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Neurogenetic Answers™

Single exon resolution copy number analysis significantly increases clinical sensitivity of NGS

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Introduction

The detection of copy number variants (CNVs) using next generation sequencing (NGS) data is part of MNG's comprehensive approach to increase the sensitivity of our phenotype-driven sequencing panels. Typically, an array-based (CMA) copy number analysis is used to detect CNVs across the genome even before sequencing is ordered. While CMA offers a cheaper and more complete approach for identification of large genomic CNVs, NGS sequencing data offers better resolution in the coding regions. In our custom NGS panels, we seek to provide a more comprehensive diagnosis through the detection of CNVs down to a single exon resolution. Using a custom designed capture reagent and the EXCAVATOR copy number analysis software, we are able to detect CNVs of greater than 5 exons across all genes included in our panels. Additionally, we are able to detect CNVs at single exon resolution in the regions of previously described pathogenic CNVs. We accomplish this through the use of supplementary intronic probes for all genes with known pathogenic CNVs of ten exons or fewer. Since the inclusion of copy number analysis with our custom NGS panels in September 2016, we have seen a 1.6% increase in the total number of positive reports issued. Here we present multiple case studies in which single exon resolution copy number analysis was crucial in reaching a diagnosis across a variety of phenotypes.

Methodology

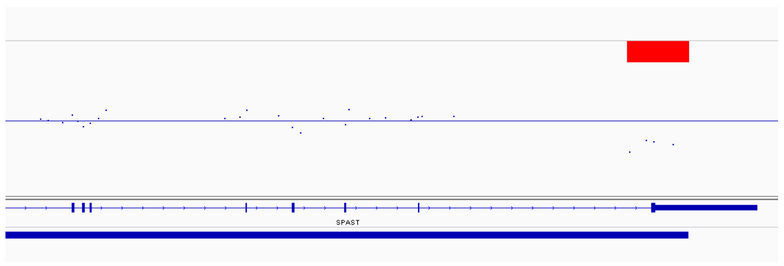
Capture Design:

Regions of interest are captured using a custom designed Agilent SureSelect reagent that is padded with intronic baits around exons that are part of pathogenic copy number variants smaller than 10 exon in size. We update our reagent every six months to include novel pathogenic SNVs and CNVs based on the latest release of ClinVar and relevant publications. This custom capture produces a highly reproducible coverage profile both within and across batches allowing for accurate and reproducible copy number analysis.

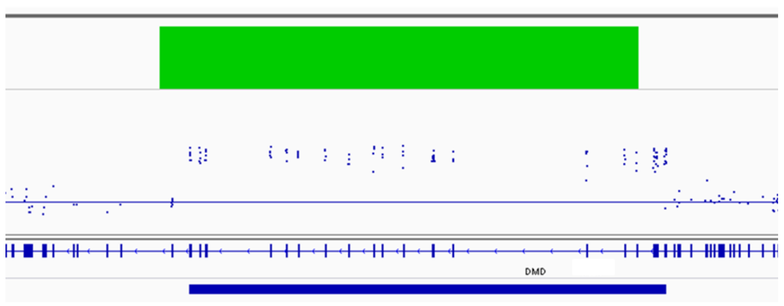
Copy Number Analysis:

Patients are run in batches of approximately 24 per lane on the Illumina Hiseq 2500., with each batch containing a similar proportion of males and females. CNVs are identified using the EXCAVATOR software in a Linux environment based on comparison of each individual with the sex-matched batch controls. Results are visualized and reviewed in IGV.

FIG 1: Examples of Single Gene CNVs Detected

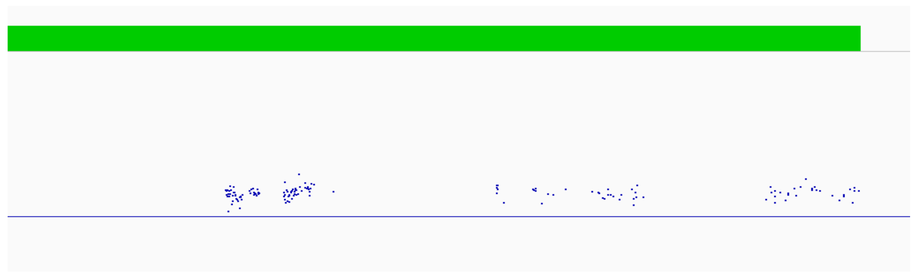


I: SPAST exon 17 deletion in a 49 year old patient presenting with hereditary spastic paraplegia. This result was verified using MLPA.

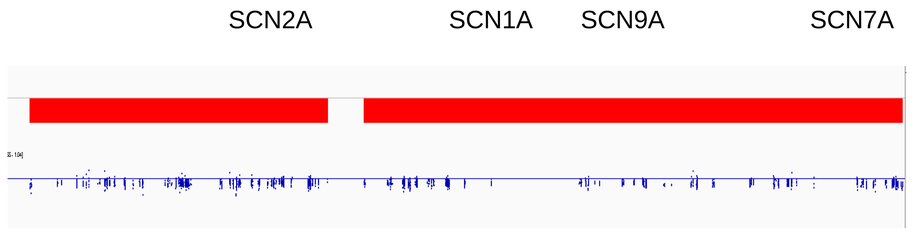


II: DMD exons 35-59 duplication in a 41 year old patient presenting with lower limb weakness and prominent quadriceps.

FIG 2: Examples of Multigene CNVs Detected



I: Partial chromosome 2 duplication including SCN1A, SCN2A, SCN7A and SCN9A genes in a 1 month old presenting with intractable seizures.



II: Mosaic Turner syndrome in a 4 year old female with epilepsy. Mosaic Turner syndrome has been identified in multiple individuals, and is considered an incidental finding.

Table 1: Positive CNVs detected (n=32)

CNVs	Number Detected
Smaller than one gene	**11**
One gene	**7**
Larger than one gene	**14**

I: Description of all 32 CNVs detected that resulted in a positive report.

Phenotype	Single Gene CNVs
Ataxia	APTX, CACNA1A
Bartter/Gitelman	CLCNKB
Charcot-Marie-Tooth	PMP22
Epilepsy	CNTNAP2, KCNQ2, SCN1A
Muscular Dystrophy/Myopathy	DMD
Parkinson's	SNCA
Spastic Paraplegia	SPAST

II: Description of CNVs detected that were one gene or smaller resulting in a positive report. Genes are listed in association with the phenotype of the patient.

Table 2: Indeterminate CNVs Detected (n=46)

CNVs	Number Detected
Smaller than one gene	**12**
One gene	**15**
Larger than one gene	**19**

I: Description of all 46 CNVs detected that resulted in an indeterminate report.

Phenotype	Single Gene CNVs
Ataxia	BBS9, NPHP1, **PNKD**
Charcot-Marie-Tooth	KIF1A
Dystonia	ACTB, **PNPT1**
Epilepsy	**AKT2**, **ASPA**, **CACNA1H**, GABRD, **KDM6A**, **MMAA**, NRXN1, PANK2, **TUBB4A**
Intellectual Disability	**DNAJC6**, RAF1, TMLH3.
Muscular Dystrophy/Myopathy	DHTKD1, **GAA**, **LARGE1**, **MPZ**, **MYH8**, NIPA1, **PNPLA2**

II: Description of CNVs detected that were one gene or small resulting in an indeterminate report. Genes are listed in association with the phenotype of the patient. Novel CNVs are **bolded**.

Table 3: Positive Report Metrics

Phenotype	CNVs Reported	Percentage
Ataxia	2	**1.1%**
Bartter/Gitelman	2	**10%**
Charcot-Marie-Tooth	2	**2.1%**
Epilepsy	12	**3.3%**
Intellectual Disability	2	**1.4%**
Muscular Dystrophy/Myopathy	9	**2.2%**
Parkinson's	2	**11.8%**
Spastic Paraplegia	1	**1.6%**

I: Percentage of reports that were positive due to a CNV for phenotype-driven panels.

Total	Percentage
All Positives	**10.6%**
All Tests	**1.6%**

II: Percentage of reports that were positive due to a CNV out of all positive reports (n=302) and all tests ordered (n=2052).

Table 4: Indeterminate Report Metrics

Phenotype	CNVs Reported	Percentage
Ataxia	4	**2.3%**
Charcot-Marie-Tooth	1	**1.1%**
Dystonia	3	**3.3%**
Ehlers Danlos	1	**2%**
Epilepsy	14	**3.9%**
Intellectual Disability	5	**3.5%**
Muscular Dystrophy/Myopathy	15	**3.6%**
Neuronal Migration	1	**6.7%**
Spastic Paraplegia	2	**3.2%**

I: Percentage of reports that were indeterminate due to a CNV for phenotype-driven panels.

Total	Percentage
All Indeterminate	**4.9%**
All Tests	**2.2%**

II: Percentage of reports that were positive due to a CNV out of all positive reports (n=933) and all tests ordered (n=2052).

Conclusion

Single exon resolution copy number analysis allows for the detection of small CNVs that cannot be detected by traditional CMA. The addition of this analysis to NGS panels results in a more comprehensive diagnosis for patients, which is demonstrated by the significant increase in positive and indeterminate reports observed across a variety of phenotypes. These results suggest that the role of small CNVs in disease is underestimated. Clinicians seeking diagnosis for their patients should consider requesting NGS panels that provide single exon resolution copy number analysis.

References

1. Magi A, et al. EXCAVATOR: detecting copy number variants from whole-exome sequencing data. Genome Biology 2013 Dec;14:R120.