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**IMPG2-Associated Retinitis Pigmentosa Displays Relatively Early Macular Involvement**

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**Genetics**

Retinitis pigmentosa (RP) is a group of diseases featuring progressive degeneration of rod and cone photoreceptor cells and RPE, and is considered the most commonly inherited retinal dystrophy with an estimated prevalence of approximately 1:4000.1-3 Retinitis pigmentosa typically starts with night blindness followed by loss of the peripheral visual field that leads to tunnel vision, whereas visual acuity often remains normal until the late stages.3,4 Hallmark fundus abnormalities in RP are bone-spicule pigmentation, a waxy pale optic disc, and attenuation of retinal vessels. Electroretinography (ERG) responses reveal rod and cone dysfunction, where rod abnormalities often are observed earlier in the course of the disease. However, wide variability in terms of disease onset, progression rate, and degeneration patterns are observed in RP.4,5

The genetic background underlying RP is also very heterogeneous. Inheritance modes observed in RP include autosomal-recessive (50% of patients), autosomal-dominant (20%), X-linked (10%), mitochondrial (<1%) patterns, and a few cases of digenic RP.6-9 However, the remaining 40% of patients are isolated cases.10 Autosomal recessive RP is currently associated with mutations in 42 different genes (RetNet, available in the public domain at https://sph.uth.edu/retnet/), which provide a molecular genetic explanation for approximately 50% of all recessive RP cases.11 The proteins encoded by these genes are involved in a broad range of cellular processes.
functions, including phototransduction, the visual (retinoid) cycle, transport along the connecting cilium, cell-to-cell signaling or synaptic interaction, gene regulation, cell or cytoskeletal structure, cell-cell interactions, and outer segment phagocytosis.\textsuperscript{3,10,12}

Recently, mutations in the IMPG2 gene have been implicated in autosomal recessive RP\textsuperscript{13} This gene encodes the interphotoreceptor matrix proteoglycan-2 (IMPG2), formerly known as IPM 200 or SPACRCAN,\textsuperscript{14} which is localized in the retinal extracellular matrix (also known as the interphotoreceptor matrix [IPM]). The IPM is a viscous substance mainly composed of glycoproteins and proteoglycans that fills the space between individual photoreceptor cells and between photoreceptors and the RPE.\textsuperscript{15,16} For many years, the IPM was considered merely a fixating medium,\textsuperscript{17} but in the past few decades multiple functions of the IPM have been reported, including important functions in intercellular communication, regulation of neovascularization, cell survival, membrane turnover, photoreceptor differentiation and maintenance, retinoid transport, matrix turnover, and the precise alignment of the photoreceptor cells to the optical light path.\textsuperscript{14,18,19} Both rod and cone photoreceptor cells synthesize IMPG2 and secrete the protein into the IPM,\textsuperscript{20} where it binds to other proteins, such as hyaluronan, and also seems to be anchored in the plasma membrane of the photoreceptor cells, thereby fixating the photoreceptors in the IPM.\textsuperscript{21} Additionally, IMPG2 is thought to have calcium-binding potential, which suggests it has an important role in sequestering extracellular calcium released by photoreceptors in response to light to light.\textsuperscript{21}

Knowledge of the natural course of IMPG2-related RP is of significant importance for prognosis counseling as well as genetic counseling. Furthermore, this knowledge is vital in the view of emerging therapy trials, in terms of patient selection and the assessment of treatment effects. In this international collaborative study, we aim to provide a detailed overview of the clinical findings in patients with IMPG2-associated RP.

**METHODS AND PATIENTS**

**Subjects and Genetic Analysis**

The specialized ophthalmogenetic centers of the Radboud University Medical Center (RAcVH, CBH, and BJk), the Rotterdam Eye Hospital (LiJvDB), the Erasmus University Medical Center Rotterdam (CCWK), the Hadassah-Hebrew University Medical Center in Jerusalem (EB), the Seconda Università degli Studi di Napoli (FS), and the Shifa College of Medicine in Islamabad (RQ) participated in this study. As described previously,\textsuperscript{15} six families of Israeli, Palestinian, Pakistani, Italian, or Dutch origin were found to carry causative mutations in IMPG2 (families A–F, Fig. 1). Additionally, we selected four more families after identification of causative IMPG2 mutations in a targeted next-generation sequencing experiment in 100 Dutch RP probands (family G),\textsuperscript{22} or whole exome sequencing (families H, I, J, K, Fig. 1). Exome sequencing was performed using the 5500x1 Genetic Analyzer of Life Technologies (Applied Biosystems, Foster City, CA, USA) and the Agilent SureSelectXT Human All Exon 50-Mb amplification kit (Agilent Technologies, Inc., Santa Clara, CA, USA). Data were analyzed with LifeScope software (version 2.1; Life Technologies, Applied Biosystems). All mutations were confirmed with Sanger sequencing.

We adhered to the tenets of the Declaration of Helsinki and obtained approval for this study from the Institutional Ethics Committee from the Radboud University Medical Center. Approval included permission to use the documented medical data and, when indicated, clinically reassess affected individuals and to obtain blood for the purposes of DNA extraction and genetic analysis. We obtained informed consent from all participants before the collection of blood samples and additional ophthalmologic examinations.

**Clinical Analysis**

We collected the available clinical data from the medical files of all patients. Nine patients were clinically reevaluated after the identification of causative IMPG2 mutations. Medical history was registered with a focus on the age at onset, initial symptoms, and the overall course of the retinal disorder. The age at onset was defined as the age at which the initial symptom was noticed by the patient. Additionally, we questioned patients about the presence of syndromic features, which generally occur in 20% to 30% of RP patients.\textsuperscript{4} This questionnaire included the presence of hearing and balance abnormalities, renal failure, cardiac and respiratory anomalies, polydactyly, obesity, cognitive impairment, fertility disorders, hypogonadism, and dental anomalies.

Clinical examination included best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, and ophthalmoscopy. Additional examinations were performed if feasible. Goldmann (kinetic) perimetry was performed in 11 patients using targets V-4e, III-4e, I-4e, I-3e, I-2e, and I-1e. In two patients (F-II:1 and F-II:2) perimetry was restricted to analysis of the central 30° of the visual field with the Humphrey perimeter (Carl Zeiss Meditec, Jena, Germany). In all but one patient (F-II:2), full-field ERG was performed according the guidelines of the International Society for Clinical Electrophysiology of Vision.\textsuperscript{23} Results were compared to the local reference values. We evaluated color vision in six patients using the Farnsworth Dichotomous Test (Panel D-15) and/or the Hardy-Rand-Rittler test. Fundus photographs of the central retina (Topcon TRC50IX; Topcon Corporation, Tokyo, Japan) were obtained in 15 patients. Fundus autofluorescence images (Spectralis; Heidelberg Engineering, Heidelberg, Germany) of the central retina were acquired in eight patients using a confocal scanning laser ophthalmoscope with an optically pumped solid state laser (488 nm) for excitation. Spectral-domain optical coherence tomography (SD-OCT, Spectralis; Heidelberg Engineering) could be performed in 13 patients. In three patients, a high-resolution OCT was not available and a time-domain OCT (Stratus; Carl Zeiss Meditec) was obtained. No OCT images were available for the remaining four patients. In eight Dutch patients with high-resolution SD-OCT images (mean age: 51 years; range, 23–67 years), we quantified thickness of the total retina at the fovea and at 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mm eccentricity from the fovea in the right eye using the thickness graphs in the Heidelberg Eye Explorer Software (version 1.6.4.0; Heidelberg Engineering). In seven of these patients, we quantified the foveal volume by measuring the retinal volume within the central 3 mm² using the thickness map in the Heidelberg Eye Explorer Software (version 1.6.4.0; Heidelberg Engineering). A normal dataset of retinal thickness and foveal volume in individuals without (vitreo)retinal disease was obtained from 25 age-matched Dutch individuals (mean age: 46 years; range, 27–62 years) for reference purposes.

**RESULTS**

Ten families with a total of 17 affected individuals were included in this study. The pedigrees of all families are depicted in Figure 1. An overview of the clinical findings in all 17 patients is provided in Table 1.
Clinical Evaluation

The most recent examination of the 17 RP patients was performed at a mean age of 49 years (range, 23–67 years). The mean age at onset was 10.5 years (range, 4–20 years), and night blindness was the most frequent initial symptom, occurring in 10 patients (59%). Other initial symptoms were decrease in visual acuity (35%) and loss of visual field (6%). In the patients who initially revealed a decreased visual acuity, normal BCVA was measured before the decrease in visual acuity, which excludes refractive amblyopia. In one patient (A-II:6), the diagnosis of RP was made during a routine ophthalmologic consultation when he was 12 years old. At that time, he had not noticed any symptoms associated with RP, but later in the course of the dystrophy, night blindness manifested as the first symptom.

Figure 1. Pedigrees of the families that were included in this study. Where relatives were available (families A, B, C, F, G, and H), the mutation segregates with the disease. Plus signs denote the wild-type allele, square boxes indicate men, circles indicate women, and affected individuals are pointed out in black. The arrows indicate the probands. Double lines point out consanguineous marriages, the numbers indicate the degree of consanguinity. The dagger (†) indicates the patients diagnosed with RP not included in this study due to the lack of clinical data.
### Table 1. Clinical Findings in Patients With IMPG2-Associated RP at Their Most Recent Visit

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient ID/ Age, y/ Sex</th>
<th>Initial Symptom</th>
<th>Visual Acuity†</th>
<th>SER (D)‡</th>
<th>Lens Status</th>
<th>Ophthalmoscopy Results</th>
<th>ERG Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>OS</td>
<td>OD</td>
<td>OS</td>
<td></td>
</tr>
<tr>
<td>A (W01-299)</td>
<td>A-II:1/8/59/F</td>
<td>Decrease in visual acuity</td>
<td>LP (2.7)</td>
<td>LP (2.7)</td>
<td>−4.00</td>
<td>−4.00</td>
<td>PSC cataract; extracted at 37 y (RE) and 38 y (LE)</td>
</tr>
<tr>
<td>A (W01-299)</td>
<td>A-II:5/14/45/M</td>
<td>Loss of visual field</td>
<td>20/200 (1.0)</td>
<td>LP (2.7)</td>
<td>−0.25</td>
<td>−0.25</td>
<td>PSC cataract; extracted at 24 y (LE) and 32 y (RE)</td>
</tr>
<tr>
<td>A (W01-299)</td>
<td>A-II:6/12/44/M</td>
<td>None, after diagnosis night blindness</td>
<td>20/120 (0.8)</td>
<td>20/400 (1.5)</td>
<td>plan</td>
<td>−0.25</td>
<td>PSC cataract; extracted at 32 y (both eyes)</td>
</tr>
<tr>
<td>B (MOL0764)</td>
<td>B-II:1/ Childhood/66/F</td>
<td>Decrease in visual acuity</td>
<td>LP (2.7)</td>
<td>LP (2.7)</td>
<td>−12.00</td>
<td>−11.75</td>
<td>PSC and nuclear cataract</td>
</tr>
<tr>
<td>B (MOL0764)</td>
<td>B-II:2/ Childhood/66/F</td>
<td>Decrease in visual acuity</td>
<td>HM (2.3)</td>
<td>HM (2.3)</td>
<td>−8.25</td>
<td>−5.50</td>
<td>PSC cataract</td>
</tr>
<tr>
<td>B (MOL0764)</td>
<td>B-II:3/ Childhood/52/F</td>
<td>Decrease in visual acuity</td>
<td>CF (1.9)</td>
<td>CF (1.9)</td>
<td>−3.00</td>
<td>−3.25</td>
<td>Mild PSC cataract</td>
</tr>
<tr>
<td>C (W08-1378)</td>
<td>C-II:2/8/33/M</td>
<td>Night blindness</td>
<td>20/50 (0.4)</td>
<td>20/50 (0.4)</td>
<td>−5.50</td>
<td>−7.00</td>
<td>Clear</td>
</tr>
<tr>
<td>C (W08-1378)</td>
<td>C-II:3/8/36/M</td>
<td>Night blindness and decreased color vision</td>
<td>20/50 (0.4)</td>
<td>CF (1.9)</td>
<td>−4.00</td>
<td>−4.50</td>
<td>Small opacities</td>
</tr>
<tr>
<td>D (NAP1)</td>
<td>D-II:1/20/65/F</td>
<td>Night blindness</td>
<td>HM (2.3)</td>
<td>HM (2.3)</td>
<td>NP</td>
<td>NP</td>
<td>PSC cataract</td>
</tr>
</tbody>
</table>
# Investigative Ophthalmology & Visual Science

## Table 1. Extended

<table>
<thead>
<tr>
<th>Family*</th>
<th>Patient ID/ Age, y/ Sex</th>
<th>Goldmann Perimetry</th>
<th>OCT Results</th>
<th>Autofluorescence Results</th>
<th>Follow-up, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (W01-299) A-II:1/8/59/F</td>
<td>NP</td>
<td>Severe thinning of the retina, generalized loss of the outer retina, central RPE residue with parafoveal RPE atrophy</td>
<td>NP</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>A (W01-299) A-II:5/14/45/M</td>
<td>Constricted to 10° with moderately sensitivity loss.</td>
<td>Generalized loss of reflectance of the outer retinal layers, irregular reflective spots just above the RPE reflective band, lowered parafoveal RPE reflectance, ERM with minimal traction inferior of the fovea.</td>
<td>Irregular hypoAF in the macula, normofluorescent aspect surrounding the macula giving the impression of a hyperAF ring, mottled aspect of hypoAF in the midperiphery.</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>A (W01-299) A-II:6/12/44/M</td>
<td>Constricted to 10° with moderately sensitivity loss.</td>
<td>Generalized loss of the outer retinal layers, RPE reflectance fairly intact, except in the temporal parafovea.</td>
<td>Granular hypoAF aspect of the macula, perifoveal deep hypoAF signal giving the impression of a bull’s eye-like aspect, normofluorescent aspect surrounding the macula giving the impression of a hyperAF ring, mottled aspect of hypoAF and larger hypoAF lesions in the midperiphery.</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>B (MOL0764) B-II:1/ Childhood/66/F</td>
<td>Constricted VF up to less than 5°.</td>
<td>Severe thinning of central retina.</td>
<td>NP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B (MOL0764) B-II:2/ Childhood/60/F</td>
<td>Constricted VF up to 5°.</td>
<td>Severe thinning of central retina, atrophy of the choriocapillaris.</td>
<td>HypoAF macula and peripapillary region, granular hypoAF aspect of midperiphery.</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B (MOL0764) B-II:3/ Childhood/52/F</td>
<td>Peripheral island</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C (W08-1378) C-II:2/8/33/M</td>
<td>Mildly decreased sensitivity with absolute nasal parafoveal scotomas</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C (W08-1378) C-II:3/8/36/M</td>
<td>Mildly decreased sensitivity, relative and absolute scotomas in the midperiphery, temporal parafoveal scotoma.</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D (NAPI) D-II:1/20/65/F</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Patient ID/ Age/ Sex</td>
<td>Initial Symptom</td>
<td>Visual Acuity†</td>
<td>SER (D)‡</td>
<td>Lens Status</td>
<td>Scotopic Ophthalmoscopy Results</td>
</tr>
<tr>
<td>----------------------</td>
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<td>-------------------------------</td>
</tr>
<tr>
<td>E (NP75) F (RP49) 6/23/F</td>
<td>Night blindness</td>
<td>20/120 (0.8) 20/40 (0.3)</td>
<td>-1.00</td>
<td>NP</td>
<td>Macular atrophy, ERM, waxy pallor of the tilted optic disc, attenuated vessels, peripheral RPE atrophy and bone spicules.</td>
</tr>
<tr>
<td>F (RP49) 6/17/59/F</td>
<td>Night blindness</td>
<td>20/120 (0.8) 20/100 (1.2)</td>
<td>-2.00</td>
<td>NP</td>
<td>BEM, ERM, waxy disc, bone spicule pigmentations, waxy optic disc, bone specular degeneration, choroidal bone spicules anterior to retinal macula.</td>
</tr>
<tr>
<td>G (NP75) F (RP49) 6/21/69/F</td>
<td>Night blindness</td>
<td>20/50 (0.2) 20/40 (0.3)</td>
<td>-6.00</td>
<td>CN cannot</td>
<td>Small central area with spared RPE, bone spicule pigmentations anterior, severely attenuated vessels, periphery bone spicules and drusenoid deposits.</td>
</tr>
<tr>
<td>H (RP49) 6/17/59/F</td>
<td>Decrease in visual acuity</td>
<td>20/20 (1.0) 20/40 (0.3)</td>
<td>-5.75</td>
<td>Cataract extracted at age 35 (RE) and 39 (LE)</td>
<td>Medial macular, moderate pallor, bone spicules and drusenoid deposits.</td>
</tr>
</tbody>
</table>

**Clinical Characteristics of IMPG2-Associated RP**
<table>
<thead>
<tr>
<th>Family*</th>
<th>Patient ID/ Age, y/ Sex</th>
<th>Goldmann Perimetry</th>
<th>OCT Results</th>
<th>Autofluorescence Results</th>
<th>Follow-up, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>E-II:4/4/65/F</td>
<td>NP</td>
<td>Thinned retina, increased beam penetrance in the fovea due to RPE loss (TD-OCT).</td>
<td>NP</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>F-II:1/12/23/M</td>
<td>Constriction to 20° or midperipheral scotoma (only central 30° was tested), parafoveal scotoma in LE, mildly decreased sensitivity (Humphrey)</td>
<td>Severe thinning of central retina (TD-OCT).</td>
<td>NP</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>F-II:2/10/32/F</td>
<td>Constriction to 20° or midperipheral scotoma (only central 30° was tested), moderately decreased sensitivity (Humphrey)</td>
<td>Severe thinning of central retina (TD-OCT).</td>
<td>NP</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>G-II:1/17/60/M</td>
<td>Constricted to 20° with inferior residue, severely decreased central sensitivity.</td>
<td>Generalized loss of the bands corresponding to the photoreceptor inner and outer segments, a thinned ONL, patchy loss/thinning of the foveal RPE, profound loss of the outer retina, RPE and choroid reflectance in the midperiphery.</td>
<td>HypoAF macula, granular hypoAF aspect with large confluent hypoAF lesions in the midperiphery, peripapillary hypoAF</td>
<td>54</td>
</tr>
<tr>
<td>G</td>
<td>G-II:2/16/59/F</td>
<td>Constricted to 20° with residues inferior. Sensitivity very mildly decreased.</td>
<td>Fairly intact foveal laminar retinal architecture, confluence of the bands corresponding to the ellipsoid inner segments and the RPE, ELM reflectance is present only in the fovea, thinned ONL outside the fovea, minimal CME in the LE, the RPE seems fairly intact except for irregular thinning the fovea and RPE loss in the peripapillary region, profound loss of the outer retinal, RPE, and choroid reflectance in the midperiphery.</td>
<td>2 small hypoAF spots in the otherwise hyperAF macula, subtle hyperAF ring around macula, granular aspect with sporadic hypoAF lesion just anterior of the vascular arcades, prominent blockage of AF by bone spicules.</td>
<td>34</td>
</tr>
<tr>
<td>H</td>
<td>H-II:6/17/46/F</td>
<td>Constricted to &lt;10°, moderately decreased central sensitivity, small inferotemporal residues</td>
<td>Severely thinned central retina, small central residue of the ONL, fairly intact RPE reflectance in the fovea.</td>
<td>HyperAF macula, mottled aspect of hypoAF and larger hypoAF lesions just anterior of the vascular arcades, peripapillary hypoAF</td>
<td>16</td>
</tr>
<tr>
<td>J</td>
<td>H-II:6/17/46/F</td>
<td>Constricted to 40° with temporal residual VF, mildly decreased central sensitivity in RE, severely decreased central sensitivity in LE.</td>
<td>Loss of the bands corresponding to the photoreceptor inner and outer segments, severely thinned ONL outside the fovea, irregular reflectance of presumably the ELM in the foveola, RPE reflectance intact in central retina.</td>
<td>Irregular hyperAF in macula (LE &gt; RE), sporadic small hypoAF spots just anterior of the vascular arcades.</td>
<td>14</td>
</tr>
</tbody>
</table>
**Table 1. Continued**

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient ID/ Age, y/Sex</th>
<th>Initial Symptom</th>
<th>Visual Acuity†</th>
<th>SER (D)‡</th>
<th>Lens Status</th>
<th>Ophthalmoscopy Results</th>
<th>ERG Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>K-II:1/4/67/F</td>
<td>Decrease in visual acuity</td>
<td>20/400 (1.3) 20/400 (1.3)</td>
<td>-4.25 -5.50</td>
<td>Pseudophakia in RE, mild cataract in LE</td>
<td>BEM, pallor of the optic disc, peripapillary pigmentation, severely attenuated vessels, RPE atrophy, and intraretinal bone spicule pigmentsations in the midperiphery</td>
<td>SR (age 24) WNL (age 24)</td>
</tr>
</tbody>
</table>

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**Table 1. Continued Extended**

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient ID/ Age, y/Sex</th>
<th>Goldmann Perimetry</th>
<th>OCT Results</th>
<th>Autofluorescence Results</th>
<th>Follow-up, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>K-II:1/4/67/F</td>
<td>NP</td>
<td>Loss of the bands corresponding to the photoreceptor inner and outer segments, severely thinned ONL in the central retina, RPE reflectance intact except for in the parafovea.</td>
<td>Mottled hypoAF aspect of the perifoveal region, hyperAF in the fovea, mottled hypoAF aspect just anterior of the vascular arcades.</td>
<td>41</td>
</tr>
</tbody>
</table>
symptom at age 24. In eight patients, an extended clinical follow-up period varying from 14 to 48 years was available (mean: 29 years). The course of the BCVA for each of these patients during follow-up is represented in Figure 2. None of the patients showed extraocular abnormalities that are indicative of syndromic RP.

Refractive errors included mild to high myopia (range of spherical equivalents: plan to +12.00 diopters; Table 1). Significant lens opacities were observed in 13 patients, most often subcapsular posterior cataracts (seven patients [41%], Table 1). Six patients had experienced cataract surgery, most often in the fourth decade (five of six patients). Ophthalmoscopy revealed the classic RP features, including bone spicule pigmentation at the (mid)periphery, attenuated vessels, waxy pallor of the optic disc, and atrophy of the RPE and choriocapillaris in all RP patients. In one patient (A-II:5), marked sheathing of the peripheral retinal vessels was noted (Fig. 3A). In addition, all patients revealed macular abnormal-

![Graph showing the change in visual acuity (y-axis) related to the age in years (x-axis) in patients carrying mutations in the IMPG2 gene.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932990/)

**Figure 2.** Graph showing the change in visual acuity (y-axis) related to the age in years (x-axis) in patients carrying mutations in the IMPG2 gene. Snellen visual acuity was transformed into logMAR for visualization purposes. A logMAR value of 1.9 was assigned to counting fingers (CF), 2.3 to hand movements (HM), and 2.7 to light perception (LP). When the visual acuity differed in both eyes, the visual acuity of the best eye was used. The improvement in visual acuity in patient A-II:5 was seen after cataract surgery; subsequently, the decrease in visual acuity was probably due to cystoid macular edema, which was successfully treated. The cause of the improvement in patient J-II:1 is unclear, because refractive, optical, or retinal causes seemed absent.

![Fundus photographs and FAF imaging of patients carrying mutations in IMPG2.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932990/)

**Figure 3.** Fundus photographs and FAF imaging of patients carrying mutations in IMPG2. (A and F) Fundus photograph of temporal superior region of the retina (A), showing sheathing of the retinal vessels, and FAF imaging of the central retina (G), revealing irregular FAF signal in the macula and a granular hypofluorescent aspect of the RPE surrounding the posterior pole, in patient A-II:5 (age 45). (B and H) Fundus photograph composition (G), showing macular atrophy, and FAF images (I), revealing a hypofluorescent macula, a granular aspect with large confluent hypofluorescent lesions in the midperiphery, and peripapillary hypofluorescence, of patient G-II:1 (age 60). (C and E) Fundus photograph (C) and FAF image (F) of the central retina in patient A-II:6 (age 44). The fundus photograph reveals a BEM. The FAF image shows granular aspect of the macula, whereas deeper perifoveal hypofluorescence gives the impression of a BEM, mottled aspect of midperiphery with larger hypofluorescent lesions. (D and G) Fundus photograph (D) and FAF image (H) of the central retina in patient K-II:1 (age 67). The fundus photograph shows a BEM, whereas FAF reveals a mottled hypofluorescent aspect of the perifoveal region, hyperautofluorescence in the fovea, and a mottled aspect just anterior of the vascular arcades.
ities ranging from subtle changes, such as mottling of the macular RPE as observed in C-II:2 and J-II:1, to profound macular atrophy as was observed in seven patients (mean age: 57 years; range, 36–66, Fig. 3B). A bull’s eye maculopathy (BEM) was a distinctive ophthalmoscopic feature in six patients, which mainly was observed during the fifth and sixth decades of life (mean age: 51 years; range, 32–67 years, Figs. 3C, 3D, 4A). Since the exact onset of the BEM could not be ascertained, we were unable to define the interval in which the BEM had been present in these patients. Progression of a BEM to atrophy covering the whole fovea was observed in the follow-up data of patient G-II:1.

Perimetry revealed visual field constriction resulting in tunnel vision of 20° or less in nine patients (mean age: 48 years; range, 23–66 years; Fig. 5). Macular involvement was apparent by a gradual decrease in central sensitivity, which we observed in 10 patients (mean age: 40 years; range, 23–66 years). We observed paracentral scotomas in two patients (C-II:2 and C-II:3) with a relatively intact (mid)peripheral visual field (Fig. 5C). Additionally, paracentral scotomas were present in patients F-II:1 and F-II:2, in whom only the central 30° was analyzed with the more sensitive static perimeter (Table 1). Electroretinographic responses were nonrecordable in 11 patients (mean age: 51 years). In five patients (mean age: 31 years), a severely reduced photoreceptor dysfunction was seen in a rod-cone pattern. Evaluation of color vision resulted in an isolated tritan defect in patients A-II:6, G-II:1, and G-II:2 at ages 24, 40, and 39, respectively, whereas patients C-II:2 and C-II:3 demonstrated strong protan, deutan, and tritan defects at ages 33 and 36, respectively. In patient A-II:5, we did not detect color vision defects.

Fundus autofluorescence (FAF) images revealed macular involvement in all eight patients for whom FAF imaging was available. The macular aspects varied from hyperautofluorescence to profound hypoautofluorescent RPE lesions (mean age: 51 years; range, 23–67 years, Figs. 3E–G, 3I, 4B). Midperipheral changes include granular or mottled hypoautofluorescent changes that are spread to the vascular arcades. In some patients, large (confluent) hypoautofluorescent lesions were observed just anterior of the vascular arcades (Figs. 3E, 3H). Evaluation of the central retinal structure with SD-OCT revealed loss of photoreceptors before RPE cell loss, which eventually result in moderate to severe retinal thinning (Fig. 6A). The foveal volume in seven Dutch RP patients (mean age: 49 years; range, 23–67) was significantly lower compared with the foveal volume in 25 age-matched Dutch healthy controls (P < 0.0001; Fig. 6B). In all patients with high-resolution SD-OCT, except in G-II:2, the bands corresponding to the photoreceptor inner and outer segments were lost. The outer nuclear layer, containing the photoreceptor cell bodies, was concentrically lost and severely thinned where present. We observed concentric atrophy of the layer that corresponds to the RPE cells that progressed from the midperiphery. The central abnormalities in the RPE layer started in the parafoveal region.

Figure 4. Multimodal imaging of the central retina in patient G-II:2 at the age of 59. (A) Fundus photograph showing a BEM, attenuated vessels, peripapillary atrophy, pale optic disc, bone-spicule pigmentation, and chorioretinal atrophy in the midperiphery. (B) Autofluorescence imaging shows a subtle hyperautofluorescent ring around the macula, spots of decreased macular and peripheral autofluorescence, and absence of autofluorescence corresponding with the peripapillary atrophy. (C) Spectral domain OCT reveals loss of the bands corresponding to the photoreceptor inner and outer segments in the macula. The external limiting membrane is present only in the fovea. The RPE layer seems fairly intact, except for irregular thinning in the fovea and loss of RPE in the peripapillary region. (D) Infrared en face image reveals the location of the SD-OCT image (green line).
as was observed in four patients who also revealed a BEM (Fig. 6D). In patient J-II:1, who displayed mottling in the macula, the RPE appeared normal on SD-OCT (Fig. 6E). In later stages of the disease, we observed loss of the foveal RPE layer, which was highlighted by an increased beam penetration and choroidal reflection in patients E-II:4 and G-II:1 (Fig. 6F). Spectral-domain OCT scans in the midperiphery revealed loss of the photoreceptor-RPE complex and intraretinal pigment deposits in patients G-II:1 and G-II:2 at ages 60 and 59, respectively (Fig. 6G), whereas patient J-II:1 at the age of 23 revealed only photoreceptor loss (Fig. 6H).

Mutation Analysis

A description of the molecular genetic findings in families A to F were reported earlier. In summary, sequence analysis of all 19 coding exons of IMPG2 led to the identification of 10 different pathogenic variants in these 17 patients (Table 2). In the families with available family members, the homozygous or compound heterozygous mutations segregated completely with the RP phenotype (Fig. 1). In addition to the mutations described earlier, four new pathogenic variants in IMPG2 were identified. In family G, a targeted next-generation sequencing approach that covered 111 blindness genes resulted in two compound heterozygous mutations: a nonsense mutation (p.Arg127*), which is predicted to cause premature truncation of the IMPG2 protein, and a 4-base-pair deletion (c.3423-8_c.3423-5del) that affects the splice acceptor site (Table 2).

Reverse transcriptase PCR analysis on RNA isolated from patients' lymphoblastoid cells revealed that, instead of the regular splice acceptor site, a second splice site located upstream in the same intron is used that results in the inclusion of 80 additional nucleotides to IMPG2 mRNA, subsequently leading to a frameshift and premature termination of IMPG2 (Supplementary Fig. S1). The cDNA products generated from RNAs isolated from cells grown under nonsense-mediated decay (NMD)-suppressing conditions show subtle differences compared with those generated from RNAs isolated from cells grown under normal conditions. Growing cells under NMD-suppressing conditions did not yield an obvious difference in the amount of aberrantly spliced IMPG2, indicating that a truncated protein may be produced. The other novel mutations include a nonsense mutation (p.Tyr171*) and a missense mutation (p.Ser379Pro). The nonsense mutation is predicted to cause a premature truncation of IMPG2. The p.Ser379Pro missense mutation changes a highly conserved amino acid and is unanimously predicted to be pathogenic by multiple in silico prediction tools (SIFT: deleterious [score: 0], Polyphen-2: probably damaging [score: 1.000], Align GVGD: Class C65, MutationTaster: disease causing [probability: 0.994], Grantham score: 74, PROVEAN prediction: deleterious [score: −2.972]).

DISCUSSION

The Phenotype of IMPG2-Associated RP

In this study, we provide a detailed clinical description of the RP associated with mutations in IMPG2, a gene that recently was added to the long list of genes that may cause autosomal recessive RP when mutated. Most of the patients with IMPG2-associated RP demonstrated the classic RP symptoms: night blindness and progressive concentric loss of the visual field. However, 6 of 17 patients mentioned a decrease in BCVA that could not be attributed to amblyopia as the initial symptom. Loss of vision as the initial symptom is not just a result of our electrically illuminated nighttime environment that compensates for an impaired night vision, but a consequence of macular abnormalities that are a prominent feature of this type of RP. Overall, the BCVA progressively decreased during the first 4 decades of life, and subsequently deteriorates to levels lower than 20/500 during the fifth and sixth decades of life. The only exception was patient G-II:2, who still enjoyed a BCVA of 20/30 at the age of 59.

Thirteen of the 17 RP patients included in this study showed significant macular abnormalities: a BEM was observed in six patients (mean age: 51 years) and profound macular atrophy in seven patients (mean age: 57 years). We hypothesize that the perifoveal atrophy, manifesting as a bull’s eye pattern,
eventually progresses to macular atrophy with profound
degeneration of photoreceptors and RPE, although this was
observed only in patient G-II:1 because longitudinal data of the
other patients with macular atrophy were not available. The 13
patients with macular involvement displayed a decreased
central sensitivity and/or paracentral scotomas on visual field
examination in addition to the concentric constriction that is
typically seen in RP (Table 1; Fig. 5). Spectral-domain OCT of
the macula showed early loss of photoreceptor inner and outer
segments before loss of the RPE layer in the central retina. On
FAF imaging, macular hypoautofluorescence was observed,
whereas hypoautofluorescence in the midperiphery generally
had a granular aspect. In contrast to the significant macular
involvement that was observed in most patients, subtle
macular FAF abnormalities appeared in patients C-II:2 and J-
II:1. However, subtle macular FAF abnormalities have been
observed in other forms of RP with intact central vision and
therefore do not automatically predate loss of macular
function.25

A BEM is a nonspecific reaction of the posterior pole, which
can occur in various diseases affecting the bipolar cell layer,
photoreceptor cell layer, or RPE.26 It is not often observed in
RP, but has been reported in some specific forms of syndromic
and nonsyndromic RP,27–31 and is associated with a faster
deterioration of the visual acuity compared with RP without
specific macular lesions.32 Concerning the BEM in IMPG2-
associated RP, multimodal imaging revealed abnormalities in
the photoreceptor and RPE cell layers. However, it is unclear
why RPE abnormalities initially predominate in the perifoveal
region, as the preceding abnormalities in the photoreceptor
layer are ubiquitously present. Possible explanations may
include topographical differences in metabolism and cell
densities,33 the higher vulnerability of S ("blue") cone
photoreceptors to retinal disease compared with M and L
cones,34 and the higher vulnerability of parafoveal rods to
aging and light-induced damage.35–37 In patient G-II:2 (age 59),
we observed a prominent BEM due to hypopigmentation
rather than atrophy of the perifoveal RPE, as there were only
minor RPE changes on FAF imaging (Fig. 4B) and mild changes
of the band corresponding to the photoreceptor outer
segment–RPE complex (Fig. 4C). By contrast, other patients
with BEM revealed perifoveal hypoafluorescence indicating

Figure 6. Optical coherence tomography examinations in patients with IMPG2-associated RP. (A) Thickness of the total retina in eight Dutch
patients (mean age: 49 years; range, 25–67). Shaded areas: normal limits (mean ± 2 SDs) as measured in 25 controls (mean age: 46 years). (B)
Foveal volume (mm3) measured in the central 3 mm2 in these patients except patient A-II:1. The foveal volume in seven Dutch IMPG2-associated RP
patients (mean age: 49 years; range, 25–67) was significantly lower than in 25 age-matched healthy Dutch controls (P < 0.0001). The horizontal bars
indicate the median of the corresponding cohort. (C) Spectral-domain OCT scan of a normal central retina (age 25). The hyporeflective bands
correspond to the external limiting membrane (1), the ellipsoid photoreceptor inner segments (2), the photoreceptor outer segment/RPE contact
cylinder region (3), and the RPE (4).24 (D) Spectral-domain OCT of patient A-II:6 (age 44) that reveals generalized loss of the outer retinal layers,
whereas the RPE reflectance is fairly intact except for the irregular signal and thinning in the temporal parafovea (white arrowhead). (E/H)
Spectral-domain OCT in patient J-II:1 (age 25). The central retina (E) revealed normal bands corresponding to the RPE, whereas the photoreceptor
bands are absent outside the fovea. The midperipheral retina (H) reveals loss of the photoreceptor inner and outer segment bands. However, no
profound RPE atrophy or intraretinal pigment deposits were observed yet. (F and G) Spectral-domain OCT of patient G-II:1 (age 60). The central
retina (F) reveals generalized loss of photoreceptor inner and outer segments, a thinned outer nuclear layer, and patchy loss/thinning of the foveal
RPE. The midperipheral retina (G) shows profound loss of the outer retinal layers, RPE and choriocapillaris reflectance, as well as intraretinal
pigment deposits.
Table 2. Mutations Identified in the IMPG2 Genes in the Patients With Inherited Retinal Disease Included in This Study

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Nonsense mutations change a DNA codon for an amino acid in a stop codon, inducing a premature truncation of the protein. Deletions remove one or more nucleotides from the DNA, which can alter the reading frame. In these cases, the deletions cause frameshifts and prematurely truncated proteins. Missense mutations change a DNA codon for an amino acid in a codon for another amino acid. These mutations can have structural or functional effects on the protein, depending on the domain in which the mutation occurs. Missense mutations generally have less-severe effects on protein function when compared with nonsense mutations or frameshift-inducing deletions. The p.Arg906* mutation was identified frequently in the Dutch patients included in this study (9/22 alleles, 41%) and may be a Dutch founder mutation. AR, autosomal recessive; NI, not identified; del, deletion; *, premature stop.
perifoveal RPE atrophy (Figs. 3E, 3G). In late stages of the disease, profound macular hypoaufotofluorescence developed, which is indicative of RPE atrophy (Fig. 3H). In the light of future therapeutic options for retinal dystrophies, knowledge of the natural course of IMPG2-associated retinal disease is necessary to select patients amenable for treatment and to correctly interpret the effect of therapeutic intervention.

**Genotype-Phenotype Correlation**

Bandah-Rosenfeld et al. identified mutations only with severe effects on the IMPG2 protein in RP patients, whereas a homoygous mild missense mutation was identified in one patient with a mild maculopathy. In families G to K, we identified two novel truncating nonsense mutations (families G and H), a deletion causing a splice defect (family G), and a missense mutation that is unanimously predicted to be pathogenic (family K). The function of IMPG2 is vital for retinal survival and function, as most IMPG2 mutations that are identified in our patients can be considered true loss-of-function alleles. Until now, only one homoygous mild (missense) mutation in IMPG2 has been described in a single patient with an isolated maculopathy and a mildly affected visual function. This might indicate that a minimal loss of function of the IMPG2 protein may result in mild or even absent retinal disease.

Each of the seven nonsense mutations lead to either mRNA breakdown because of nonsense-mediated decay, or predicted truncated IMPG2 proteins that all lack the transmembrane domain and the cytoplasmatic tail. The in-frame deletion identified in family A (c.888-1554_908-274del; absence of seven amino acids) is thought to result in a nonfunctional IMPG2 protein, as in a cellular transfection assay this mutant protein was retained in the endoplasmic reticulum, whereas IMPG2 is physiologically located in the cell membrane. The 4-base-pair deletion of the splice acceptor site in family G (c.3423-8_c.3423-5del) was found to result in the use of an alternative splice acceptor site, and thereby also predicted to result in the generation of a truncated protein that most likely has reduced or no remaining function. Interestingly, the p.Arg906Stop was present in 8 of 20 alleles (40%) in 10 Dutch patients, which may imply a founder mutation in the Dutch population.

Because the identified mutations cause (near-)complete loss of IMPG2 function, the clinical variation is limited in IMPG2-associated RP. The patients in family G, however, retained slightly better visual acuity and visual field compared with the other patients (Fig. 2; Table 1). This could imply that the splice defect in this family results in an IMPG2 protein with some residual function. However, functional assays are needed to reveal the true effect of the c.3423-8_c.3423-5 deletion, as there also is evidence of modifying factors in this family (Table 1; Figs. 2, 3B, 3H, 4) that influence the intrafamilial differences.

The IMPG2 protein (SPACR) is highly homologous to SPACR1, the product of the interphotoreceptor matrix proteoglycan 1 (IMPG1) gene, which has been linked to benign concentric annular macular dystrophy (BACM) and vitelliform macular dystrophy (VMD). Interestingly, BCAMD includes parafoveal hypopigmentation and RP-like fundoscopic changes in the end-stage disease, although visual acuity is generally better preserved than in the IMPG2-associated RP patients in this report. The IMPG1-associated vitelliform dystrophy is also associated with macular pathology, although this is characterized by accumulation of lipofuscin rather than a BEM.

In conclusion, severe mutations in IMPG2 are the cause of an autosomal recessive RP phenotype that manifests in the early teens and is accompanied by atrophic maculopathy often in a bull's eye pattern. In early disease stages, the maculopathy is characterized by mild RPE alterations, but in later stages of the disease, a BEM and profound macular choriotiretal atrophy may occur. In most patients, the RP phenotype arising from mutations in the IMPG2 gene is severe, because of the unfortunate combination of progressive constriction of the visual fields and maculopathy that occurs relatively early in the course of the disease.

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**References**


