Complementary Testing Methods Improve the Clinical Sensitivity of Genetic Testing

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INTRODUCTION

Next-generation sequencing (NGS) has been adopted by molecular laboratories as the preferred technology for diagnosing an increasing number of genetic diseases. Targeted, phenotype-based NGS panels are powerful tools, but the diagnostic rate varies between 10-50% depending on the panel. This means that more than half of the families that are searching for a cause of their disease fail to receive a definitive diagnosis. A comprehensive approach should be taken as traditional panel sequencing alone may not be enough. Including additional test methodologies to targeted phenotype-based NGS panels might significantly increase the sensitivity of genetic testing.

We have evaluated the improvement in clinical sensitivity of our panels as a result of adding copy number variation analysis (CNVs), repeat expansion testing, and mitochondrial DNA (mtDNA) sequencing and deletion analysis with the clinically appropriate MNG panels.

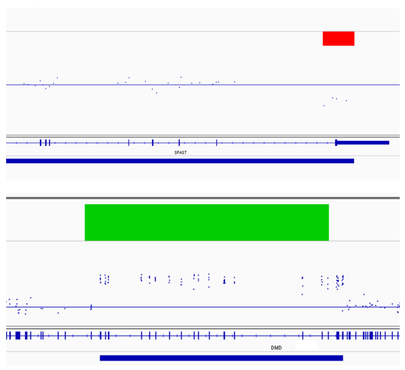
METHODOLOGY 1: COPY NUMBER VARIANT ANALYSIS (CNV)

The detection of copy number variants (CNVs) using NGS data is part of MNG's comprehensive approach to increase the sensitivity of our phenotype-driven sequencing panels. While CMA can identify large genomic CNVs, NGS 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 in the regions of previously described pathogenic CNVs. We are able to detect CNVs of greater than 10 exons across all genes included in our panels, and provide single exon resolution for known pathogenic CNVs.

Methodology: 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 exons in size. This custom capture produces a highly reproducible coverage profile both within and across batches allowing for accurate and reproducible copy number analysis. CNVs are identified using the EXCAVATOR software[1] in a Linux environment based on comparison of each individual with the sex-matched batch controls. Results are visualized and reviewed in IGV.

COPY NUMBER VARIANT DETECTION INCREASES TESTING SENSITIVITY

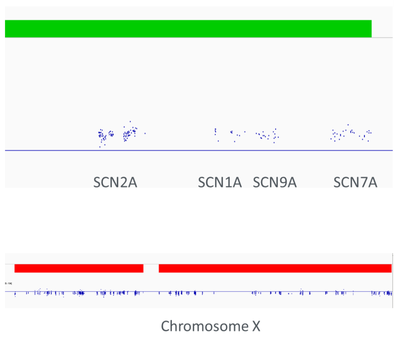
Figure 1



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

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

Figure 2



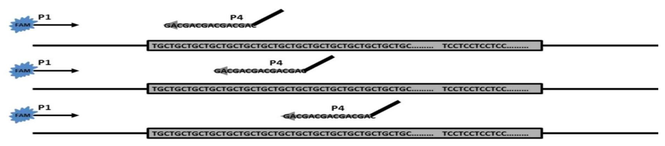
I: Partial chromosome 2 duplication including *SCN1A*, *SCN2A*, *SCN7A* and *SCN9A* genes in a 1 month old presenting with intractable seizures.

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.

METHODOLOGY 2: REPEAT EXPANSION TESTING

To date, more than 20 disorders have been found to be caused by repeat expansions[2,3]. While NGS panels can precisely identify SNPs and CNVs, due to the current limitations of sequencing technology, NGS cannot reliably detect large repeat expansions.

Figure 3: Repeat Primed PCR and capillary electrophoreses are used to detect repeat expansions

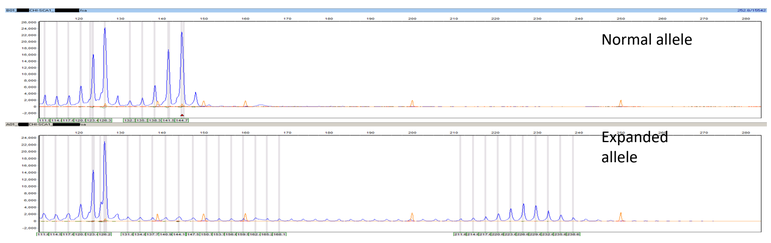


REPEAT EXPANSION TESTING INCREASES TESTING SENSITIVITY

The inclusion of appropriate repeat expansion testing with targeted phenotype-based sequencing panels increases the clinical sensitivity:

Ataxia Testing: Repeat expansion testing for eleven of the most common spinocerebellar ataxias is included with our comprehensive sequencing panel (SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, SCA36, and DRPLA). Of 503 individuals tested, pathogenic repeat expansions were detected in 48 (9.5%).

Figure 4: The top image shows a patient with two SCA1 alleles within the normal range and the bottom image shows a patient with an expanded allele.



Amyotrophic Lateral Sclerosis (ALS) Testing: C9orf72 repeat expansion is included with our ALS sequencing panel. Of 126 individuals tested, pathogenic repeat expansions were detected in 13 (10%).

Table 1: Summary of ALS Results (n=126)

Type of ALS Report	Number	Percentage
Positive by sequencing	11	8.7%
Indeterminate by sequencing	34	27.0%
Negative by sequencing	81	64.3%
Positive for C9orf72 repeat expansion	13	10.3%

METHODOLOGY 3: MITOCHONDRIAL DNA SEQUENCING

Epidemiological studies have estimated the incidence of clinical mitochondrial diseases to be approximately 1 in 5000[4]. Additionally, a survey of newborn cord bloods found that ~1 in 200 infants harbored common pathogenic mtDNA variants[5,6]. The inclusion of mitochondrial DNA (mtDNA) sequencing and deletion analysis in appropriate phenotype-based sequencing panels increases the clinical sensitivity of these tests.

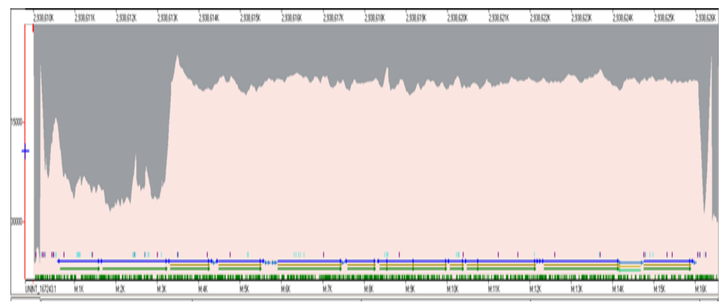
Methodology: A long-range PCR of 16,476 base pairs of the mitochondrial genome followed by Next Generation Sequencing and detection of deletions based on the identification of the regions flanking the deletion is performed. With this methodology, the mitochondrial genome is sequenced at an average depth of approximately 5,000X and heteroplasmy can be detected at levels as low as 1%.

MITOCHONDRIAL DNA ANALYSIS INCREASES TESTING SENSITIVITY

Figure 5: Heteroplasmy as low as 1% can be detected by this method. The 3243 A>G MELAS mutation was detected at low levels in this patient. This variant is often found at low levels in the blood and higher levels in other tissues.

MT-*TL1* mutation associated with MELAS

Figure 6: Deletion assessment is based on the identification of regions flanking a deletion using LUMPY[7]. A visualization of a large mtDNA deletion is shown below. Disorders associated with mtDNA deletions include Kearns-Sayre syndrome, Pearson syndrome, and progressive external ophthalmoplegia.



INCREASE IN SENSITIVITY OF NGS PANELS DUE TO COMPLEMENTARY TESTS

Complementary Methodologies	Positive Reports Due to Complementary Tests / All Positive Reports	Increase in Sensitivity
Copy number variant calling (n = 3134)	58/495	**11.7%**
Repeat expansion testing for SCAs (n = 503)	48/230	**20.8%**
Repeat expansion testing for C9orf72 (n = 126)	13/96	**13.5%**
Mitochondrial genome sequencing and deletion analysis (n = 2,428)	43/284	**15.1%**

Table 2:
Since the inclusion of CNV analysis with our custom NGS panels, an 1.8% increase in total positive reports has been observed (n=3,134).

Initial findings have shown that adding repeat expansion testing with our neuromuscular and movement disorder panels has increased positive results by 9.7% (n=629).

Inclusion of mtDNA sequencing and deletion analysis increased positive reports by 1.8% (n=2,428).

These data highlight the overall increased clinical sensitivity of next generation sequencing panels bundled with additional testing methods.

The inclusion of mtDNA sequencing, repeat expansions, and copy number variant analysis with appropriate sequencing panels, has allowed for the identification of pathogenic variants in patient samples having no definitively pathogenic nuclear sequencing variants. This increased sensitivity has been particularly beneficial in NGS panels associated with neurological disorders. The increased sensitivity of the NGS panels and the ease of ordering a single comprehensive test instead of multiple individual tests provides added value for both clinicians and their patients.

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