The Spectrum of Structural and Functional Abnormalities in Female Carriers of Pathogenic Variants in the \textit{RPGR} Gene

Mays Talib,\(^1\) Mary J. van Schooneveld,\(^2\) Caroline Van Cauwenbergh,\(^3,4\) Jan Wijnholds,\(^1\) Jacoline B. ten Brink,\(^5\) Ralph J. Florijn,\(^5\) Nicole E. Schalij-Delfos,\(^1\) Gislin Dagnelie,\(^6\) Maria M. van Genderen,\(^7\) Elfride De Baere,\(^4\) Magda A. Meester-Smoor,\(^8\) Julie De Zaytijd,\(^3\) Frans P. M. Cremers,\(^9\) L. Ingeboor van den Born,\(^10\) Alberta A. Thiadens,\(^8\) Carel B. Hoyng,\(^11\) Caroline C. Klaver,\(^8,11,12\) Bart P. Leroy,\(^3,4,13\) Arthur A. Bergen,\(^5,14\) and Camiel J. F. Boon\(^1,2\)

\(^1\)Department of Ophthalmology, Leiden University Medical Center, Leiden, The Netherlands
\(^2\)Department of Ophthalmology, Amsterdam UMC, University of Amsterdam, The Netherlands
\(^3\)Department of Ophthalmology, Ghent University and Ghent University Hospital, Ghent, Belgium
\(^4\)Center for Medical Genetics, Ghent University and Ghent University Hospital, Gent, Belgium
\(^5\)Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, The Netherlands
\(^6\)Wilmer Eye Institute, Johns Hopkins University, Baltimore, Maryland, United States
\(^7\)Bartiméus, Diagnostic Centre for Complex Visual Disorders, Zeist, The Netherlands
\(^8\)Department of Ophthalmology, Erasmus Medical Center, Rotterdam, The Netherlands
\(^9\)Department of Human Genetics and Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands
\(^10\)Rotterdam Eye Hospital, Rotterdam, The Netherlands
\(^11\)Department of Ophthalmology, Radboud University Medical Center, Nijmegen, The Netherlands
\(^12\)Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands
\(^14\)The Netherlands Institute for Neuroscience (NIN-KNAW), Amsterdam, The Netherlands

Correspondence: Camiel J. F. Boon, Leiden University Medical Center, Department of Ophthalmology, Albinusdreef 2, Leiden 2333 ZA, The Netherlands; c.j.f.boon@lumc.nl.

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**PURPOSE.** The purpose of this study was to investigate the phenotype and long-term clinical course of female carriers of \textit{RPGR} mutations.

**METHODS.** This was a retrospective cohort study of 125 heterozygous \textit{RPGR} mutation carriers from 49 families.

**RESULTS.** Eighty-three heterozygotes were from retinitis pigmentosa (RP) pedigrees, 37 were from cone-/cone-rod dystrophy (CORD) pedigrees, and 5 heterozygotes were from pedigrees with mixed RP/CORD or unknown diagnosis. Mutations were located in exon 1-14 and in ORF15 in 42 of 125 (34%) and 83 of 125 (66%) subjects, respectively. The mean age at the first examination was 34.4 years (range, 2.1 to 86.0 years). The median follow-up time in heterozygotes with longitudinal data \((n = 62)\) was 12.2 years (range, 1.1 to 52.2 years). Retinal pigmentary changes were present in 73 (58%) individuals. Visual symptoms were reported in 51 (40%) cases. Subjects with both symptoms and pigmentary fundus changes were older than the other heterozygotes \((P = 0.01)\) and had thinner foveal outer retinas \((P = 0.006)\). Complete expression of the RP or CORD phenotype was observed in 29 (23%) heterozygotes, although usually in milder forms than in affected male relatives. Best-corrected visual acuity (BCVA) was <20/40 and <20/400 in at least one eye in 45 of 116 (39%) and 11 of 116 (9%) heterozygotes, respectively. Myopia was observed in 74 of 101 (73%) subjects and was associated with lower BCVA \((P = 0.006)\). Increasing age was associated with lower BCVA \((P = 0.002)\) and decreasing visual field size \((P = 0.012; I4e isopter)\).

**CONCLUSIONS.** \textit{RPGR} mutations lead to a phenotypic spectrum in female carriers, with myopia as a significantly aggravating factor. Complete disease expression is observed in some individuals, who may benefit from future (gene) therapeutic options.

Keywords: retinitis pigmentosa GTPase regulator, retinitis pigmentosa, gene therapy, disease progression

Pathogenic variants in the \textit{RPGR} gene are associated with a wide variety of severe X-linked retinal degenerations in male patients, including retinitis pigmentosa (RP3), cone-rod dystrophy (CORD), and isolated cone dystrophy (COD).\(^{1-14}\) Female carriers of X-linked RP usually experience no or mild symptoms and signs of ocular involvement.\(^{5,6}\) However, variable degrees of disease expression have been reported,\(^{2,7-10}\) and a few studies have shown a correlation between fundus...
features and retinal function measures. A pathognomonic retinal feature in carriers of X-linked RP is the tapetal-like reflex, a golden metallic-luster sheen of the perimacular retina, but this is often absent or only seen in a subset of female carriers of RPGR mutations.

Although a wide spectrum of severity has been described in female carriers from RP pedigrees, female carriers from RPGR-associated COD/CORD pedigrees usually display no or mild fundus abnormalities. Genotype–phenotype correlations in female carriers of pathogenic variants in RPGR, linking the genotype to the degree of disease expression, have rarely been described, as large cohorts of female heterozygotes are scarce.

The recent initiation of a human gene therapy trial for RPGR-associated retinal dystrophies in male patients offers a promising therapeutic perspective (ClinicalTrials.gov number: NCT03116113). An optimal insight into the clinical characteristics and variability of disease expression in female carriers of pathogenic RPGR variants becomes increasingly important to further understand the phenotypic spectrum. The purpose of this study was to expand our knowledge of the initial and longitudinal clinical characteristics of a large cohort of female carriers of pathogenic RPGR variants and to assess whether upcoming RPGR gene therapy trials may consider the inclusion of affected female carriers.

Materials and Methods

Study Population
This study identified female carriers of disease-causing RPGR variants who underwent at least one clinical examination. Inclusion criteria were a molecular confirmation of an RPGR mutation or an obligate carrier status. Additionally, two subjects had not undergone molecular genetic analysis, but an RPGR mutation was molecularly confirmed in a first-degree relative, along with the presence of a tapetal-like reflex in the female subject, which is considered a pathognomonic sign of XLRP carriership. Obligate carriers were defined as daughters of an affected father, mothers of at least two affected sons, or mothers of one affected son along with at least one other affected male patient or confirmed carrier in the family, to exclude the possibility of a de novo mutation.

Heterozygotes were collected from the database (Delleman database) for genetic eye diseases at the Academic Medical Center (AMC) in Amsterdam, from various other Dutch medical centers within the framework of the RD5000 consortium, and from the Ghent University Hospital in Belgium. Of the 125 included female carriers of pathogenic RPGR variants, 21 were from a large Dutch pedigree that has been previously described, and additional follow-up data since the publication of that study were available in three subjects.

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center for the Dutch subjects and by the Ethics Committee of Ghent University Hospital for the Belgian subjects and adhered to the tenets of the Declaration of Helsinki. Dutch participants provided informed consent for the use of their clinical data for research purposes. In Dutch subjects who were no longer traceable, the subject-specific information was deleted after data collection and the subjects were pseudonymized. For Belgian subjects, the local Ethics Committee waived the need for informed consent on the condition of pseudonymization.

Genetic Analysis

Of the 125 heterozygotes, 108 had received molecular confirmation of their carrier status through Sanger direct sequencing (n = 71), linkage analyses (n = 35), or whole exome sequencing (n = 2). 15 were obligate carriers with no further molecular analysis, and 2 nonobligate carriers had a tapetal-like reflex and were first-degree relatives to patients who had received genetic confirmation of an RPGR mutation. Mutation analyses were performed at the AMC in Amsterdam, The Netherlands (n = 77), the Radboud University Medical Center in Nijmegen, The Netherlands (n = 2), Ghent University Hospital, Ghent, Belgium (n = 14), or at the Manchester Centre for Genomic Medicine, Manchester, United Kingdom (n = 15).

Clinical Data Collection
Medical records were reviewed for symptoms, best-corrected visual acuity (BCVA), biomicroscopy, fundus examination, full-field dark- and light-adapted single flash and 30 Hz electroretinography (HERG) according to the International Society for Clinical Electrophysiology of Vision standards, Goldmann visual field (GVF) examination, spectral-domain optical coherence tomodraphy (SD-OCT), and fundus autofluorescence (FAF) where available. Color vision testing was performed in 45 subjects, using Hardy-Rand-Rittler plates (n = 24), Ishihara plates (n = 26), Farnsworth Panel D15 testing (n = 35), and Farnsworth Trian plates (n = 25). GVF areas of the V4e and I4e target were digitized and converted to seeing retinal areas in mm², using a method described by Dagnelie. Not all subjects underwent all clinical examinations. All FAF images and most SD-OCT images (n = 41) were obtained with the Heidelberg Spectralis (Heidelberg Engineering, Heidelberg, Germany). For these patients, retinal thickness measurements and segmentation analyses were obtained using the Spectralis software, measuring the total central retinal thickness (from the inner limiting membrane to the basal membrane), outer nuclear layer thickness, and the outer retinal thickness + PRE complex (from the external limiting membrane to the basal membrane). In the other three subjects, SD-OCT images were taken with the Topcon (n = 1; 3D OCT-1000; Topcon Medical Systems, Tokyo, Japan) or Zeiss CIRRUS version 6.0 (n = 2; Carl Zeiss Meditec, Inc., Dublin, CA, USA).

We categorized fundus status based on previously used criteria using clinical notes and fundus photographs: grade 0 (no fundus abnormalities); grade 1 (a tapetal-like reflex without pigmentary changes in the retina); grade 2 (regional pigmentary changes, e.g., bone-spicule-like pigmentation, involving at least two quadrants, and/or macular RPE alterations, with or without a tapetal-like reflex); grade 3 (at least three quadrants of pigmentary changes or RPE atrophy in the periphery).

Statistical Analysis
Data were analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). BCVA was divided into the following categories, based on the World Health Organization criteria, adding a category of “mild visual impairment” (BCVA <20/40 and ≥20/67): normal or (subnormal) visual acuity (BCVA ≥20/40), low vision (BCVA <20/67 and ≥20/200), severe visual impairment (BCVA <20/200 and ≥20/400), and blindness (BCVA <20/400). For statistical analysis, we converted visual acuities to logMAR. We used the unpaired t-test, Mann-Whitney test, and χ² tests to compare means, medians, and proportions, respectively. Longitudinal changes in BCVA and seeing retinal area were analyzed using linear mixed models.

Results
One hundred twenty-five female carriers from 49 families were investigated. Genetic analysis yielded 39 distinct pathogenic
Phenotypic Spectrum in RPGR Heterozygotes

Full-Field ERG and Visual Field Findings

ERGs, available for 59 heterozygotes, showed significantly reduced amplitudes in various patterns in 42 of 59 (71%) heterozygotes (Table), with no significant age difference between heterozygotes with normal and those with attenuated
**Table. Clinical Characteristics of RPGR Heterozygotes**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n = 125)</th>
<th>Origin RP Pedigree (n = 83)*</th>
<th>Origin COD/CORD Pedigree (n = 37)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1-14, n (%)</td>
<td>42 (34)*</td>
<td>38 (46)</td>
<td>-</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ORF15, n (%)</td>
<td>83 (66)</td>
<td>45 (54)</td>
<td>37 (100)</td>
<td></td>
</tr>
<tr>
<td>Median refractive error (IQR; range), D†</td>
<td>–3.6</td>
<td>–5.5</td>
<td>–1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>High myopia (&lt; −6D), n (%)</td>
<td>36/102* (35)</td>
<td>31/68 (46)</td>
<td>5/29 (17)</td>
<td>0.007</td>
</tr>
<tr>
<td>Moderate myopia (−3 D &gt; SER ≥ −6 D), n (%)</td>
<td>22/102 (22)</td>
<td>17/68 (25)</td>
<td>4/29 (14)</td>
<td></td>
</tr>
<tr>
<td>Mild myopia (−0.75 D &gt; SER ≥ −3 D), n (%)</td>
<td>16/102 (16)</td>
<td>6/68 (9)</td>
<td>8/29 (28)</td>
<td></td>
</tr>
<tr>
<td>Vascular attenuation, n (%)</td>
<td>33/107 (31)</td>
<td>27/72 (38)</td>
<td>5/52 (16)</td>
<td>0.02</td>
</tr>
<tr>
<td>Fundus grade assessable</td>
<td>117*</td>
<td>79</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Grade 0, n (%)</td>
<td>21 (17)</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Grade 1, n (%)</td>
<td>11 (9)</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Grade 0 or 1, n (%)‡</td>
<td>12 (10)</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Grade 2, n (%)</td>
<td>43 (34)</td>
<td>34</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Grade 3 (%)</td>
<td>17* (14)</td>
<td>15</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Grade 2 or 3, n (%)‡</td>
<td>13* (10)</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Pigment changes, any type, n (%)</td>
<td>75/117* (62)</td>
<td>53/79 (67)</td>
<td>17/34 (50)</td>
<td>0.10</td>
</tr>
<tr>
<td>Bone spicule-like/round, n (%)</td>
<td>46* (37)</td>
<td>39</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Salt-and-pepper, n (%)</td>
<td>4 (4)</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Macular RPE alterations/atrophy, n (%)</td>
<td>40 (32)</td>
<td>31</td>
<td>8</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean age at macular RPE involvement ± SD, y</td>
<td>43.6 ± 17.5</td>
<td>44.1 ± 18.2</td>
<td>43.2 ± 15.8</td>
<td>0.90</td>
</tr>
<tr>
<td>(range)</td>
<td>(12.2–77.7)</td>
<td>(12.2–77.7)</td>
<td>(21.4–59.3)</td>
<td></td>
</tr>
<tr>
<td>RPE alterations in periphery, n (%)</td>
<td>37 (29)</td>
<td>23</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Tigroid/tessellated fundus, n (%)</td>
<td>15 (12)</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>ERG pattern‡</td>
<td>59*</td>
<td>44</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>No abnormalities, n (%)</td>
<td>11 (19)</td>
<td>7 (11)</td>
<td>4 (36)</td>
<td>0.06</td>
</tr>
<tr>
<td>Subnormal/low-to-normal amplitudes, n (%)</td>
<td>6 (10)*</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Electronegative, n (%)§</td>
<td>2 (3)</td>
<td>2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Rod-cone, n (%)</td>
<td>9 (15)</td>
<td>9</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Reduced rods and cones, no predominance</td>
<td>19* (32)</td>
<td>17*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>specified, n (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Cone-rod, n (%)</td>
<td>8 (14)</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Isolated cone reduction, n (%)</td>
<td>2 (3)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nondetectable, n (%)</td>
<td>2 (3)</td>
<td>2</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Significant P values (<0.05) are indicated in bold. SER, spherical equivalent of the refractive error; SPC, subcapsularis posterior cataract.

* Five heterozygotes with mutations in exon 1-14 (n = 4) or ORF15 (n = 1) were not included in the columns distinguishing based on pedigree (RP or COD/CORD), as the diagnosis in the pedigrees could not be established or confirmed in four subjects, and one heterozygote with a mutation in exon 10 (deletion after exon 10) was from a mixed RP/CORD pedigree.

† Averaged between eyes.

‡ In 18 (20%) carriers, no distinction between fundus grades 0 and 1 (n = 9; 10%) or 2 and 3 (n = 9; 10%) could be made based on the notes in the medical record. In seven (8%) heterozygotes, no sufficient data on their fundus appearance were available to estimate the fundus grade.

§ One of the heterozygotes with nondetectable responses had previously documented rod-cone responses. One heterozygote with anisometropia had significantly reduced rod and cone amplitudes in her highly myopic eye and normal amplitudes in her mildly myopic eye.

|| Fisher’s exact test.

¶ Markedly reduced b-wave with a response resembling an electronegative ERG.

# One heterozygote had significantly reduced rod and cone amplitudes in one eye (OS), but normal amplitudes in OD. Other measures of visual function (GVE BCVA) in this subject showed marked asymmetry in favor of OD.

Between heterozygotes, little intrafamilial variability was seen, as heterozygotes with abnormal ERGs tended to cluster within families. However, intrafamilial differences between heterozygotes and male hemizygotes were observed: five heterozygotes from different pedigrees had a cone- or cone-rod pattern on ERG, whereas their affected male relatives had RP.

Goldmann visual fields, available for 53 heterozygotes (106 eyes), showed variable degrees of sensitivity reduction in 46 of
53 subjects (87%; Supplementary Fig. S2; Supplementary Table S3), with a unilateral sensitivity reduction in 2 subjects and relatively more severely affected superior hemifields in 13 subjects. Age was associated with the I4e isopter size (decline rate 4.9%/y; $P = 0.012$), but not with the V4e isopter size ($P = 0.06$).

Of the 90 eyes with variable degrees of sensitivity reduction, 69 (77%) had peripheral retinal pigment changes or chorioretinal atrophy. Heterozygotes had a better preserved visual field than their age-matched affected male relatives, with three exceptions from one family (Supplementary Fig. S2).

**Findings on Retinal Imaging**

SD-OCT imaging of the macula in 47 heterozygotes showed a preservation of all retinal layers in 22 subjects (15 of 22 with peripheral retinal pigment changes; 4 of 22 with central RPE alterations), but showed outer retinal attenuation in 25 heterozygotes (53%), with relative foveal sparing in 23 of these subjects. There was no significant age difference between heterozygotes with outer retinal attenuation on SD-OCT (mean, 44.7 years; SD, 20.3; range, 11.7 to 80.0 years) and those with normal outer retinas on SD-OCTs (mean, 37.2 years;
FIGURE 2. Multimodal imaging illustrating the phenotypical spectrum of RPGR heterozygotes from pedigrees of RP. (A–C) The right eye of a 48-year-old heterozygote (ORF15: c.2200G>T) with optimal BCVA, sectorial pigmentary changes consistent with RP (A). On FAF with a 30° field of view (B), a radial pattern of different AF is clearly visible. FAF further shows small hyper-AF spots surrounded by a hypo-AF border that colocalize with small hyporeflective ellipsoid zone irregularities on SD-OCT (C, green arrowheads). The appearance of a thick outer plexiform layer may indicate a non–well-aligned scan. (D–F) The left eye of a 65-year-old highly myopic (−11 D) heterozygote (exon 3: c.248-28_248-10del) with light perception vision OU, showing a posterior staphyloma (D), an atrophic retina with partially obliterated vessels, and bone-spicule pigmentations in the midperiphery (not shown). FAF with a 30° field of view (E) shows extensive patches of absent AF, but areas of normo-AF and hyper-AF are
visible. SD-OCT (F) shows atrophy of all retinal layers, and the ellipsoid zone and external limiting membrane are not discernible. The markedly thinned, almost absent chorioid points to an important myopic degenerative factor. (G-I) The left eye of a 46-year-old moderately myopic heterozygote (ORF15: c.2536G→T) with RP and a decimal BCVA of 0.3/0.5. Her fundus shows vascular attenuation, bone-spicule pigmentation in the midperiphery, and alterations of the RPE in the macula (G). FAF imaging with a 55° field of view (H) shows an oval zone of mottled hypo-AF around the fovea and more coarsely mottled hypo-AF in the perimacula and midperiphery, mainly nasal to the optic disc. The corresponding SD-OCT scan (I) shows generalized severe attenuation of the outer retinal layers, with only scattered granular remnants of the ellipsoid zone. The ONL is nearly absent outside of the fovea. (J, K) The right eye of a 54-year-old highly myopic (−16 D) heterozygote (ORF15: c.2010del), whose fundus photograph (J) shows a paracentral patch of retinal atrophy, corresponding on FAF imaging with a 55° field of view (K) with a patch of absent AF. FAF further shows a ring of relative hyper-AF surrounding a normal fovea and mottled areas of hypo-AF around the vascular arcade, more distinctly in the inferior retina.

DISCUSSION

In this retrospective cohort study, we describe the phenotypic spectrum of 125 female carriers of pathogenic RPGR variants from 49 pedigrees of RP and COD/CORD. This study is one of the largest studies on female RPGR carriers to date, and we identified a number of novel structural and functional characteristics, also in comparison to affected male relatives. Signs and/or symptoms were common (70%) both in the RP and COD/CORD carrier groups in carriers of mutations in exon 1-14 and in carriers of ORF15 mutations, with complete expression of RP or CORD in 29 heterozygotes (25%). Although the longitudinal results are probably biased, as the follow-up was significantly longer and more frequent in heterozygotes with myopia or pigmented changes, our results indicate that clinically severe phenotypes are not rare in heterozygous female RPGR mutation carriers.

Female carriers of X-linked retinal dystrophies have been reported to express RP characteristics, 21,22 sometimes partial or sectorial, albeit in small cohorts.7,11,17,25 This variability has challenged the ability to identify the accurate disease inheritance mode in families with affected female patients.24-26 In the early stages of genetic counseling of an RP pedigree, the presence of affected female subjects should raise suspicion of both an autosomal-dominant and an X-linked inheritance mode. In fact, typical RP fundus features, such as bone-spicular or nummular intraretinal pigmentation, optic disc pallor, and vascular attenuation, were more prevalent in the cohort, expectedly more so in heterozygotes from RP pedigrees (49%, 44%, and 56%, respectively; Table) than in those from COD/CORD pedigrees (18%, 18%, and 18%, respectively).

Previous reports have shown that female carriers of X-linked COD/CORD usually show either no or minimal fundus changes,14,27 but cases of heterozygotes displaying macular RPE alterations have been described,19 and our cohort further expands this phenotypic spectrum, showing the frequent involvement of macular retina (32%; Table), including cases of full COD or CORD expression.

Myopia was associated with a lower BCVA, and high myopia (below −6 D) was found in a similar incidence as in the male cohort we previously investigated (35% in heterozygotes versus 38% in male hemizygotes), with 12% showing a tessellated fundus, four cases of posterior staphyloma, and eight cases of highly myopic patchy chorioretinal atrophy. Although the reasons for the association between RPGR mutations and high myopia remain unclear, a recent study analyzing refractive errors in inherited retinal dystrophies has postulated that the transport area between the inner and outer segment (i.e., the location of protein RPGR), is one of the critical sites for refractive error development.28 Mutations in RP1 and RP2, encoding an outer segment protein and another connecting cilium protein, respectively, have also been linked to high myopia,29,30 further corroborating this hypothesis. However, not all genes encoding connecting cilium proteins are associated with myopia, and some have been associated with hyperopia (e.g., CEP290 and RPGRIP1), and biallelic mutations in these are generally associated with much more extreme visual loss due to Leber congenital amaurosis.31,32 The presence of (high) myopia in RPGR heterozygotes with no photoreceptor dysfunction on ERG supports the notion that photoreceptor degeneration and high myopia might be two parts of the same disease, although the association between high myopia and reduced rod/cone amplitudes would support the concept of scleral remodeling stimulation by signals from dysfunctional photoreceptors and/or more frequent near-focusing due to reduced BCVA. However, the interpretation of the latter is confounded by the association of high and degenerative myopia with reduced ERG amplitudes.33

The occurrence of a BCVA below 20/200 in at least one eye (15%; Fig. 1) in this cohort was higher than in an earlier longitudinal report in a cohort of XLRP carriers from a combination of genotyped and ungenotyped families, which reported this BCVA in 2%.10 In keeping with earlier reports, abnormal GVF (46 of 53; 87%) and ERG (42 of 59; 71%) results
FIGURE 3. Multimodal imaging illustrating the phenotypical spectrum of RPGR heterozygotes from pedigrees of CORD. (A–C) The left eye of a 32-year-old heterozygote (ORF15: c.2426_2627del) with optimal BCVA, a tapetal-like reflex on fundus photography (A) and FAF with a 30° field of view (B), and a normal appearance of the retina on SD-OCT (C). (D–F) The right eye of a highly myopic (~13.5 D) 36-year-old heterozygote (ORF15: c.3092del) with a CORD phenotype and a BCVA of 0.5/0.1, showing peripapillary atrophy and a paravascular patch of chorioretinal atrophy. On FAF with a 30° field of view (E), mottled and granular hypo-AF changes are seen, predominantly along the vascular arcade, with a granular hypo-AF ring around the central macula and a sharply demarcated patch of absent AF along the upper vascular arcade. A radial pattern can be discerned in the
perimacular region around the hypo-AF ring. SD-OCT (F) shows severe extrafoveal and foveal attenuation of the outer retinal layers, ellipsoid zone, and external limiting membrane, with some granular and mottled remnants of the hyperreflective outer retinal bands. Outside of the fovea, the ONL is barely discernible. (G–I) The right eye of a 63-year-old mildly myopic heterozygote (ORF15: c.2575G>T) with COD. Fundus photography (G) shows extensive outer retinal atrophy in the posterior pole and (mid)periphery, with patches of relatively preserved retina, and bone-spicule pigmentation in the (mid)periphery, encroaching on the posterior pole. FAF with a 55° field of view (H) shows a large zone of hypo-AF in the macula, encompassing the peripapillary region and extending into the midperiphery, surrounded by a hyper-AF border. The corresponding SD-OCT scan (I) shows an overall attenuation of the outer retina, with granular remnants of the ellipsoid zone. The ONL is discernible in the fovea and direct parafovea but is nearly absent in the perimacula. (J, K) The right eye of a 56-year-old highly myopic (−14.5 D) heterozygote (ORF15: c.3092delC; maternal aunt of the subject in D–F with COD). With a BCVA of 0.1, FAF with a field of view of the right eye (J) shows peripapillary atrophy, sharply demarcated patches of absent AF along the upper and lower vascular arcade and below the vascular arcade, and a mottled decrease of AF in the posterior pole and below the vascular arcade. Some radial pattern can be detected outside of the vascular pole. SD-OCT (K) shows an atrophic ONL, a scarcely visible external limiting membrane, and a granular ellipsoid zone. As in Figure 2E, the thinned choroid points to an important myopic degenerative factor.

were more common than pigmentary changes (73 of 117; 62%) or BCVA impairment (13%, 5%, and 9% for low vision, severe visual impairment, or blind in one eye, respectively). However, GVF and ERG were consistently performed only in a subset of heterozygotes, as was the case in this retrospective cohort, and the availability of these test results may thus be subject to bias, as additional testing may have mostly been performed in heterozygotes with symptoms or signs of disease. However, even when assuming that all heterozygotes without additional testing would have no abnormalities, our study shows that a relatively large proportion of heterozygotes experience variable degrees of visual field constriction (46 of 125; 37%) or significant amplitude reduction on ERG (42 of 125; 34%).

Although there was noticeable intrafamilial variability in certain cases, heterozygotes with signs and/or symptoms of disease tended to originate from the same pedigrees. However, this aggregation may be overestimated, as possible (non-obligate) heterozygotes without signs or symptoms may never seek specialist advice from a geneticist or ophtalmologist. The intra- and interfamilial spectrum of severity in heterozygotes of X-linked retinal disorders has been at least partially attributed to the role of X-chromosome inactivation. Based on this X-chromosome inactivation model, Dobyns et al. proposed a penetrance and severity index for X-linked diseases, as opposed to using the terminology of X-linked dominant, semidominant, or recessive inheritance, as this concept is not based on X-inactivation in humans but on the X-transcription speed and dosage compensation in the Droso phila model. Random X inactivation is proposed to occur early in embryonic development, in each cell independently, and would result in a mosaic pattern of cells expressing the normal and mutated gene. Our finding of a radial pattern on the available FAF images in 65% of the examined carriers, even in those who had no tapetal-like reflex on fundoscopy, indicates that FAF may be particularly helpful in detecting such retinal mosaicism. This radial pattern on FAF would support the earlier suggestion that X inactivation might not occur in each cell independently, but in clusters of cells in a similar pattern, followed by a centrifugal radial growth of the neuroretina during embryonic development. However, random X inactivation does not fully explain the clustering of nearly complete penetrance in several families in this cohort. There may be a role for skewed X inactivation, in which the mutant allele is disproportional in active. Extremely skewed X inactivation has been shown in specific mutations in some nonretinal X-linked disease genes and has been in favor of the nonmutated allele. Secondary X inactivation may manifest through a selective (dis)advantage for cells with the mutation-containing X-chromosome, which may be hereditary. The relative family-based, rather than mutation-based, aggregation of affected heterozygotes in this cohort, along with cases of intrafamilial variability, points to a highly likely role of genetic and/or environmental modifiers, as well as potentially skewed X inactivation. Intraindividual asymmetry was a recurring finding in this cohort. Left-right asymmetry in X-chromosome inactivation in several paired structures of the body, such as the retina, has been observed, but the biological basis remains unclear. Other than few reports on asymmetrical ERG responses in small numbers of heterozygotes from X-linked RP or COD/CORD pedigrees, this clinical asymmetry has remained largely unexplored. We found a high general correlation between the right and left eye in this cohort, but notable asymmetry in fundus phenotype (9%), in V4e or I4e size on GVF (20%), BCVA (26%), and refractive error (33%) was still observed.

As 23% of heterozygotes display a complete RP or COD/CORD phenotype, with a yearly BCVA decline rate of 1.9%, the question ensues if these heterozygotes may benefit from future therapeutic options. Recently, a submacular gene therapy trial for male patients with RPGR-associated RP commenced, and preclinical studies optimizing subretinal RPGR gene therapy have focused on the typical male severe phenotype, which includes an early onset. Inclusion of heterozygotes in gene therapy trials may be challenging until safety and efficacy data are available in male subjects, because male subjects are consistently more severely affected. Nonetheless, in female cases with full disease expression, the macular RPE was frequently altered or atrophic (90% vs. 32% of the total cohort), rendering the concept of gene therapy in affected heterozygotes a possibly appropriate treatment option in the future. Serious concerns in the inclusion of female subjects would include the limited comparability to male patients, and the definition of a window of therapeutic opportunity, further complicated by the variability in disease onset and progression. The mean age at which macular RPE involvement was first documented was 45 years, and blind heterozygotes were in their sixth or seventh decade of life, which would translate to a wider window of opportunity in female patients than in the male cohort.

Limitations of this study include its retrospective design, which means that not all subjects had undergone all clinical examinations, as not all clinical examinations were considered relevant or feasible in the clinical setting. Although the gathered data are useful, even from subjects with single visits, potential bias should be taken into consideration. Furthermore, as a subgroup of subjects (37 of 125) originated from COD/CORD pedigrees, this compromises the generalizability of the reported findings.

In summary, the findings in this study provide useful insights for the genetic and clinical counselling of female carriers and may have implications for ongoing and future trials. This study displays the wide phenotypic spectrum for RPGR heterozygotes and shows that signs and/or symptoms of disease are common, although the visual prognosis is favorable compared with affected males. Myopia is a significant concern due to its effect on BCVA, as it is in affected male patients. A substantial proportion of heterozygotes displays a complete RP
or COD/CORD phenotype, and further prospective natural history studies are necessary to determine whether inclusion in future (gene) therapeutic trials would be feasible and useful in this subset of severely affected female RPGR mutation carriers and which outcome measures would provide the most sensitive detection of treatment effect.

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References


