Improved Genetic Diagnostics of *RPGR* ORF15 - associated Retinal Dystrophy

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**Summary**
- The exon ORF15 of *RPGR* is a mutational hotspot for XLRP accounting for two-thirds of all disease-causing mutations.
- We have developed an NGS-based diagnostic test with 100% sensitivity for variants in the difficult-to-sequence region in the ORF15.
- Clinical validation study of 16 patients with a clinical suspicion of XLRP and a negative result from previous genetic testing revealed a truncating variant in the ORF15 in 25% of the cases (4/16).
- In an unselected set of 386 patients analyzed with the BpG 266-gene Retinal Dystrophy Panel, the *RPGR* molecular diagnosis was found in 6.6% of the patients.

**Background**

Retinitis pigmentosa (RP) is the most common form of inherited retinal degeneration affecting around 1,300 individuals worldwide. Classical RP is characterized by progressive peripheral vision loss and night vision difficulties that can lead to central vision loss. RP is clinically and genetically heterogeneous. The majority of the X-linked RP, associated with a severe phenotype, is caused by mutations in the *RPGR* gene. All known mutations causing *RPGR*-related retinal dystrophies are found to affect the *RPGR*ORF15 isoform, which contains a unique C-terminal 567-aa exon called ORF15 (Figure 1). ORF15 is a mutational hotspot for *RPGR*-associated RP, accounting for two-thirds of all disease-causing mutations. The exon ORF15, however, includes a highly repetitive, purine-rich sequence, which generally performs poorly in next-generation sequencing (NGS)-based assays. To address the clinical importance of the *RPGR* ORF15 and the lack of high quality NGS-based diagnostics, we have developed a novel test to detect variants in the ORF15 region.

![Figure 1. Schematic representation of the *RPGR* isoform NM_001034853.1. The 567-aa ORF15 repetitive domain (glutamic acid/glycine-rich) is a mutational hotspot for XLRP. (Fig from Megaw et al. 2015 Exp Eye Res)](image)

**Results**

Blueprint Genetics WES assay shows improved coverage in the exon ORF15 of *RPGR* compared to other NGS technology (Figure 2). A custom Sanger sequencing assay was developed for the 400-600-bp difficult-to-sequence region. This diagnostic test combines NGS analysis with Illumina NovaSeq 6000 platform and Sanger sequencing.

Diagnostic assay was validated with seven samples with a known variant in the ORF15 region and showed 100% sensitivity to detect variants in this region (Table 1 and Figure 3).

Clinical validation with 16 patients with a clinical suspicion of XLRP and a negative result from a previous genetic testing revealed a truncating variant in ORF15 in 25% (4/16) of the patients (Table 1). Since 1st of March 2018, the BpG 266-gene Retinal Dystrophy Panel has identified likely pathogenic or pathogenic variant in *RPGR* in 25 out of 386 patients (6.6%).

### Table 1. *RPGR* ORF15 variants confirmed in assay validation cases (7/7) and identified in clinical validation cases (4/16).

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>Variant (NM_001034853.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>n/a</td>
<td>c.2625dupA, p.(Gly876Argfs*203)</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>n/a</td>
<td>c.2176G&gt;T, p.(Glu726*)</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>n/a</td>
<td>c.214G&gt;T, p.(Glu716*)</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>n/a</td>
<td>c.2236_2237del, p.(Glu746Argfs*23)</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>25 years</td>
<td>c.2442_2445del, p.(Gly817Lysfs*2)</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>32 years</td>
<td>c.2601_2602del, p.(Glu868Glyfs*210)</td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>30 years</td>
<td>c.2548del, p.(Glu850Lysfs*239)</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>8 years</td>
<td>c.2993_2997del, p.(Glu998Glyfs*79)</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>4 years</td>
<td>c.2763_2764del, p.(Glu922Glyfs*156)</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>11 years</td>
<td>c.2655_2656del, p.(Glu886Glyfs*192)</td>
</tr>
<tr>
<td>11</td>
<td>Male</td>
<td>4 years</td>
<td>c.2867del, p.(Glu956Glyfs*133)</td>
</tr>
</tbody>
</table>

**Figure 2. Coverage at the ORF15 region of *RPGR*. The BpG WES assay shows improved coverage at ORF15 compared to another NGS technology.**

**Figure 3. Validation of the ORF15 diagnostic test, an example of *RPGR* c.2601_2602del, p.(Glu868Glyfs*210). NGS data (A), Sanger validation (B).**

**Methods**

The WES capture was performed with IDT xGen Exome Research Panel assay and sequencing was performed at Blueprint Genetics (BpG) using an Illumina NovaSeq sequencing system. Performance of the assay was assessed by using reference samples with high-quality sequence variant calls. The validation of the detected ORF15 variants was performed with custom Sanger sequencing method. The difficult-to-sequence region was amplified with long-range PCR followed by nested PCR using a custom reaction mixture and analyzed with ABI 3500xL genetic analyzer.

Conflict of interest statement: All authors are employed by Blueprint Genetics.