study had a confirmed genetic diagnosis of *PDE6A*-associated retinitis pigmentosa. If possible, validation of homozygosity, compound heterozygosity, and biallelism was performed by familial segregation analysis, allelic cloning, or assessment of next-generation sequencing-read data. Whole-exome sequencing to exclude the presence of pathogenic variants in other genes associated with inherited retinal degeneration was performed for 23 cases. Standards and guidelines provided by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology were applied to classify the identified variants.[16] The potential pathogenicity of missense changes was assessed using 5 online prediction software tools, namely SIFT, PolyPhen-2, Mutation Taster, Mutation Assessor, and Provean.[17-21] Assessment of variants potentially affecting splicing was performed with the Alamut Genova soft-

ware version 1.4 (Sophia Genetics) using default parameters. Variant designation is based on the National Center for Biotechnology Information reference sequence for *PDE6A* (NM_000440.3; GRCh38) involving 22 coding exons.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics for Windows, version 26.0 (IBM). Normal distribution was tested using the Shapiro-Wilk test. Differences between genetic subgroups were tested using regression analysis.

Results

Fifty-seven patients from across Europe were included. Of the 57 patients (114 eyes) in this analysis, 25 (44%) were male and 32 (56%) were female. The mean (SD) age at baseline was 40 (14) years (range, 12-78 years). The mean (SD) follow-up time was 2.9 (2.1) years. Forty of the 57 patients (70%) were followed up as part of the study. The mean (SD) follow-up time was 2.9 (2.1) years.

Genetic Findings

All 57 affected individuals harbored rare and potentially disease-causing variants compatible with autosomal recessive inheritance in the *PDE6A* gene (**Table 1**). Thirty patients were homozygous for disease-causing alleles. Validation of true homozygosity by parental segregation analysis was possible in 9 cases. Twenty-seven patients were heterozygous for 2 different *PDE6A* variants each. In these, compound heterozygosity of variants could be validated in 19 cases by segregation analysis. In addition, in 2 cases, transconfiguration of variants was established by assessing independent next-generation sequencing-read data or allelic cloning, respectively.

The sequence variation spectrum included 33 different alleles, 17 of which have not been reported to date, to our knowledge. They included 12 missense variants, 8 canonical splice-site variants, 7 nonsense variants, 5 frameshift variants, and 1 in-frame deletion or insertion variants. Recurrent alleles were frequently observed: 16 patients carried the c.304C>A;p.(R102S) variant on 1 or both alleles, making it the most common allele in the cohort (23 alleles), followed by c.2053G>A;p./(V685M) (12 alleles). The splice-site variant c.998 + 1G>A;p.? accounted for 14 alleles in the cohort. However, this high number is associated with 6 siblings from the same family having homozygosity for this variant (**Figure 1**). All variants were classified according to their pathogenicity based on the ACMG guidelines (**Table 2**).[16]

Ophthalmological Findings

The mean (SD) BCVA was 0.43 (0.48) logMAR (range, −0.10 to 2.30; 114 eyes; Snellen equivalent, 20/50; range, hand movement to 20/16). Visual impairment was mild in 15 of 114 eyes (13.2%), moderate in 19 of 114 eyes (16.7%), and severe in 7 of 114 eyes (6.1%), and 4 of 114 eyes (3.5%) met the criteria for legal blindness as defined by the World Health Organization with respect to BCVA. The BCVA findings were highly symmetrical in right and left eyes (R^2, 0.786).

Kinetic visual field testing with object III4e of Goldmann allowed for evaluation in 107 of 114 eyes (93.9%). The median visual field area in these 107 eyes was 660 (5th-95th percentiles, 76-11 019; 25th-75th percentiles, 255-3923) square degrees (**Figure 2**). In 3 eyes, object III4e was not recognized. Kinetic visual field testing obtained with object I4e allowed for evaluation in 92 of 114 eyes (80.7%). The median visual field area in these 92 eyes was 150 (range, 2-2833) square degrees (Figure 2). In 16 eyes, object I4e was not recognized. Kinetic visual field measurements were highly symmetrical in right and left eyes (R^2, 0.951). Progression of visual field defects with increasing age or disease duration is shown in Figure 2.

Optical coherence tomography (OCT) images were available in 108 of 114 eyes (94.7%). As is typical for RP, OCT imaging revealed thinning of the outer retinal layers from the periphery to the center of the retina (macula or fovea), with disruption or loss of the ellipsoid zone in advanced disease. In the cohort, 69 of 108 eyes (63.9%) showed additional findings on OCT: cystoid macular edema (CME) in 27 of 108 eyes (25.0%), CME and macular atrophy in 2 of 108 (1.9%), macular atrophy in 18 of 108 (16.7%), epiretinal membrane (ERM) in 8 of 108 (7.4%), ERM with traction in 6 of 108 (5.6%), ERM with a lamellar hole in 2 of 108 (1.9%), an isolated lamellar hole in 1 of 108 (1%), a full-thickness macular hole in 1 of 108 (0.9%), and posterior staphyloma in 4 of 108 (3.7%) (eFigure 1 in the Supplement).

Full-field ERG findings were available in 108 of 114 eyes (94.7%). In 4 of 108 eyes (3.7%), dark-adapted and light-adapted ff-ERG responses were within normal limits. In 8 of 108 (7.4%) each, light-adapted ff-ERG showed some measurable, albeit subnormal responses, whereas dark-adapted ff-ERG showed either residual or no responses. In 88 of 108 (81.5%), responses for both dark-adapted and light-adapted ff-ERGs were absent. Multifocal ERG findings were available in 90 of 114 eyes (78.9%). In 53 of 90 eyes (88%), multifocal electroretinography showed no responses.

We also evaluated whether the clinical findings differed between patients harboring 1 of the common disease-causing *PDE6A* variants in a homozygous state, comparing the c.304C>A;p.(R102S) (group 1), and c.998 + 1G>A;p.? (group 2). Seven patients each were homozygous for either the variant c.304C>A;p.R(102S) or the variant c.998 + 1G>A;p.?. Visual field maps, OCT, and fundus autofluorescence findings of right eyes are shown in eFigure 1 in the Supplement. The respective groups (1 and 2) differed in their mean (SD) ages at baseline (46 [17] vs 38 [6] years). The mean (SD) visual acuity was worse in group 2 when compared with group 1 (0.50 [0.37] vs 0.19 [0.27] logMAR; Snellen equivalent: 20/60 vs 20/30). Similarly, the mean (SD) VF area was worse in group 2 when compared with group 1 (1178 [1304] vs 5548 [5040] square degrees). When corrected for age, these differences were statistically significant. The BCVA mean difference was −0.31 (95% CI, −0.70 to 0.07; P = .01), and the VF area mean difference was 4370 (95% CI, −644 to 9384; P = .003).

Table 1. Genetic and Clinical Findings[a]

Patient No.	Key	Decade of life	Variant 1		Variant 2			Visual acuity, logMAR		Visual acuity, Snellen		Visual field object III4e, square degrees	
			Sequence variation	HGMD[b]	Sequence variation	HGMD[b]	Segregation	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye
34	ARRP 322	5	c.304C>A/p.R102S	CM994742	c.304C>A/p.R102S	CM994742	No	−0.1	0	20/16	20/20	1520	3249
50	SRP 1101	2	c.1957C>T/p.R653*	CM161562	c.998 + 2T>G/p.?	Novel	Yes	0	0	20/20	20/20	3538	3540
110	ARRP 415	3	c.304C>A/p.R102S	CM994742	c.1689C>A/p.H563Q	CM1825637	No	0	0	20/20	20/20	7522	6252
102	ARRP 150	3	c.304C>A/p.R102S	CM994742	c.304C>A/p.R102S	CM994742	No	0	0	20/20	20/20	13 123	12 422
49	SRP 1064	3	c.304C>A/p.R102S	CM994742	c.2053G>A/p.V685M	CM106644	No	0	0	20/20	20/20	10 889	10 754
101	ARRP 150	3	c.304C>A/p.R102S	CM994742	c.304C>A/p.R102S	CM994742	No	0	0	20/20	20/20	12 569	11 105
36	ARRP 324	4	c.1705C>A/p.Q569K	CM994743	c.1705C>A/p.Q569K	CM994743	No	0	0	20/20	20/20	3914	6895
104	SRP 578	4	c.1684C>T/p.R562W	CM1510052	c.2053G>A/p.V685M	CM106644	Yes	0	0	20/20	20/20	NA	NA
111	ARRP 410	2	c.1927-1G>T/p.?	Novel	c.2332_2335del/p.D778Lfs*42	Novel	No	0.1	0	20/25	20/20	6074	8234
48	SRP 1024	4	c.1683G>A/p.W561*	CM950913	c.1263 + 1G>A/p.?	Novel	Yes	0.1	0	20/25	20/20	2070	2599
23	ARRP 291	3	c.998 + 1G>A/p.?	CS994749	c.998 + 1G>A/p.?	CS994749	Yes	0.6	0	20/80	20/20	3759	4526
6	SRP 976	4	c.1620 + 1G>A/p.?	Novel	c.1705C>A/p.Q569K	CM994743	Yes	0	0.1	20/20	20/25	4246	5036
3	SRP 150	5	c.304C>A/p.R102S	CM994742	c.1689C>A/p.H563Q	CM1825637	Yes	0	0.1	20/20	20/25	375	220
17	ARRP 128	2	c.769C>T/p.R257*	CM066961	c.769C>T/p.R257*	CM066961	Yes	0.1	0.1	20/25	20/25	11 132	10 740
8	SRP 977	4	c.998 + 1G>A/p.?	CS994749	c.998 + 1G>A/p.?	CS994749	No	0.1	0.1	20/25	20/25	795	777
105	SRP 125	4	c.304C>A/p.R102S	CM994742	c.1966G>T/p.E656*	CM1717824	No	0.1	0.1	20/25	20/25	NA	NA
47	ARRP 322	5	c.304C>A/p.R102S	CM994742	c.304C>A/p.R102S	CM994742	No	0.1	0.1	20/25	20/25	336	355
29	ARRP 316	4	c.304C>A/p.R102S	CM994742	c.2053G>A/p.V685M	CM106644	Yes	0.2	0.1	20/30	20/25	244	203
46	SRP 1009	5	c.304C>A/p.R102S	CM994742	c.1705C>A/p.Q569K	CM994743	Yes	0.2	0.1	20/30	20/25	440	464
109	SRP 1080	6	c.1610T>C/p.I537T	Novel	c.743T>A/p.V248D	Novel	No	0.4	0.1	20/50	20/25	171	142
14	SRP 159	4	c.1862T>G/p.L621R	Novel	c.2053G>A/p.V685M	CM106644	Yes	0.1	0.2	20/25	20/30	1008	1528
15	SRP 894	4	c.304C>A/p.R102S	CM994742	c.2053G>A/p.V685M	CM106644	Yes	0.2	0.2	20/30	20/30	1728	2226
21	ARRP 290	4	c.1957C>T/p.R653*	CM161562	c.1957C>T/p.R653*	CM161562	No	0.2	0.2	20/30	20/30	434	426
12	ARRP 291	5	c.998 + 1G>A/p.?	CS994749	c.998 + 1G>A/p.?	CS994749	Yes	0.2	0.2	20/30	20/30	163	187
16	SRP 552	5	c.304C>A/p.R102S	CM994742	c.2053G>A/p.V685M	CM106644	Yes	0.2	0.2	20/30	20/30	2383	2593
30	ARRP 316	3	c.304C>A/p.R102S	CM994742	c.2053G>A/p.V685M	CM106644	Yes	0.3	0.2	20/40	20/30	86	107
26	ARRP 291	4	c.998 + 1G>A/p.?	CS994749	c.998 + 1G>A/p.?	CS994749	Yes	0.3	0.2	20/40	20/30	1837	1585
27	SRP 829	7	c.304C>A/p.R102S	CM994742	c.304C>A/p.R102S	CM994742	No	0.3	0.2	20/40	20/30	1680	3923
41	SRP 986	6	c.612del/p.K205Rfs*16	CD192430	c.612del/p.K205Rfs*16	CD192430	No	0.5	0.2	20/60	20/30	187	117
33	ARRP 307	7	c.1705C>A/p.Q569K	CM994743	c.1065 + 2T>A/p.?	Novel	Yes	0.6	0.2	20/80	20/30	70	124
42	SRP 1089	2	c.1926 + 1G>A/p.?	Novel	c.1926 + 1G>A/p.?	Novel	No	0.3	0.3	20/40	20/40	1967	2961
5	ARRP 399	3	c.769C>T/p.R257*	CM066961	c.769C>T/p.R257*	CM066961	No	0.3	0.3	20/40	20/40	6737	7446

(*continued*)

Table 1. Genetic and Clinical Findings[a] (continued)

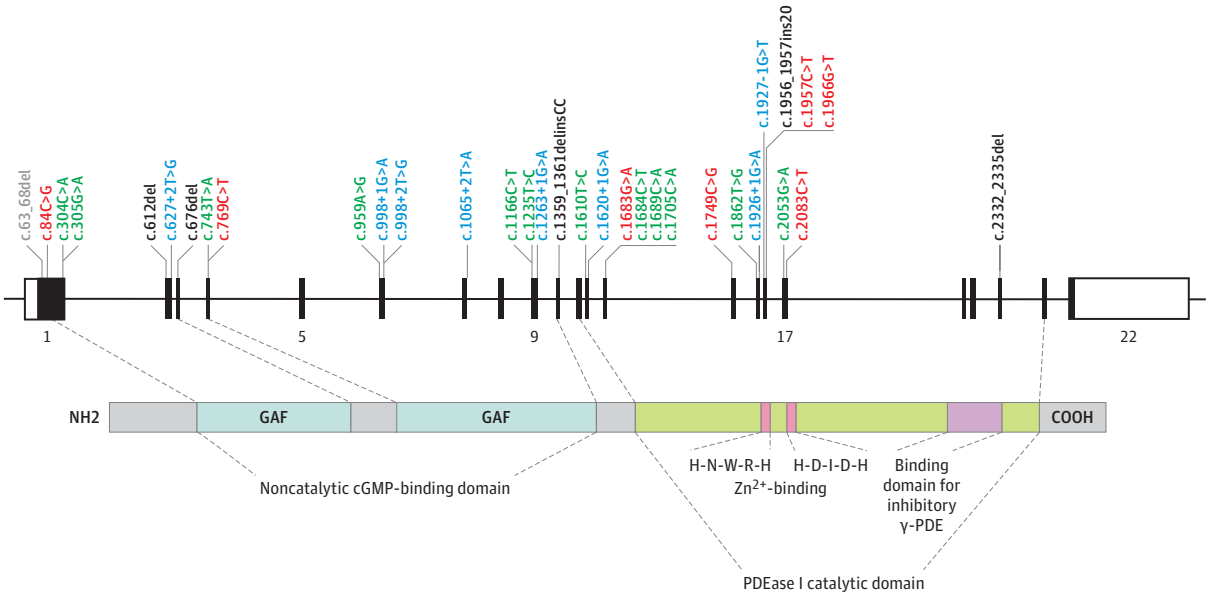
Patient No.	Key	Decade of life	Variant 1		Variant 2		Segregation	Visual acuity, logMAR		Visual acuity, Snellen		Visual field object III4e, square degrees	
			Sequence variation	HGMD[b]	Sequence variation	HGMD[b]		Right eye	Left eye	Right eye	Left eye	Right eye	Left eye
19	SRP 905	5	c.1957C>T/p.R653*	CM161562	c.2332_2335del/ p.D778Lfs*42	Novel	Yes	0.3	0.3	20/40	20/40	562	1299
4	ARRP 399	3	c.769C>T/p.R257*	CM066961	c.769C>T/p.R257*	CM066961	No	0.4	0.3	20/50	20/40	8937	7597
45	ARRP 341	7	c.959A>G/p.D320G	Novel	c.1749C>G/p.Y583*	CM950914	No	0.4	0.3	20/50	20/40	660	523
1	ARRP 254	8	c.304C>A/p.R102S	CM994742	c.304C>A/p.R102S	CM994742	No	1	0.3	20/200	20/40	140	107
39	SRP 928	5	c.1957C>T/p.R653*	CM161562	c.1956_1957ins20/ p.R653*	Novel	Yes	0.4	0.4	20/50	20/50	702	683
20	ARRP 290	4	c.1957C>T/p.R653*	CM161562	c.1957C>T/p.R653*	CM161562	No	0.5	0.4	20/60	20/50	374	267
108	SRP 973	4	c.2083C>T/p.Q695*	Novel	c.1065 + 2T>A/p.?	Novel	No	1	0.4	20/200	20/50	444	751
28	ARRP 314	4	c.305G>A/p.R102H	CM994741	c.305G>A/p.R102H	CM994741	No	0.2	0.5	20/30	20/60	753	576
7	ARRP 219	6	c.304C>A/p.R102S	CM994742	c.304C>A/p.R102S	CM994742	No	0.2	0.5	20/30	20/60	8076	9060
103	ARRP 223	3	c.1166C>T/p.P389L	CM112500	c.1166C>T/p.P389L	CM112500	No	0.3	0.5	20/40	20/60	7812	9453
31	ARRP 307	6	c.1705C>A/p.Q569K	CM994743	c.1065 + 2T>A/p.?	Novel	Yes	0.5	0.5	20/60	20/60	221	167
2	SRP 564	4	c.63_68del/ p.K21_Y23delinsN	Novel	c.1926 + 1G>A/p.?	Novel	Yes	0.3	0.7	20/40	20/100	324	305
107	ARRP 320	3	c.769C>T/p.R257*	CM066961	c.769C>T/p.R257*	CM066961	No	0.7	0.7	20/100	20/100	6644	6108
106	SRP 764	7	c.676del/p.H226Tfs*2	CD140529	c.2053G>A/p.V685M	CM106644	No	0.9	0.7	20/160	20/100	255	322
24	ARRP 291	5	c.998 + 1G>A/p.?	CS994749	c.998 + 1G>A/p.?	CS994749	Yes	1	0.7	20/200	20/100	496	365
44	ARRP 317	6	c.627 + 2T>G/p.?	Novel	c.627 + 2T>G/p.?	Novel	No	0.5	0.8	20/60	20/125	318	292
11	ARRP 60	5	c.2053G>A/p.V685M	CM106644	c.2053G>A/p.V685M	CM106644	Yes	0.7	0.9	20/100	20/160	163	204
22	ARRP 291	5	c.998 + 1G>A/p.?	CS994749	c.998 + 1G>A/p.?	CS994749	Yes	0.7	0.9	20/100	20/160	592	568
25	ARRP 291	4	c.998 + 1G>A/p.?	CS994749	c.998 + 1G>A/p.?	CS994749	Yes	1	1	20/200	20/200	394	441
10	ARRP 60	5	c.2053G>A/p.V685M	CM106644	c.2053G>A/p.V685M	CM106644	Yes	1.2	1	20/300	20/200	129	90
32	ARRP 307	7	c.1705C>A/p.Q569K	CM994743	c.1065 + 2T>A/p.?	Novel	Yes	1.1	1.1	20/250	20/250	49	33
35	ARRP 323	5	c.84C>G/p.Y28*	Novel	c.84C>G/p.Y28*	Novel	No	1.2	1.2	20/300	20/300	436	583
40	SRP 929	6	c.305G>A/p.R102H	CM994741	c.1359_1361delinsCC/ p.V454Qfs*5	Novel	Yes	1.3	1.3	20/400	20/400	NA	9
18	SRP 250	6	c.1235T>C/p.F412S	Novel	c.1966G>T/p.E656*	CM1717824	Yes	1/35 at 1 m	HM	1/35 at 1 m	HM	218	20
38	ARRP 306	7	c.1705C>A/p.Q569K	CM994743	c.1705C>A/p.Q569K	CM994743	No	HM	HM	HM	HM	NA	NA

Abbreviations: ARRP, autosomal recessive retinitis pigmentosa; CD, deletion, coding; CM, substitution, coding; CS, splicing; HGMD, Human Gene Mutation Database; HM, hand movements; NA, not applicable; SRP, sporadic retinitis pigmentosa.

[a] Patients are sorted by age.

[b] The codes CD, CM, and CS refer to variants in the HGMD Accession number definitions.

Figure 1. Genomic and Protein Structure of PDE6A and Location of Variants



The exon and intron organization is shown to scale at the top; the polypeptide and its functional domains, below. Blue indicates splice-site variants; black, frameshift variants; red, nonsense variants; gray, in-frame deletion or insertion; green, missense variants. cGMP indicates cyclic guanosine monophosphate; GAF, cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA; PDE and PDEase, phosphodiesterase.

Discussion

In this study, we examined 57 patients from across Europe with RP associated with pathogenic, biallelic variants in the *PDE6A* gene. Given that *PDE6A*-associated RP is such a rare genetic subtype of RP, the size of this cohort is uniquely large.[6] From comprehensive genetic analyses in association with this study of *PDE6A* in patients with a clinical diagnosis of RP, we conclude that there is a frequency of *PDE6A*-associated RP of 1.6% in Germany (1100 patients residing in Germany, of whom 17 were found to harbor biallelic variants in *PDE6A* [data not shown]).

Clinical Findings

All patients with *PDE6A*-associated RP in our study exhibited highly symmetrical findings typical for RP with respect to visual acuity, visual field, and other modalities. When comparing BCVA with VF area in terms of symmetry, the VF area was more symmetrical in right and left eyes. Disease symmetry presents a strong case for using the untreated eye as the control eye in an interventional study with small patient numbers. The untreated eye may serve as a control to assess possible negative adverse effects as well as positive outcomes of therapy (such as gene supplementation therapy; ie, the preservation of VF area or the deceleration of VF area loss).

The greatest (numberwise) loss in VF area was approximately −7900 square degrees in a 23-year-old patient with a VF area of 8900 square degrees at baseline 2 years earlier. This change reflects the natural course of disease, characterized by a slow progression of VF defects before young adulthood, typically followed by a sudden loss of large or larger VF areas in young adulthood and subsequent slow progression of VF defects the smaller the remaining VF area gets.

Kinetic visual field testing with object III4e allowed for evaluation in more than 90% of eyes. However, kinetic visual field testing is a set of psychophysical examinations with advantages but also limitations. The limitations are that the condition of the patient affects the results of the examination, in that a patient who is alert will achieve better results than a patient with fatigue, and the same is true for the technician performing the examination and encouraging the patient. Thus, VF findings can vary between visits, and comparisons between patients, as well as within the same patient at different points, may yield misleading results. However, given the remarkable symmetry of the VF area in right and left eyes that we observed in this cohort and the fact that VF area is a measure of visual function rather than an anatomical end point, we judge VF testing as a suitable marker and end point for a phase I or II safety and efficacy trial.

Macular OCT findings may well limit a patient's eligibility to participate in a gene therapy trial, since the preferred injection site for the therapeutic vector to date is the central retina. Generally, macular findings in patients with RP include cystoid macular edema in 6% to 25%, ERM in 1% to 27%, and macular holes in 1% to 5% of patients with RP.[30-32] In our cohort, CME was present in 25%, ERM in 7%, and macular holes in 4%. Thus, the frequency of macular OCT findings in *PDE6A*-associated RP was similar to that in the general population of patients with RP. Mild CME may not be as much of a limitation for a patient to participate in a gene therapy trial, and the same applies to mild ERMs without traction. Yet, extensive

Table 2. *PDE6A* Sequence Variants Identified in This Study

Nucleotide (NM_000440.3)	PDE6A protein (NP_000431.2)	Initial known description	Localization of missense variants	Consensus prediction for missense variants[a]	American College of Medical Genetics and Genomics		gnomAD minor allele frequency
					Categories[b]	Prediction, final classification	
Splice site variants							
c.627 + 2T>G	NA (p.D159_E209del)[c]	This study	NA	NA	PVS1; PM2	Likely pathogenic	None
c.998 + 1G>A	NA (p.I313Sfs*4)[c]	Dryja et al,[5] 1999	NA	NA	PVS1; PM2; PP1	Pathogenic	0.00002830
c.998 + 2T>G	NA (p.I313Sfs*4)[c]	This study	NA	NA	PVS1; PM2; PM3	Pathogenic	None
c.1065 + 2T>A	NA (p.N334Ffs*5)[c]	Khateb et al,[6] 2019	NA	NA	PVS1; PM2; PM3	Pathogenic	None
c.1263 + 1G>A	NA (p.K372_E421del)[c]	This study	NA	NA	PVS1; PM2; PM3	Pathogenic	None
c.1620 + 1G>A	NA (p.Q492_E540del)[c]	This study	NA	NA	PVS1; PM2; PM3	Pathogenic	0.000007958
c.1926 + 1G>A	NA (p.S614Afs*2)[c]	This study	NA	NA	PVS1; PM2; PM3	Pathogenic	0.000003978
c.1927-1G>T	NA (p.S643Efs*13)[c]	This study	NA	NA	PVS1; PM2	Likely pathogenic	None
Frameshift deletions and insertions							
c.612del	p.K205Rfs*16	Jespersgaard et al,[22] 2019	NA	NA	PM2; PVS1	Likely pathogenic	None
c.676del	p.H226Tfs*2	Glöckle et al,[23] 2014[d]	NA	NA	PM2; PVS1	Likely pathogenic	None
c.2332_2335del	p.D778Lfs*42	This study	NA	NA	PM2; PVS1; PM3	Pathogenic	0.00002475
c.1359_1361 delinsCC	p.V454Qfs*5	This study	NA	NA	PM2; PVS1; PM3	Pathogenic	None
c.1956_1957ins20	p.R653*	This study			PM2; PVS1; PM3	Pathogenic	None
Nonsense variants							
c.84C>G	p.Y28*	This study	NA	NA	PM2; PVS1	Likely pathogenic	0.000003980
c.769C>T	p.R257*	Riazuddin et al,[24] 2006	NA	NA	PM2; PVS1; PM3	Pathogenic	0.00003890
c.1683G>A	p.W561*	Huang et al,[2] 1995	NA	NA	PM2; PVS1; PM3	Pathogenic	0.000003977
c.1749C>G	p.Y583*	Huang et al,[2] 1995	NA	NA	PM2; PVS1	Likely pathogenic	None
c.1957C>T	p.R653*	Perez-Carro et al,[25] 2016	NA	NA	PM2; PVS1; PM3	Pathogenic	0.00002787
c.1966G>T	p.E656*	Soens et al,[26] 2017	NA	NA	PM2; PVS1; PM3	Pathogenic	0.000007079
c.2083C>T	p.Q695*	This study	NA	NA	PM2; PVS1	Likely pathogenic	0.000003981
In-frame deletion and insertion							
c.63_68del	p.K21_Y23delinsN	This study	NA	NA	PM2; PM3; PM4	Likely pathogenic	None
Missense variants							
c.304C>A	p.R102S	Dryja et al,[5] 1999	GAF domain	Damaging	PM2; PM3; PM5; PS3	Pathogenic	0.0001556
c.305G>A	p.R102H	Dryja et al,[5] 1999	GAF domain	Damaging	PM2; PM3; PM5; PS3	Pathogenic	0.00002122
c.743T>A	p.V248D	This study	GAF domain	Damaging	PM2	VUS	None
c.959A>G	p.D320G	This study	GAF domain	Damaging	PM2	VUS	None
c.1166C>T	p.P389L	Collin et al,[27] 2011	GAF domain	Damaging	PM2; PS3	Likely pathogenic	0.000007954
c.1235T>C	p.F412S	This study	GAF domain	Damaging	PM2; PM3	VUS	None
c.1610T>C	p.I537T	This study	Catalytic domain	Damaging	PM1; PM2	VUS	None
c.1684C>T	p.R562W	Sothilingam et al,[11] 2015[d]	Catalytic domain	Damaging	PM1; PM2; PM3; PS3	Pathogenic	0.00001193
c.1689C>A	p.H563Q	Birtel et al,[28] 2018	Catalytic domain	Damaging	PM1; PM2; PM3	Likely pathogenic	0.000007954
c.1705C>A	p.Q569K	Dryja et al,[5] 1999	Catalytic domain	Damaging	PM1; PM2; PM3	Likely pathogenic	0.0001344
c.1862T>G	p.L621R	This study	Catalytic domain	Damaging	PM1; PM2; PM3	Likely pathogenic	None
c.2053G>A	p.V685M	Corton et al,[29] 2010	Catalytic domain	Damaging	PM1; PM2; PM3; PS3	Pathogenic	0.00004957

Abbreviations: NA, not applicable; GAF, cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA; gnomAD, Genome Aggregation Database; PM, pathogenic, moderate; PP, pathogenic, supporting; PS, pathogenic, strong; PVS, pathogenic, very strong; VUS, variant of uncertain significance.

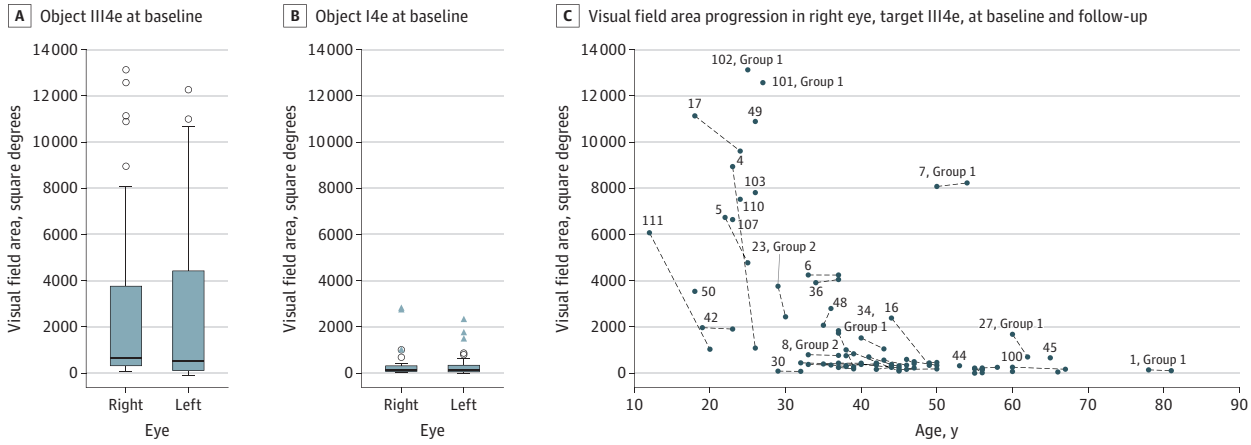
[a] The American College of Medical Genetics and Genomics categories PM, PP, PS, and PVS are further subdivided and ranked from 1 to 6 for PM, with lower numbers higher in influence. It is the state-of-the-art standard classification of genetic variants.[16]

[b] Consensus of 4 or more prediction algorithms.

[c] Consequence of putative exon skipping.

[d] The patient described is the same as in the present study.

Figure 2. Visual Field Data



A and B, Visual field area at baseline for objects III4e and I4e. C, Visual field area of right eyes at baseline, and, if available, at last follow-up. Note the drop in visual field area that is most rapid in the second and third decades of life.

CME, as well as marked ERM with traction and any type of macular hole, would be expected to increase the risk of complications attributable to the surgical procedure, irrespective of the investigational new drug, thus having a potential effect on safety and efficacy observations.

Among the most frequent variants found in our cohort, the variant c.998 + 1G>A;p.? led to a more severe phenotype with respect to VF and BCVA when present in a homozygous state and when compared with individuals homozygous for the c.304C>A;p.(R102S) variant. The third most frequent variant c.2053G>A;p.(V685M) was mostly present in a (compound) heterozygous state, making it difficult to compare this genotype with the 2 other most frequent variants mentioned with respect to the severity of the phenotype. Our clinical findings are thus in line with the fact that c.998 + 1G>A;p.? is considered a null allele, in contrast with c.304C>A;p.(R102S).

Genetic Findings of Pathogenicity of Identified Variants

Because we were unable to validate biallelism in all of the patients, the pathogenicity of single variants rather than genotypes will be discussed. Of the 33 putatively pathogenic variants identified in this study, 7 are nonsense variants and 5 are frameshift variants. These are considered to constitute loss-of-function alleles. Furthermore, we identified 8 canonical splice variants, 7 of which affect invariable donor sites and 1 of which affects an invariable acceptor site.

Because nonsense, canonical splice site, and frameshift variants have a strong weight in the ACMG scoring system, these classes of variants are consequently classified either as pathogenic or likely pathogenic (Table 2). On the other hand, missense variants that lack segregation data and functional analyses to support a damaging outcome are always classified as variants of uncertain significance per the ACMG guidelines. The potential pathogenicity of the missense variants affecting codon 102 and 389 of the *PDE6A* gene (c.304C>A;p.R102S, c.305G>A;p.R102H, and c.1166C>T;p.P389L) is supported by the fact that corresponding sequence variations in *PDE6C* have been shown to have a causal association with *PDE6C*-associated autosomal recessive achromatopsia.[33] Their effect on PDE6 function has further been substantiated by functional in vitro studies performed on the catalytic subunit of cone photoreceptor phosphodiesterase PDE6C, the PDE6 paralog in cone photoreceptors.[33] Grau and coworkers[33] demonstrated that the corresponding *PDE6C* missense variants c.310C>T;p.R104W and c.1172C>T;p.P391L led to a highly significant reduction in phosphodiesterase activity to almost baseline levels. Since codons 104 and 391 of *PDE6C* are homologous to codons 102 and 389 of *PDE6A*, the results obtained from functional studies performed on PDE6C can be transferred to PDE6A. Evidence for the pathogenicity of the missense variants c.1684C>T;p.R562W and c.2053G>A;p.V685M is derived from the functional analysis of *Pde6a* mouse models: Sothilingam and coworkers[11] showed that homozygous (R562W/R562W and V685M/V685M) and compound heterozygous (R562W/V685M) *Pde6a* mutant animals expressed only residual amounts of Pde6a protein, resulting in photoreceptor cell death. Functional studies or animal models are not available for the remaining 7 missense variants identified in this study. Yet, the respective amino acid residues are located either between cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA (GAF) domains (c.743T>A;p.V248D), within the second GAF domain (c.959A>G;p.D320G, c.1235T>C;p.F412S), or in the catalytic domain of PDE6A (c.1610T>C;p.I537T, c.1689C>A;p.H563Q, c.1705C>A;p.Q569K, c.1862T>G;p.L621R). Specifically, the variants c.1684C>T;p.R562W and c.1689C>A;p.H563Q affect the Zn^{2+}-binding motif, which is invariant among phosphodiesterases. All affected amino acid residues are highly conserved among vertebrates, not only for PDE6A, but also within PDE6B and PDE6C (eFigure 2 in the Supplement). Consequently, in silico assessment using 5 different prediction tools predicted damaging outcomes on protein function for all 7 variants (Table 2). However, without functional data supportive of damaging outcomes, their pathogenicity cannot be unequivocally proven.

Last, we have identified a novel in frame deletion/insertion variant, c.63_68del;p.(K21_Y23delinsN). It localizes near the N-terminus of PDE6A, upstream of the first GAF domain, and affects 3 amino acid residues that are conserved among vertebrate PDE6A and partially conserved among human PDE6B and PDE6C polypeptides. It has been shown that dimerization of PDE6 is mediated by multiple regions in the N-terminal domain.[34] We therefore hypothesize that the c.63_68del;p.(K21_Y23delinsN) variant may impair dimerization, thereby leading to loss of function of PDE6A.

Limitations

True homozygosity and the absence of pathogenic variants in other genes that are known to cause inherited retinal dystrophies are key eligibility criteria for inclusion in a gene therapy trial. One limiting factor with respect to the clinical aspect (and course) of *PDE6A*-associated RP and subretinal gene therapy in our cohort may be macular pathology, which would increase the risk of complications attributable to the surgical procedure if the injection site is the central retina. However, given that PDE6 is rod specific, from a mechanistical standpoint, it would be more promising to treat regions outside the macula with high rod density early in life.

Conclusions

In summary, we observed and described the genetic and ophthalmologic characteristics in 57 patients with RP associated with pathogenic biallelic variants in the *PDE6A* gene. Regarding the genetic findings, 17 of the *PDE6A* variants found in these patients appeared to be novel. Regarding the clinical findings, disease was highly symmetrical between right and left eyes and visual impairment was mild or moderate in 90% of patients, providing a window of opportunity for gene therapy. These data suggest that individuals with *PDE6A*-associated RP may be eligible for gene therapy.

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Statistical analysis: Kuehlewein.
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