In-House Targeted Gene Sequencing Reveals Rare Variants Linked to FEVR, Norrie Disease and Persistent Fetal Vascular Syndrome

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Introduction

Familial Exudative Vitreoretinopathy (FEVR), Norrie Disease (ND), and Persistent Fetal Vascular Syndrome (PFVS) belong to a family of retinopathies that are often present at birth and are associated with irregular development of the retinal microvasculature and subsequently decreased vision[1]. All three conditions have been linked to variants in the *NDP* gene located on the X chromosome, which encodes for Norrin — the ligand for the Frizzled-4 Wnt receptor[2]. FEVR has been linked to variants in at least 7 genes *(NDP, FZD-4, LRP-5, TSPAN-12, ZNF408, CTNNB1, KIF11),* and sequencing of affected patients suggests that it is a multigenic condition with incomplete penetrance [3,4]. Custom, targeted, next generation sequencing panels can play a significant role in identifying novel variants in a way that is efficient and economical, which ultimately benefits the patient, researchers, and clinicians involved in their care.

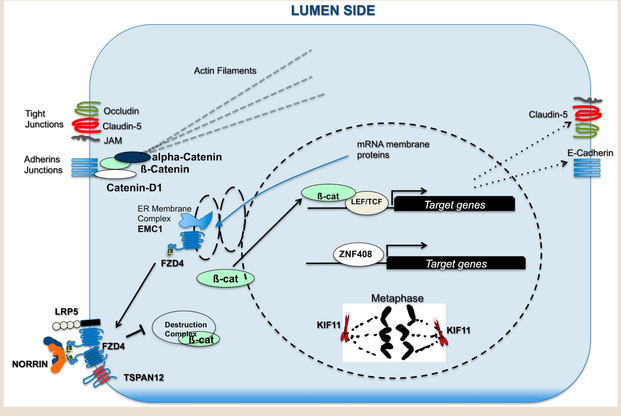

Figure 1. Roles of NDP, FZD-4, LRP-5, TSPAN-12, ZNF408, CTNNB1, and KIF11 in the cell[4]. NDP, FZD-4, LRP-5, and TSPAN-12 make up the canonical Wnt-signaling complex, while ZNF408, CTNNB1, and KIF11 encode a zinc-finger transcription factor, ß-catenin, and kinesin motor protein, respectively. (From graphical abstract [4] , Le et al., 2023)

Methods

- Patients seen by physicians at Associated Retinal Consultants, PC were consented, and a sample of their blood was obtained.
- DNA was extracted and quantified from blood, followed by the creation of custom Ampliseq gene libraries.
- Quality of libraries was confirmed via the Agilent 4150 Tapestation System.
- Sequencing was carried out with the Illumina iSeq-100 instrument and VCF files were analyzed via the Ensembl Variant Effect Predictor Database.
- Variant consequences were assessed via ClinVar.

Patient ID	Diagnosis	Sex	Age	Gene	Variant	Coding Consequence	Frequency	Consequence
1	ND	M	3 months	NDP	NP_000257.1:p.Arg37Ter	stop gained	0.00000	likely pathogenic
				LRP5	NP_002326.2:p.Leu20dup	inframe insertion	0.02077	benign
2	PFVS	M	6 Months	NDP	NP_000257.1:p.Met32TrpfsTer9	frameshift, stop gained	0.00000	likely pathogenic
				FZD4	NP_036325.2:p.Pro335er	missense	0.01735	likely benign
				FZD4	NP_036325.2:p.Pro168Ser	missense	0.01836	benign
				LRP5	NP_002326.2:p.Leu20dup	inframe insertion	0.02077	benign
3	ND	M	4 Months	NDP	NP_000257.1:p.Leu13_Met19del	inframe deletion	0.00000	likely pathogenic
				ZNF408	NP_079017.1:p.Val194_Val197del	inframe deletion	0.15560	benign
4	Poss FEVR	M	78	ZNF408	NP_079017.1:p.Ser225Phe	missense	0.00000	uncertain significance
5	FEVR	F	20	ZNF408	NP_079017.1:p.Ser225Phe	missense	0.00000	uncertain significance
6	FEVR	F	9 months	TSPAN12	NP_036450.1:p.Val123Leu	missense	0.00795	benign
				LRP5	NP_002326.2:p.Leu7_Leu18dup	inframe insertion	0.00000	uncertain significance
				LRP5	NP_002326.2:p.Leu8_Leu20del	inframe deletion	0.00000	likely benign
7	Poss FEVR	F	3	LRP5	NP_002326.2:p.Leu20dup	inframe insertion	0.02077	benign
8	Poss FEVR	M	51	LRP5	NP_002326.2:p.Leu20dup	inframe insertion	0.02077	benign
				ZNF408	NP_079017.1:p.Val194_Val197del	inframe deletion	0.13070	benign
				LRP5	NP_002326.2:p.Ala1330Val	missense	0.13400	benign
9	Poss FEVR	M	36	LRP5	NP_002326.2:p.Val667Met	missense	0.03400	likely benign
				LRP5	NP_002326.2:p.Ala1330Val	missense	0.13400	benign
10	Poss FEVR	F	56	LRP5	NP_002326.2:p.Leu20dup	inframe insertion	0.02077	benign
11	Poss FEVR	M	6 months	ZNF408	NP_079017.1:p.Arg337Pro	missense	0.00609	benign
				ZNF408	NP_079017.1:p.Val194_Val197del	inframe deletion	0.13070	benign
				NDP	NP_000257.1:p.Asp210Glu	missense	0.00400	benign
12	Poss FEVR	M	5	LRP5	NP_002326.2:p.Leu20dup	inframe insertion	0.02077	benign
13	FEVR	F	1	LRP5	NP_002326.2:p.Val667Met	missense	0.03400	likely benign
				LRP5	NP_002326.2:p.Ala1330Val	missense	0.13400	benign
				NDP	NP_000257.1:p.Met40Val	missense	0.00000	uncertain significance
14	FEVR	M	8 months	LRP5	NP_002326.2:p.Val667Met	missense	0.03400	Likely-benign
				LRP5	NP_002326.2:p.Ala1330Val	missense	0.13400	benign
				ZNF408	NP_079017.1:p.Val194_Val197del	inframe deletion	0.13070	benign
15	Poss FEVR	M	6 months	ZNF408	NP_079017.1:p.Arg337Pro	missense	0.00086	benign
				ZNF408	NP_079017.1:p.Val194_Val197del	inframe deletion	0.13400	benign
16	FEVR	F	24	LRP5	NP_002326.2:p.Ala1330Val	missense	0.13400	benign
				ZNF408	NP_079017.1:p.Val194_Val197del	inframe deletion	0.13070	benign
17	FEVR	M	2	n/a	no protein changes			
18	FEVR	F	1 year 8 months	n/a	no protein changes			
19	Poss FEVR	F	2 months	n/a	no protein changes			
20	ND or FFVS	M	1 month	n/a	no protein changes			

Figure 2. Results of sequencing run. Out of 20 patients sequenced, 16 presented with variants resulting in missense, in-frame insertions, in-frame deletions, or premature stop codons. Diagnoses were provided by physicians at Associated Retinal Consultants, PC. Two patients with ND and PFVS (1,2) presented with rare variants resulting in early termination codons in *NDP,* and another patient with ND (3) presented with a novel in-frame deletion in *NDP.* A young female diagnosed with FEVR (5) was found to have a novel missense variant in *ZNF408.* Her father (4), who is not a patient but agreed to sequencing, shares the same variant.

Results and Discussion

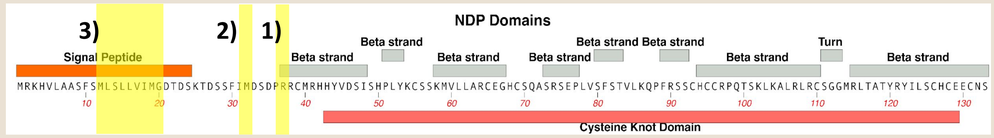

Figure 3. Novel/rare likely pathogenic variants in *NDP*. The likely pathogenic variants identified in *NDP* are highlighted in the context of *NDP*'s functional domains. Variants are labelled with their corresponding patient ID found in Figure 2. The cysteine knot domain is a highly conserved region of *NDP,* and variants that affect this motif have been associated with more severe clinical presentations[1].
1) Arg37Ter. An early stop codon renders the Norrin protein non-functional, and subsequently, this variant is likely the cause of the patient's ND. 2) Met32TrpfsTer9. A novel frameshift variant results in a premature stop codon, also rendering the Norrin protein nonfunctional. This is likely the driving factor behind this patient's PFVS. 3) Leu13_Met19del. This novel seven amino acid deletion occurs within the "signal peptide" domain of *NDP.* This variant may affect the protein's extracellular transport out of Müller cells, thus impacting its impact on the retinal vasculature during early development.

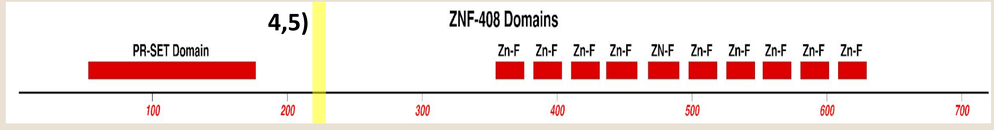

Figure 4. Novel variant of unknown significance in *ZNF408.* The variant identified in *ZNF408* is highlighted in the context of *ZNF408's* functional domains. The variant is labelled with its corresponding patient IDs found in Figure 2.
4,5) Ser225Phe. This variant does not fall in any of the major domains of *ZNF408.*

Conclusion

- 3 novel or rare variants were identified in *NDP* in patients diagnosed with ND or PFVS and are likely the cause of their condition given that 2/3 of variants resulted in a truncated Norrin protein.
- 1 novel variant was identified in *ZNF408* a daughter diagnosed with FEVR as well as her father, who shows little sign of retinopathy.
- This supports the consensus that FEVR is a multigenic condition and that pathogenesis is likely due to the contribution of variants in multiple different genes.
- Sequencing was completed entirely in-house with the use of a targeted 7-gene panel. Given FEVR's multigenic nature, expansion of said panel will be crucial to better understand the contributing factors that lead to development of this hereditary disorder.

References and Support

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