Difficult-to-sequence genes

Improving clinical sensitivity in difficult-to-sequence genes for rare hereditary disorders

The Blueprint Genetics approach to providing tailored diagnostic services is rooted in **a specialized team dedicated to troubleshooting and developing custom solutions for challenging genetic regions.** Our Clinical Research and Development (R&D) team consists of genetics and molecular biology professionals whose core focus is on genomic regions too complex to resolve with standard NGS analysis, and developing new methods to analyze technically challenging but clinically important genes.

Difficult-to-analyze genomic regions include genes that have

- Pseudogenes
- Other highly homologous regions in the genome
- Longer stretches of repetitive sequences

The Blueprint Genetics clinical R&D team's work is driven by the opportunity to tackle the most challenging parts of our genome and by the reward inherent in finding a genetic diagnosis for a patient.

Blueprint Genetics

The Blueprint Genetics approach to providing tailored diagnostic services

SMN1/SMN2 and spinal muscular atrophy

Spinal muscular atrophies (SMA) are autosomal recessive disorders characterized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy (OMIM #253300). Five types of SMA are recognized based on the age of onset, the maximum muscular activity achieved, and survivorship.[1] Homozygous loss of the survival of motor neuron 1 gene (*SMN1*), caused by deletions or point mutations, causes SMA (OMIM #253300). Homozygous absence of exon 7 of *SMN1* has been identified in approximately 95% of patients with SMA.[2]

Patient case: Patient is a 64-year-old male with spinal muscular atrophy type III diagnosed at the age of 27 years based on electromyoneurography (ENMG) and muscle biopsy findings. The patient presented with symptoms throughout childhood and adolescence, including clumsiness while running and difficulty climbing stairs. He is unable to transition independently, has generalized weakened control of body and cervical spine movements, and has shortness of breath. There is no known family history of similar disease.

Genetic testing: A **Blueprint Genetics Spinal Muscular Atrophy Panel** was requested, which tests for 30 genes, including copy number variants and assessment of known disease-causing, deep intronic variants. The **Spinal Muscular Atrophy Panel** is ideal for patients with a clinical suspicion of distal hereditary motor neuropathy or spinal muscular atrophy.

Diagnostic summary: Bioinformatic analysis developed specifically for copy number determination of the *SMN1* and *SMN2* genes identified a homozygous deletion including at least exon 7 of the *SMN1* gene, which is considered a marker for whole gene deletion. In addition, ≥3 copies of *SMN2* were detected. Considering the current literature and well-established role of *SMN1* deletions as pathogenic in association with autosomal recessive SMA, this finding was consistent with the patient's phenotype.

Diagnostic implications

- The diagnosis of SMA was confirmed
- Genetic counseling and family member testing are now available to family members to clarify their risk of carrying or developing the disease
- Because the genetic etiology of this patient's SMA and the copy number of *SMN2* has been identified, they may be eligible to participate in gene therapy clinical trials for patients with multiple copies of *SMN2*

PKD1 and autosomal dominant polycystic kidney disease

Patient information: Patient is a 25-year-old female diagnosed with polycystic kidney disease at 4 years of age. There is a family history of ADPKD in multiple generations.

Genetic testing: A **Blueprint Genetics Polycystic Kidney Disease Panel** was requested, which tests for 10 genes, including assessment of noncoding variants. The Polycystic Kidney Disease Panel is ideal for patients suspected of having autosomal dominant or autosomal recessive polycystic kidney disease.

Diagnostic summary: The patient was diagnosed as having a pathogenic heterozygous nonsense variant c.4957C>T, p.(Gln1653*) in exon 15 of *PKD1*. This variant was confirmed with Sanger sequencing using primers that were designed to maximize their *PKD1* specificity over several highly homologous pseudogenes. The variant is rare and causes a premature stop codon and is thus predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. The variant has been listed as pathogenic in the *PKD1* Mutation Database, where it has been reported in several families with ADPKD.

Diagnostic implications

- The diagnosis of ADPKD was confirmed
- Genetic counseling and family member testing are now available to family members to clarify their risk of developing the disease
- The molecular diagnosis allows for testing of asymptomatic family members to identify those at risk of developing disease in addition to identifying related kidney donors

RPGR and X-linked retinitis pigmentosa

X-linked retinitis pigmentosa (XLRP) accounts for 10%-20% of families with retinitis pigmentosa (RP) and is the most severe form of RP. Males with XLRP become symptomatic in early childhood and most have complete vision loss by the end of their third decade. Female carriers have a broad spectrum of fundus appearances, ranging from normal to extensive retinal degeneration. Typically, retinal disease in females with XLRP is less severe than that seen in males.

Variants in the *RPGR* gene are associated mainly with X-linked retinitis pigmentosa (XLRP, OMIM #300029), and mutations in this gene account for over 70% of patients with XLRP. *RPGR* (OMIM *312610) encodes a protein with a series of 6 RCC1- like domains (RLDs), characteristic of the highly conserved guanine nucleotide exchange factors. This protein localizes to the outer segment of rod photoreceptors and is essential for their viability.

Patient information: Patient is a 15-year-old male with retinitis pigmentosa. His brother and maternal great-uncle are also affected.

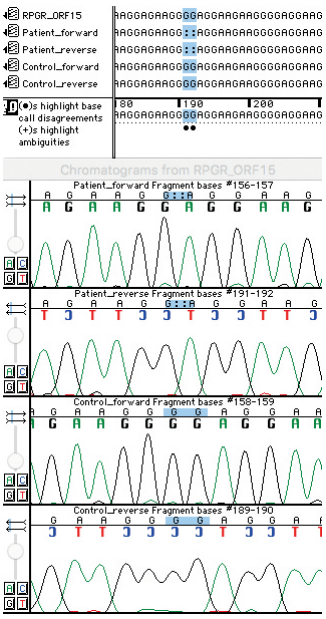
Genetic testing: A comprehensive **Blueprint Genetics Retinal Dystrophy Panel** was requested, which tests for 266 genes, including copy number variants and assessment of known disease-causing, deep intronic variants. The Retinal Dystrophy panel is ideal for patients with a clinical suspicion or diagnosis of isolated retinitis pigmentosa or isolated or syndromic retinal dystrophy.

Diagnostic summary: Sequence analysis identified a pathogenic hemizygous variant c.2601_2602del, p.(Glu868Glyfs*210) in the technically challenging *RPGR* ORF15 region, leading to the diagnosis of X-linked retinitis pigmentosa (XLRP, MIM#300029). The variant was confirmed with a custom Sanger sequencing method optimized for the ORF15 region consisting of a GA-rich repetitive sequence.

This variant is rare, results in a frameshift transcript with a premature stop codon, and is predicted to cause loss of normal protein function through protein truncation. The variant was initially reported in one family when Bader et al conducted a comprehensive screening for *RP2* and *RPGR* gene mutations including *RPGR* exon ORF15 in 58 index patients with X-linked retinitis pigmentosa (described as g.ORF15 848_849delGG,).[3] Subsequently, the variant has been reported in additional male patients with X-linked RP.

Diagnostic implications

- The diagnosis of *RPGR*-related X-linked retinitis pigmentosa was confirmed
- Genetic counseling and family member testing are now available to family members to clarify their risk of carrying the disease-causing variant or developing symptoms
- Because the genetic etiology of this patient's retinitis pigmentosa has been identified, they may be eligible to participate in gene therapy clinical trials for patients with pathogenic *RPGR* variants



Sanger confirmation of patient's variant c.2601_2602del, p.(Glu868Glyfs*210). Confirmation of variants found in *RPGR* ORF15 is made possible with a customized sequencing method, optimized for highly repetitive GA-rich sequences.

Other difficult-to-sequence and difficult-to-interpret genes by Blueprint Genetics

***ACAN*–gene is associated to, eg**, spondyloepimetaphyseal dysplasia (aggrecan type), spondyloepiphyseal dysplasia (Kimberley type), osteochondritis dissecans (short stature, and early-onset osteoarthritis). The gene has suboptimal coverage (>90% of the gene's target nucleotides are not covered at >20x with a mapping quality score of MQ>20 reads). **Analysis of this gene is improved by boosted coverage.**

For more information, visit genetic testing for malformations at Blueprint Genetics.

***FOXL2*–gene is associated to, eg**, premature ovarian failure, blepharophimosis (epicanthus inversus, and ptosis). Expansion of the polyAla-tract is an established disease mechanism in blepharophimosis, ptosis and epicanthus inversus syndrome (BPES) type II. **Analysis of this gene is improved by boosted coverage.**

For more information, visit genetic testing for ophthalmology and endocrinology at Blueprint Genetics.

***GBA*:** Blueprint Genetics custom assay has good coverage (>20x) with high mapping rates (mapping quality >40) for 100.0% of the target regions in the *GBA* gene. Our validation showed high mean coverage of 184X for the *GBA* gene. Thus, our NGS Panel is not expected to have major limitations in detecting variants in the *GBA* gene although clinical validation has not been performed at large scale for Gaucher disease. **Analysis of this gene is improved by boosted coverage.**

For more information, visit genetic testing for neurology, immunology, malformations, hematology, and metabolic disorders at Blueprint Genetics.

NCF1*:** The following exons are not included in our tests as they are not sufficiently covered with high quality sequence reads: *NCF1* (NM_000265:1,5,8,9,11). ***NCF1*– *gene is associated to, eg, chronic granulomatous disease. **Analysis of this gene is improved by boosted coverage, and a custom CNV analysis pipeline.**

For more information, visit genetic testing for immunology and hematology at Blueprint Genetics.

PKD1*:** Blueprint Genetics custom assay has good coverage (>20x) with high mapping rates (mapping quality >40) for 99.5% of the target regions in *PKD1* gene. Our validation showed high mean coverage of 199X for the *PKD1* gene. Thus, our NGS is not expected to have major limitations in detecting variants in *PKD1* gene although clinical validation has not been performed at large scale. ***PKD1*– *gene is associated to, eg, polycystic kidney disease. **Analysis of this gene is improved by boosted coverage, and a custom CNV analysis pipeline.**

For more information, visit genetic testing for neurology and gastroenterology at Blueprint Genetics.

RPGR ORF15*:** Blueprint Genetics custom assay has good coverage (>20x) with high mapping rates (mapping quality >20) for 100.0% of the target regions in *RPGR* gene. Our validation showed high mean coverage of 139X for the *RPGR* gene. Thus, our NGS Panel is not expected to have major limitations in detecting variants in the *RPGR* gene, including ORF15 exon. ***RPGR* – *gene is associated to, eg, retinitis pigmentosa, Cone-rod dystrophy (X-linked,1), macular degeneration (X-linkedatrophic), retinitis pigmentosa 3. **Analysis of this gene is improved by boosted coverage, and a custom CNV analysis pipeline.**

For more information, visit genetic testing for ophthalmology at Blueprint Genetics.

SMN1/SMN2*:** Can detect the copy number of *SMN1* exon 7, which is commonly used as a marker for copy number of the *SMN1* gene. Analysis includes only *SMN1* copy number analysis; sequence variants are not included in this test. "Silent" carriers of SMA (individuals with two copies of *SMN1* on one allele, and zero copies on the other allele) is not detected with this test. We do not include *SMN1* c.*3+80T>G as this is mostly uninformative in the general population. This variant is common in African American individuals (27% carrier frequency) where it poorly predicts *SMN1* 2+0 allele status and it is rare in Ashkenazi Jewish individuals (3.5% carrier frequency) where it reliably predicts *SMN1* 2+0 allele status (PMID: 23788250). ***SMNI/SMN2* – *gene is associated to, eg, spinal muscular atrophy. **Analysis of this gene is improved by a custom CNV analysis pipeline.**

For more information, visit genetic testing for neurology and malformations at Blueprint Genetics.

***SBDS*–gene is associated to, eg**, aplastic anemia, Shwachman-Diamond syndrome, severe spondylometaphysealdysplasia. Analysis of this gene is complicated by a pseudogene. **Analysis of this gene is improved by boosted coverage, a custom CNV analysis pipeline, and specific interpretation guidelines.**

For more information, visit genetic testing for immunology, malformations, hematology, and hereditary cancer at Blueprint Genetics.

***STRC*–gene is associated to, eg**, deafness. Exons 1-18 are not included in our panel testing as they are not sufficiently covered with high-quality sequence reads. We are able to detect variants in exons 19-29 of *STRC*, but our abilities are limited due to the high degree of homology that is shared between these exons and other regions of the genome. Whole gene deletions of *STRC* can and have been detected. **Analysis of this gene is improved by a custom CNV analysis pipeline. We also have this webinar from 2021: Navigating Genetic Diagnostics for Hereditary Hearing Loss.**

For more information, visit genetic testing for Ear, Nose, and Throat at Blueprint Genetics.

References: 1. Prior TW et al. Spinal Muscular Atrophy. GeneReviews® [Internet]. 2. Biros I, Forrest S. Spinal muscular atrophy: untangling the knot? *J Med Genet.* 1999;36(1):1-8. 3. Bader I et al. X-linked retinitis pigmentosa: RPGR mutations in most families with definite X linkage and clustering of mutations in a short sequence stretch of exon ORF15. *Invest Ophthalmol Vis Sci.* 2003;44(4):1458-63.