Prevalence and characteristics of RPGR ORF15 variants in patients with inherited retinal dystrophies

J. Sistonen¹, S. Tuupanen¹, K. Kämpjärvi¹, P. Siivonen¹, M. Mehine¹, J. Känsäkoski¹, K. Wells¹, J. Schleit¹, M. Valori¹, P. Salmenperä¹, E.M. Sankila², E. Salminen¹, T. Alastalo¹, J. Koskenvuo¹, S. Myllykangas¹

¹ Blueprint Genetics, Helsinki, Finland.
² Helsinki University Eye Hospital, Helsinki, Finland.

Introduction

Pathogenic variants in RPGR account for 80% of cases with X-linked retinitis pigmentosa (XLRP). The C-terminal 567-aa exon ORF15 is a mutational hotspot for RPGR-associated RP. However, it generally performs poorly in standard sequencing-based assays due to a highly repetitive glutamic acid/glycine-rich sequence (Figure 1). To address the clinical importance of the RPGR ORF15 and the lack of high-quality next-generation sequencing (NGS)-based diagnostics, we aimed to develop a comprehensive high-throughput clinical test for inherited retinal dystrophies (IRD), and to specifically evaluate the performance of RPGR ORF15 sequencing in a patient cohort.

Figure 1. Schematic representation of the RPGR isoform NM_001034853.1.¹

Methods

We optimized a whole-exome sequencing (WES) workflow with the Illumina NovaSeq 6000 platform to cover 266 retinal dystrophy-associated genes, including the difficult-to-sequence region in RPGR ORF15. We evaluated the prevalence and characteristics of RPGR variants in a cohort of 1587 unselected patients with IRD. Additionally, a custom confirmatory Sanger sequencing method was developed.

RPGR coverage

The whole RPGR gene and specifically the ORF15 exon showed high median coverage and excellent mapping quality (Figure 2 and Table 1).

Figure 2. Sequence coverage at the RPGR ORF15 exon. Blueprint Genetics’ WES assay, based on IDT xGen Exome Research Panel, shows improved coverage at ORF15 compared to another NGS technology.

Table 1. Coverage metrics for RPGR, complete ORF15 exon and the ORF15 central region (aa p.824 - p.1077).

<table>
<thead>
<tr>
<th>Exon</th>
<th>MQ20 median coverage</th>
<th>MQ20 % covered &gt;20x</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPGR</td>
<td>125x</td>
<td>99.4</td>
</tr>
<tr>
<td>ORF15</td>
<td>163x</td>
<td>99.9</td>
</tr>
<tr>
<td>ORF15 p.824 - p.1077</td>
<td>130x</td>
<td>99.8</td>
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Results

In our clinical cohort of 1587 IRD patients, the overall diagnostic yield was 58%. A molecular diagnosis in RPGR was identified in 5.7% (90/1587) of the patients (Figure 3A). Female patients accounted for 24% of the diagnostic cases. A majority (70%) of the pathogenic (P) / likely pathogenic (LP) variants were frameshifts (Figure 3B). Seventy-one out of 90 (79%) P/LP variants were detected in the ORF15 (31% in the most difficult-to-sequence central region p.824 - p.1077) and 19 (21%) within the exons 1-14 (Figure 3C). RPGR explains approximately 9% of cases with RP. Diagnostic variants in ORF15 were confirmed using a custom Sanger sequencing method optimized for purine-rich sequence (Figure 4).

Figure 3. A) RPGR diagnostic yield among 1587 IRD patients B) Characteristics of diagnostic variants (n = 90) C) Distribution of the diagnostic variants within RPGR. A majority (79%) of the variants were in the ORF15 region.

Figure 4. An example of a patient with a diagnostic variant in the ORF15 difficult-to-sequence region, RPGR c.2601_2602del, p.(Glu866Glyfs*210). A) NGS data B) Sanger confirmation.

Conclusions

- We have developed a high-quality diagnostic test for IRD including the difficult-to-sequence region in RPGR ORF15
- RPGR explains 5.7% of the unselected patients with IRD
- 79% of the diagnostic RPGR variants were in the ORF15
- Female patients accounted for 24% of the diagnostic cases
- NGS-based assay including the complete RPGR ORF15 region is required for successful molecular diagnostics for patients with IRD

References:


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