Custom Ampliseq Targeted Sequencing Panel for Orphan Pediatric Retinal Diseases: Norrie Disease, FEVR, and Retinoschisis

EYE RESEARCH INSTITUTE

MICHAEL SUN, WENDY DAILEY, AMANDA PETRELLI CICERONE, JENNIFER FELISKY, KAYLEE MOYER, NAOMI HAQUE, ALVARO GUZMAN, KENDRA MELLERT, KIMBERLY DRENSER, KENNETH MITTON

Eye Research Institute, Oakland University Rochester, MI, United States

Introduction:

Orphan retinal diseases such as Norrie Disease, Familial Exudative Retinopathy, and Retinoschisis, affect less than 200,000 people in the United States.[1] As a result of the rarity of these diseases, research is often difficult and sequencing to find the genetic basis of these diseases is expensive. Furthermore, sequencing for patients may not be covered by health insurance in the United States. We have developed a novel next generation iSeq 100 protocol to sequence 8 genes involved in Norrie Disease, FEVR, and Retinoschisis at a cost of $250 per person.

Aims and Objectives:

To develop a protocol to enable the sequencing and analysis of orphan diseases that is scalable for private clinic or small hospital use to aid in the diagnosis and genetic counseling of orphan disease.

Methods:

DNA was extracted from 100 uL samples of whole frozen blood using the ThermoFisher PureLink Genomic DNA Purification Kit. An Ampliseq targeted-panel (180 amplicons) for 8 genes was designed with illumina's DesignStudio Sequencing Assay Designer, distributed into three pools (PCR reactions) per patient sample for complete coverage of 83 exons with 25 bp adjacent intron sequence. Target Genes were: *NDP* (ChrX), *RS1* (Chr10); *CTNNB1* (Chr3); *TSPAN12* (Chr7); *KIF11* (Chr10), *FZD4* (Chr11), *LRP5* (Chr11), *ZNF408* (Chr11). Ampliseq libraries were quality controlled by capillary electrophoresis (Agilent Bioanalyzer) and several sample pool sizes were tested for capacity and sequencing coverage using sequencing and variant calling on the Illumina iSeq-100 platform.

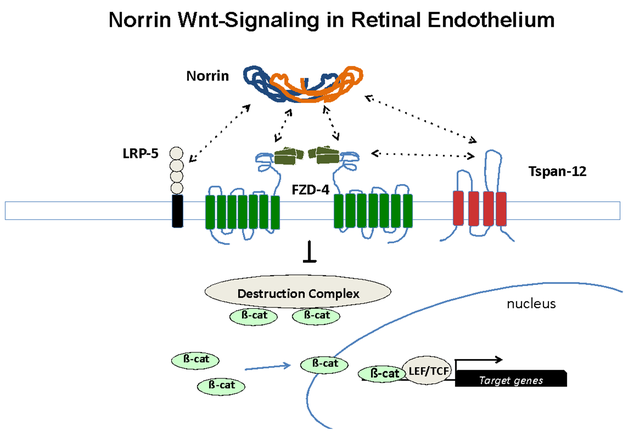

Figure-1. Norrin dimer binds to FZD-4 receptor and co-receptors LRP-5 and TSPAN-12. Activation of receptor complex causes inhibition of the β-Catenin degradation complex. β-Catenin enters the nucleus and modulates gene expression through interactions with the LEF/TCF family of transcription factors. Retinal endothelium proteins not shown: *KIF11*, motor protein, *ZNF408,* transcriptional co-factor required for normal retinal endothelial cell function, RS, extracellular matrix protein required for structural integrity.

	Average	Total
SNVs in Genes	19.31	946
SNVs in Exons	5.59	274
SNVs in Coding Regions	4.59	225
SNVs in UTR Regions	1.00	49
SNVs in Splice Site Regions	1.14	56
Stop Gained SNVs	0.04	2
Stop Lost SNVs	0.00	0
Non-Synonymous SNVs	0.92	45
Synonymous SNVs	3.63	178
Insertions in Genes	0.59	29
Insertions in Exons	0.20	10
Insertions in Coding Regions	0.16	8
Insertions in UTR Regions	0.04	2
Insertions in Splice Site Regions	0.00	0
Stop Gained Insertions	0.00	0
Stop Lost Insertions	0.00	0
Frameshift Insertions	0.04	2
Non-Synonymous Insertions	0.12	6
Deletions in Genes	0.55	27
Deletions in Exons	0.39	19
Deletions in Coding Regions	0.31	15
Deletions in UTR Regions	0.08	4
Deletions in Splice Site Regions	0.00	0
Stop Gained Deletions	0.00	0
Stop Lost Deletions	0.00	0
Frameshift Deletions	0.00	0
Non-Synonymous Deletions	0.31	15

Figure-2. Average statistics for each sample and total statistics for 48 sample run.

Results:

An average 2500-times read coverage was obtained for a pool of 16 patient libraries and 800-times for a pool of 48 patient libraries. Numerous potential disease-associated variants were detected in targeted libraries from patients diagnosed with Norrie Disease, FEVR, and Retinoschisis. Average number of variants per patient for the 48 patient pool were: 19.5 ± 1.6 SNVs, 0.6 ± 0.2 Inserts, and 0.6 ± 0.2 Deletions. Overall, 95.49% of base reads were Q30 and Amplicon mean coverage was 978. A total of 957 SNVs were found with 3 novel SNVs not found in dbSNP. A total of 83 insertions were found with 3 novel insertions not found in dbSNP. A total of 72 deletions were found with 10 novel deletions not found in dbSNP.

Conclusions:

We developed a targeted exome-sequencing protocol using Illumina Ampliseq reagents and the iSeq-100 platform for three rare pediatric retinal diseases. Coverage validated the ability to pool 40-50 patients per run for eight genes and provides for excellent base call accuracy (>Q30). Over 90 patient samples were successfully sequenced during validation. Potential mono and digenic variant contributions in FEVR patients are detectable by testing multiple genes. Research analysis costs were reduced to $250 per patient and now involve an academic eye research institute / retinal clinic research partnership.

References:

1. Center for Drug Evaluation and Research. (n.d.). Orphan products: Hope for people with rare diseases. Retrieved April 15, 2021, from https://www.fda.gov/drugs/information-consumers-and-patients-drugs/orphan-products-hope-people-rare-diseases