Custom Gene Panel Sequencing of FEVR Patients and Family Members

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Introduction

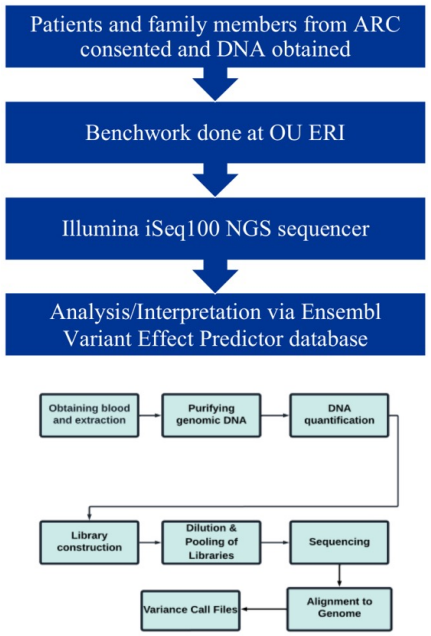
Familial exudative vitreoretinopathy, or FEVR, defines a group of inherited retinal diseases marked by abnormal blood vessel growth in the retina, causing poor vascular development in the peripheral retina. It is linked to increased vessel permeability and may result in retinal ischemia, exudative retinal detachment, and subsequent vision loss. It can be inherited through autosomal dominant, autosomal recessive, or X-linked recessive patterns. There is no definitive treatment or cure, but is treated based on staging of disease on an individual case-by-case basis. Genomics is a rapidly growing field in personalized medicine. Targeted next-generation sequencing (NGS) enables fast and cost-effective identification of genetic variations. NGS allows for the simultaneous sequencing of millions of base pairs of genetic information in just hours.

Aims and Objectives

To identify gene variants in pediatric vitreoretinal disease patients and their family members using a custom Illumina AmpliSeq orphan pediatric retinal disease gene panel. Our panel and sequencing protocol can sequence eight genes commonly associated with various inherited retinal diseases (IRDs) at greatly reduced cost and with a more streamlined sequencing approach.

Methods

Participants for DNA-sequencing analysis were referred to Associated Retinal Consultants, LLC, (ARC) of Royal Oak, Michigan, USA. Ten participants were consented under IRB approval for donation of blood to the ARC Eye Biobank, and for genetic analysis at Oakland University. A targeted 8-gene panel included 7 genes required for normal function of retinal vascular endothelial cells: NDP (ChrX), CTNNB1 (Chr3), TSPAN12 (Chr7), KIF11 (Chr10), FZD4 (Chr11), LRP5 (Chr11), ZNF408 (Chr11), and RS1 (ChrX). 180 amplicons, averaging 250 base pairs (bp), with overlapping sequence, targeted all exons and at 25 bp of adjacent intron sequence, totaling 32,000 bp. Genomic DNA was extracted from 200 uL of frozen whole blood, quantified, Illumina AmpliSeq libraries prepared, diluted, pooled, and sequenced with the Illumina iSeq-100. VCF files were analyzed with the Ensembl Variant Effect Predictor database.



Results and Conclusions

ID	Age	Gender	Family Group	Diagnosis	Gene	Coding consequence	Biotype	Amino Acids	Codons	SIFT	Poly Phen	HGVSp	AF	gnomADgAF	Significance
66984	6	Female		Stickler Syndrome	LRP5	Missense	Protein-coding	A/V	GCG/GTG	0.22	0.005	NP_002326.2:p.Ala1330Val	0.116	0.1107	benign (ClinVar)
					LRP5	In-frame insertion	Protein-coding	P/PLL	CCG/CCGCTGCTG	-	-	NP_002326.2:p.Leu19_Leu20dup	-	0.001262	(uncertain)
74746	57	Female		Possible FEVR	LRP5	In-frame insertion	Protein-coding	P/PL	CCG/CCGCTG	-	-	NP_002326.2:p.Leu20dup	0.0711	0.1014	(uncertain)
65017	58	Male	Grandfather of 35045		LRP5	Missense	Protein-coding	V/M	GTG/ATG	0	0.741	NP_002326.2:p.Val667Met	0.0184	-	benign, likely_benign (ClinVar)
41418	33	Female	Mother of	Possible FEVR	-	None	-	-	-	-	-	-	-	-	(uncertain)
38510	-	Female	Mother of 35045		CTNNB1	Missense	Protein-coding	Y/H	TAT/CAT	0	0.989	NP_00109167 9.1:p.Tyr142His	-	-	(uncertain)
					CTNNB1	Missense	Protein-coding	M/I	ATG/ATA	0.09	0.295	NP_00109167 9.1:p.Met363Ile	-	-	(uncertain)
					CTNNB1	Missense	Protein-coding	Y/F	TAT/TTT	0.08	0.671	NP_00109167 9.1:p.Tyr654Phe	-	-	(uncertain)
28857	-	Male	Father of 83597	Possible FEVR	TSPAN-12	Missense	Protein-coding	V/L	GTA/CTA	1	0.722	NP_036470.1:p.Val123Leu	0.0002	0.0007776	benign (ClinVar)
					LRP5	In-frame deletion	Protein-coding	PLLL/P	CCGCTGCTGCTG/CCG	-	-	NP_002326.2:p.Leu18_Leu20del	0.009008	0.003708	(uncertain)
86747	34	Female	Mother of 32024	Possible FEVR	LRP5	Missense	Protein-coding	T/M	ACG/ATG	0	1	NP_00127883 1.1:p.Thr271Met	-	1.31E-05	uncertain, likely pathogenic (ClinVar)
87965	59	Male		COAT	-	None	-	-	-	-	-	-	-	-	(uncertain)
62594	76	Female		Dry AMD OU	ZNF408	In-frame deletion	Protein-coding	AVVTE/A	GCAGTGGTGACAGAA/GCA	-	-	NP_00117168 0.1:p.Val186_Val189del	0.2181	0.141	benign (ClinVar)
					FZD4	Missense	Protein-coding	M/V	ATG/GTG	0.05	0.53	NP_036325.2:p.Met105Val	-	4.60E-05	pathogenic (ClinVar)
12083	41	Female	Mother of 22642	Possible FEVR	LRP5	Missense	Protein-coding	V/M	GTG/ATG	0.09	0.927	NP_00127883 1.1:p.Val86Met	0.0184	0.03475	benign, likely_benign (ClinVar)
					LRP5	Missense	Protein-coding	A/V	GCG/GTG	0.2	0.005	NM_0012919 02.2:c.2246C>T	0.116	0.1107	benign (ClinVar)

Two variants, likely pathogenic and pathogenic, were found impacting LRP5 and FZD4 respectively out of 10 samples.

Our method of custom gene panel sequencing can be done in most academic biochemistry labs and have significant impact on FEVR patients and their families. There is great potential for NGS for precision/personalized medicine and gene-mutation-specific treatments in the future. Research DNA-sequencing also provides applied clinical genetics training for medical, undergraduate, and graduate trainees. Since the completion of this sequencing, the gene panel used in the lab has been expanded to 30 total genes to include more genes implicated in inherited retinal diseases.

References

Criswick VG, Schepens CL. Familial exudative vitreoretinopathy. *Am J Ophthalmol* 1969; 68: 578–594.

Trese MT, Capone A Jr. Familial exudative vitreoretinopathy. In: Hartnett ME, ed. *Pediatric Retina*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:425-428.

Boyce T, Ramshekar A, Chau F, et al. Familial exudative vitreoretinopathy (FEVR). *Eye Wiki*. Updated July 28, 2024. Available from: https://eyewiki.aao.org/Familial_Exudative_Vitreoretinopathy_(FEVR)

Hess JF, Kohl TA, Kotrová M, et al. Library preparation for next generation sequencing: A review of automation strategies. *Biotechnology Advances*. 2020;41:107537. doi:10.1016/j.biotechadv.2020.107537.

Wang Z, Chen C, Sun L, Zhang A, Liu C, Huang L, Ding X. Symmetry of folds in FEVR: A genotype-phenotype correlation study. *Exp Eye Res*. 2019 Sep;186:107720.
Li JK, Li Y, Zhang X, Chen CL, Rao YQ, Fei P, et al. Spectrum of Variants in 389 Chinese Probands With Familial Exudative Vitreoretinopathy. *Invest Ophthalmol Vis Sci*. 2018;59(13):5368-81.

Cicerone AP, Dailey W, Sun M, et al. A Survey of Multigenic Protein-Altering Variant Frequency in Familial Exudative Vitreo-Retinopathy (FEVR) Patients by Targeted Sequencing of Seven FEVR-Linked Genes. *Genes (Basel)*. 2022;13(3):495. Published 2022 Mar 11. doi:10.3390/genes13030495

Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. Cell. 2013;155:27–38.xszxs

Gulilat, M., Lamb, T., Teft, W.A. *et al*. Targeted next generation sequencing as a tool for precision medicine. *BMC Med Genomics* 12, 81 (2019). https://doi.org/10.1186/s12920-019-0527-2

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