#390 Improving diagnostic yield in a large inherited retinal dystrophy cohort with high-throughput, NGS-based CNV calling — a clinical evaluation of detection criteria and limitations

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Purpose

Although advances in computational tools have enabled the identification of copy number variants (CNVs) from next-generation sequencing (NGS) data, specific criteria for reliable CNV detection remains largely unknown. Through a high coverage, targeted sequencing cohort, this study aims to test the limits of current NGS-based CNV calling and outline its detection criteria.

Methods

Targeted sequencing of 536 ophthalmology related genes was performed on a cohort of 512 retinal dystrophy patients. The XHMM CNV caller was used for CNV detection. Gel analysis and TaqMan qPCR were used as orthogonal screening/confirmation methods.

Results

The final dataset contained 153 CNVs across 87 genes with an estimated false positive rate of 10.5% improving overall mutation detection rate of our panel from 48.6% to 52.7%. The smallest confirmed CNV was 97bp in length and the minimum coverage required to identify a true positive CNV was 242X. The two false negative regions studied had average coverage depths less than 221X.

Conclusions

Our results have reiterated the importance of coverage depth and shown that CNVs down to 97bp are able to be identified. Careful quality control and use of orthogonal confirmation methods to manage false positives allows for highly sensitive CNV screening with finer resolution than current microarray methods.

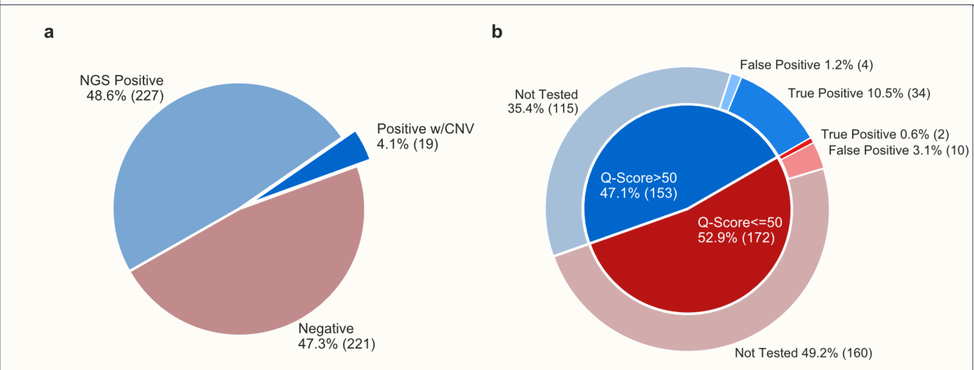

Figure 1. Clinical relevance and specificity of CNV pipeline. (a) Inclusion of CNV pipeline increases mutation detection rate from 48.6% to 52.7%. (b) Breakdown of all CNVs called by confirmation status and Q-score threshold.

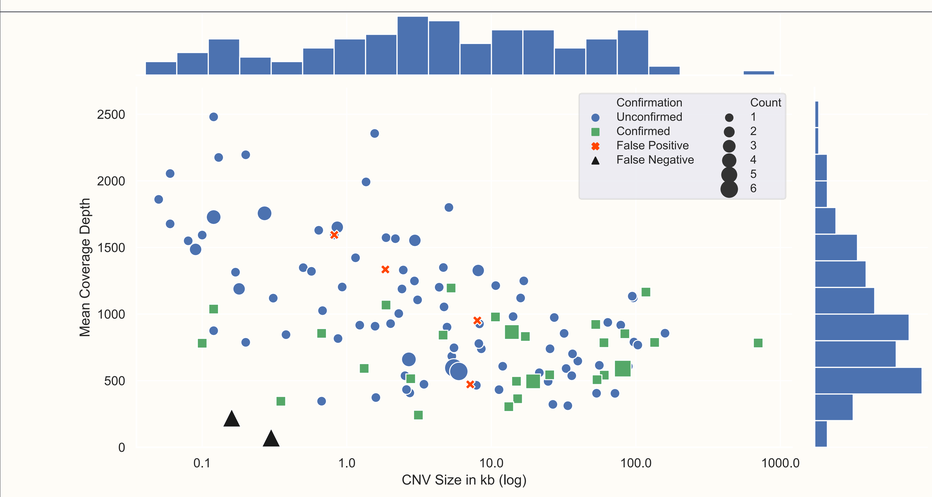

Figure 4. Size and coverage depth distribution of identified CNVs with Q-score >50. The smallest confirmed CNV was 97bp in length and the minimum coverage required to identify a true positive CNV was 242X. The two false negative regions studied had average coverage depths less than 221X. Recorded false positives were near the median values for coverage and CNV size.

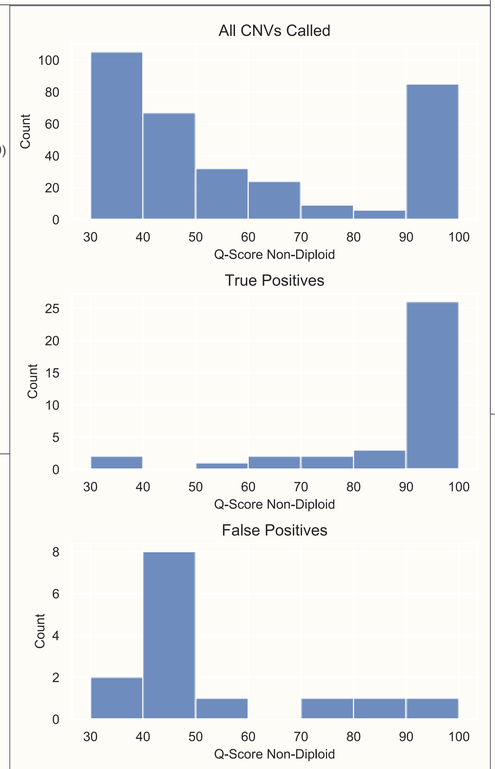

Figure 2. Distribution of Q-scores for identified CNVs. Q-score distributions of all CNVs called, true positives, and false positives. Thresholding CNVs called at Q-score >50 removes the majority of false positives while minimally affecting true positives.

Table 1. CNVs chosen for confirmation by qPCR.

Sample	Gene	Exon Interval	CNV	hg19 Interval	Size (kB)	Targets	Q-Score	Z-Score	Coverage Depth
True Positive, Causative									
CNV-174	USH2A	NM_206933.2 exon 63-64	DEL	1:215844314-215848958	4.64	2	75	-6.51	362.36
CNV-367	USH2A	NM_206933.2 exon 27-28	DEL	1:216246439-216251704	5.27	2	73	-6.44	985.91
CNV-372	USH2A	NM_206933.2 exon 12-18	DEL	1:216371657-216424440	52.78	7	99	-7.47	611.69
CNV-106	CERKL	NM_001030311.2 exon 3-9	DEL	2:182413272-182438611	25.34	7	99	-8.19	0
CNV-105	GPR98	NM_032119.3 exon 89-90	DEL	5:90449038-90459717	10.68	2	99	-7.52	498.75
CNV-271	EYS	NM_001142800.1 exon 14	DUP	6:65707475-65707596	0.12	1	61	10	1178.68
CNV-392	EYS	NM_001142800.1 exon 13-14	DEL	6:65707475-65767620	60.15	2	82	-7.6	333.63
CNV-231	RP9, BBS9	NM_203288.1 exon 1-6, NM_198428.2 exon 1-4	DUP	7:33134846-33195314	60.47	8	99	6.22	614.25
CNV-139	RP1	NM_006269.1 exon 2-3	DEL	8:55533527-55534848	1.32	2	96	-7.31	389.43
CNV-168	KIF11	NM_004523.3 exon 2-10	DEL	10:94366022-94381230	15.21	9	99	-3.45	134.22
CNV-114	BEST1	NM_001139443.1 exon 9, NM_004183.3 exon 10-11	DEL	11:61729727-61731594	1.87	2	99	-10	834.45
CNV-453	CEP290	NM_025114.3 exon 45-54	DEL	12:88442961-88457892	14.93	10	99	-5.69	272.47
CNV-420	PRPF31	NM_015629.3 exon 2-3	DEL	19:54621659-54622013	0.35	2	80	-6.67	207.17
CNV-247	PRPF31	NM_015629.3 exon 5	DEL	19:54625876-54625973	0.1	1	55	-10	352.15
CNV-074	PRPF31	NM_015629.3 exon 2-14	DEL	19:54621659-54634863	13.21	12	99	-4.8	233.64
CNV-018	C21orf2	NM_001271441.1 exon 1-6	DEL	21:45750346-45759077	8.73	6	36	-2.66	105.16
CNV-135	TIMP3	NM_000362.4 exon 5	DUP	22:33255167-33255364	0.2	1	31	7.7	2739.11
CNV-321	RPGR	NM_000328.2 exon 2-19, NM_001034853.1 exon 2-15	DEL	X:38128879-38182777	53.9	18	99	-5.87	247.76
CNV-472	RP2	NM_006915.2 exon 2	DEL	X:46712911-46713576	0.67	1	60	-10	0.46
True Positive, Non-Causative									
Sample	**Gene**	**Exon Interval**	**CNV**	**hg19 Interval**	**Size (kB)**	**Targets**	**Q-Score**	**Z-Score**	**Coverage Depth**
CNV-270	NPHP4	NM_015102.4 exon 2-4	DEL	1:6029147-6046349	17.2	3	80	-5.06	763.15
CNV-428	USH2A	NM_206933.2 exon 5-72	DUP	1:215799123-216500996	701.87	68	99	6.2	1203.8
CNV-247	NPHP1	NM_000272.3 exon 1-20	DEL	2:110881368-110962545	81.18	20	99	-4.96	186.97
CNV-321	NPHP1	NM_000272.3 exon 1-20	DUP	2:110881368-110962545	81.18	20	99	5.71	603.33
CNV-334	NPHP1	NM_000272.3 exon 1-20	DEL	2:110881368-110962545	81.18	20	99	-6.28	254.84
CNV-347	NPHP1	NM_000272.3 exon 1-20	DUP	2:110881368-110962545	81.18	20	99	4.35	599.24
CNV-465	MERTK	NM_006343.2 exon 3-19	DEL	2:112702537-112786441	83.91	15	99	-6.45	246.94
CNV-347	TYR	NM_000372.4 exon 1-5	DUP	11:88911122-89028534	117.41	5	99	9.76	1986.61
CNV-011	CACNA2D4	NM_172364.4 exon 19-26	DEL	12:1949905-1969372	19.47	8	99	-6.42	284.17
CNV-081	CACNA2D4	NM_172364.4 exon 19-26	DEL	12:1949905-1969372	19.47	8	99	-6.77	331.44
CNV-303	CACNA2D4	NM_172364.4 exon 19-26	DEL	12:1949905-1969372	19.47	8	99	-5.69	284.12
CNV-247	RPGRIP1	NM_020366.3 exon 17-19	DEL	14:21795782-21798546	2.77	3	99	-6.03	214.02
CNV-009	TRPM1	NM_001252020.1 exon 2-7	DEL	15:31355321-31369187	13.87	6	99	-8.64	335.38
CNV-095	TRPM1	NM_001252020.1 exon 2-7	DEL	15:31355321-31369187	13.87	6	99	-7.52	246.27
CNV-303	TRPM1	NM_001252020.1 exon 2-7	DEL	15:31355321-31369187	13.87	6	99	-8.57	418.9
CNV-327	TRPM1	NM_001252020.1 exon 1-26	DUP	15:31318342-31453162	134.82	24	99	7.06	1207.63
CNV-445	CA4	NM_000717.4 exon 2-7	DUP	17:58232675-58235807	3.13	6	99	4.44	501.69
False Positive									
Sample	**Gene**	**Exon Interval**	**CNV**	**hg19 Interval**	**Size (kB)**	**Targets**	**Q-Score**	**Z-Score**	**Coverage Depth**
CNV-471	SEMA4A	NM_001193300.1 exon 5-8	DUP	1:156128179-156130820	2.64	4	45	3.51	1382.06
CNV-332	HMCN1	NM_031935.2 exon 26-30	DEL	1:185969177-185976414	7.24	5	31	-2.84	442.48
CNV-309	HMCN1	NM_031935.2 exon 31-36	DEL	1:185984291-185992285	8	6	99	-3.76	728.24
CNV-309	HMCN1	NM_031935.2 exon 44-45	DEL	1:186022957-186024806	1.85	2	75	-6.25	1017.96
CNV-332	HMCN1	NM_031935.2 exon 75-77	DEL	1:186084390-186086755	2.37	3	47	-4.13	582.76
CNV-188	HMCN1	NM_031935.2 exon 106-107	DEL	1:186157015-186159010	2	2	49	-5.36	1509.7
CNV-368	SNRNP200	NM_014014.4 exon 6-8	DUP	2:96964341-96965165	0.82	3	82	5	1539.86
CNV-400	SNRNP200	NM_014014.4 exon 6-8	DUP	2:96964341-96965165	0.82	3	46	4.13	1688.21
CNV-473	PAX3	NM_181457.3 exon 3-4, NM_000438.5 exon 3-4	DUP	2:223158442-223160376	1.94	3	46	4.12	1801.84
CNV-397	PROM1	NM_001145848.1 exon 8	DEL	4:16019946-16020163	0.22	1	49	-9.37	1737.46
CNV-005	TYRP1	NM_000550.2 exon 5-6	DEL	9:12702271-12704705	2.44	2	41	-5.06	1193.32
CNV-415	PAX6	NM_001127612.1 exon 6-11	DUP	11:31814812-31823324	8.51	6	37	2.78	1151.61
CNV-352	PRPF8	NM_006445.3 exon 12-13	DEL	17:1581812-1582175	0.36	2	46	-5.48	1141.92
CNV-451	RPGR	NM_000328.2 exon 16-19	DUP	X:38128879-38136025	7.15	4	52	3.57	583.93

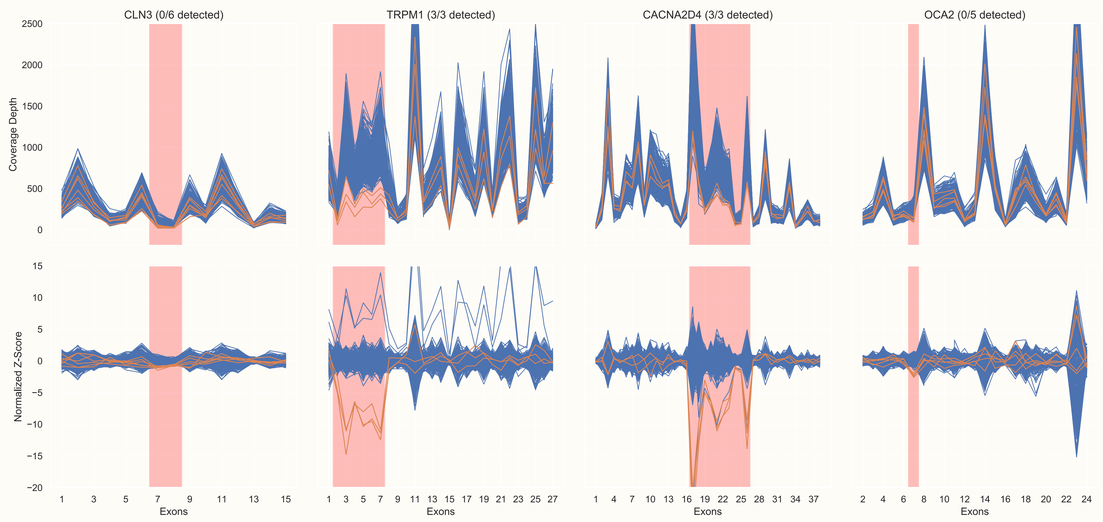

Figure 3. Detection of deletion controls identified by gel analysis. Per sample coverage depth and normalized Z-score across CLN3, TRPM1, CACNA2D4, and OCA2 exonic targets. Orange lines indicate positive controls for the deletion region highlighted in red. Blue lines indicate negative controls. The TRPM1 and CACNA2D4 deletion regions had adequate coverage and all positive controls were successfully detected. The CLN3 and OCA2 deletion regions had poor coverage and low normalized Z-score deviations with none of the positive controls identified.

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Disclosures

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