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

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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.

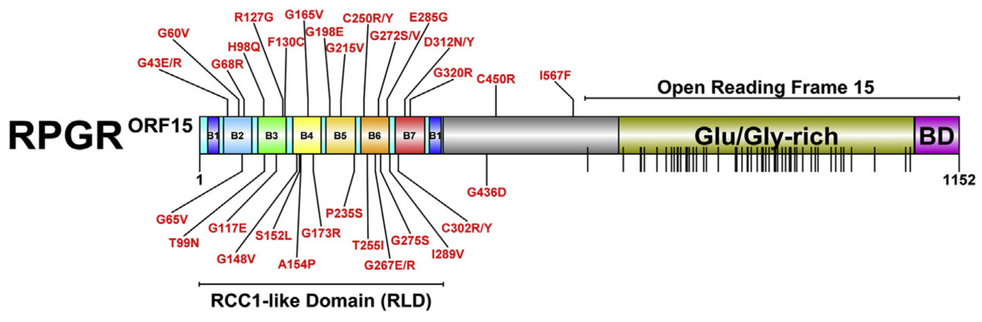

Figure 1. Schematic representation of the RPGR isoform NM_001034853.1.[1]

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**).

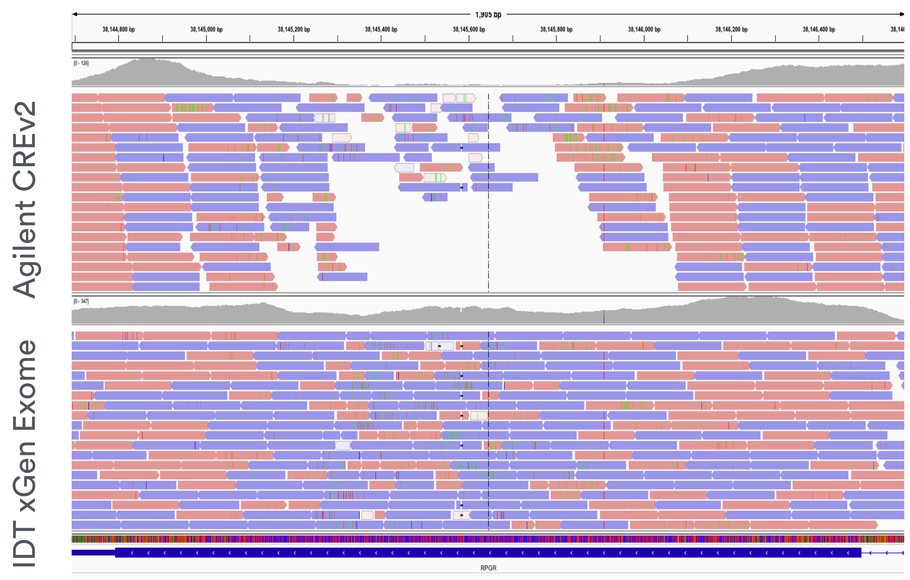

Figure 2. Sequence coverage at the *RPGR* ORF15 exon. Blueprint Genetics' WES assay, based on IDT xGen Exome Research Panel, shows improved coverage at ORF15 compared to another NGS technology.

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

	MQ20 median coverage	MQ20 % covered >20x
RPGR	125x	99.4
ORF15	163x	99.9
ORF15 p.824 - p.1077	130x	99.8

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**).

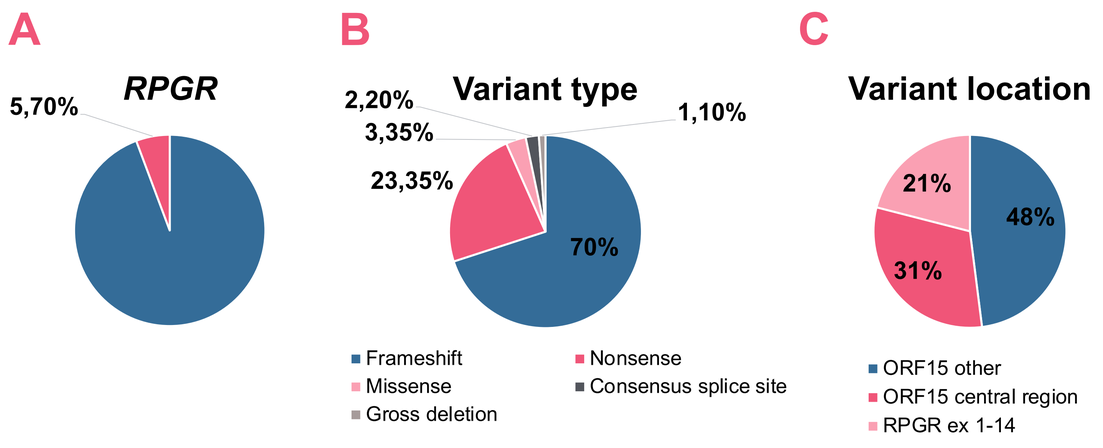

Figure 3. **A)** *RPGR* diagnostic yield among 1587 IRD patients **B)** Characteristics of diagnostic variants (n = 90) **C)** Distribution of the diagnostic variants within *RPGR*. A majority (79%) of the variants were in the ORF15 region.

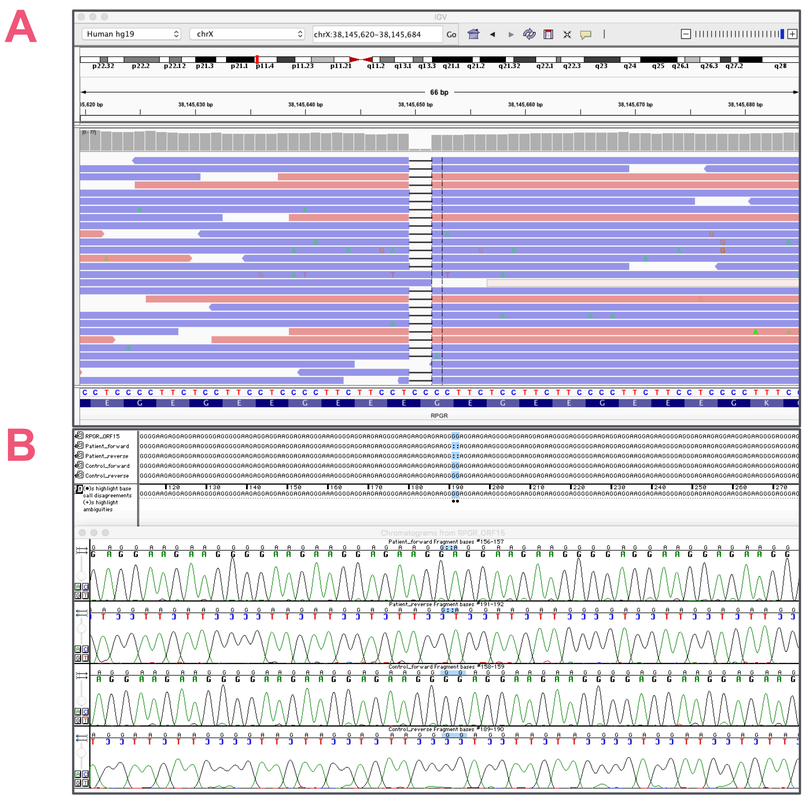

Figure 4. An example of a patient with a diagnostic variant in the ORF15 difficult-to-sequence region, *RPGR* c.2601_2602del, p.(Glu868Glyfs*210). **A)** NGS data **B)** Sanger confirmation.

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

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References:

1. Megaw RD, Soares DC, Wright AF. RPGR: Its role in photoreceptor physiology, human disease, and future therapies. *Exp Eye Res* 2015;138:32-41.

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