MNG LABORATORIES
Neurogenetic Answers™

Combining repeat expansion testing with NGS phenotype based panels provides significant diagnostic benefit of NGS

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INTRODUCTION

Targeted, phenotype-based Next Generation Sequencing (NGS) panels are a powerful tool for clinical diagnostics and have made variant calling cheaper, faster, and more accessible. The availability of targeted NGS panels has increased over the past few years to cover a range of conditions including rare neurological disorders. A number of diseases assessed by NGS panels are known to have multiple genetic causes, ranging from single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) to repeat expansions. While NGS panels can precisely identify SNPs and CNVs, due to the current limitations of sequencing technology, NGS cannot accurately detect large repeat expansions. To date, more than 20 diseases have been found to be caused by repeat expansions. By including repeat expansion testing with targeted phenotype-based NGS panels, we can increase the clinical-based sensitivity of the panels we offer. Initial findings suggest that repeat expansion testing with our neuromuscular and movement disorder panels increases our diagnostic sensitivity by 10%.

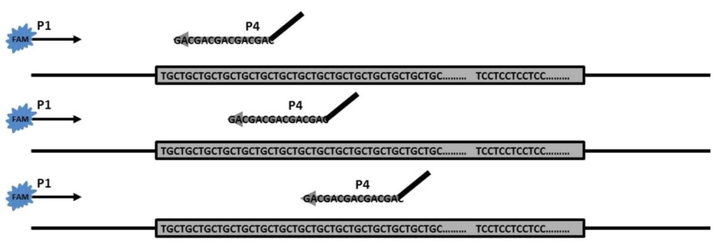
DISEASES CAUSED BY REPEAT EXPANSIONS

Diseases Caused by Repeat Expansions and Testing Offered by MNG	Gene	Repeat Unit	Repeat Location	Included with NGS Panels
Myotonic dystrophy type 1 (DM1)	DMPK	CTG	3' UTR	
Myotonic dystrophy type 2 (DM2)	ZFN9	CCTG	intron	
Huntington disease (HD)	HTT	CAG	exon	Ataxia, Dystonia, Dementia
Fragile X Syndrome	FMR1	CGG	5' UTR	Intellectual Disability
Amyotrophic Lateral Sclerosis (ALS)/ Frontotemporal Dementia	C9ORF72	GGGGCC	intron	ALS, Dementia
Friedreich ataxia (FRDA)	FXN	GAA	intron	Ataxia
Spinocerebellar ataxia 1 (SCA1)	ATXN1	CAG	exon	Ataxia
Spinocerebellar ataxia 2 (SCA2)	ATXN2	CAG	exon	Ataxia
Spinocerebellar ataxia 3 (SCA3)	ATXN3	CAG	exon	Ataxia
Spinocerebellar ataxia 6 (SCA6)	CACNA1A	CAG	exon	Ataxia
Spinocerebellar ataxia 7 (SCA7)	ATXN7	CAG	exon	Ataxia
Spinocerebellar ataxia 8 (SCA8)	ATXN8OS/ ATXN8	CTG·CAG	3' UTR	Ataxia
Spinocerebellar ataxia 10 (SCA10)	ATXN10	ATTCT	intron	Ataxia
Spinocerebellar ataxia 12 (SCA12)	PPP2R2B	CAG	5' UTR	Ataxia
Spinocerebellar ataxia 17 (SCA17)	TBP	CAG	exon	Ataxia
Spinocerebellar ataxia 36 (SCA36)	NOP56	GGCCTG	intron	Ataxia
Dentatorubral-pallidoluysian atrophy (DRPLA)	ATN1	CAG	exon	Ataxia
Spinal and bulbar muscular atrophy (SBMA)*	AR	CAG	exon	
Progressive Myoclonus Epilepsy (EPM1)*	CSTB	C4GC4GCG	promoter	
Huntington disease-like 2 (HDL2)*	JPH3	CTG	3' UTR	
Fragile XE mental retardation syndrome (FRAXE MR)*	FMR2	CGG	5' UTR	

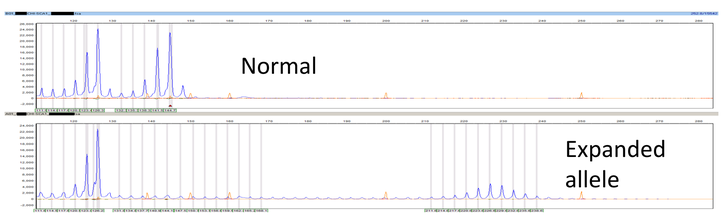
*Repeat expansion testing in currently in development

METHODOLOGY

Repeat primed PCR and capillary electrophoresis are used to detect repeat expansions.



SCA1



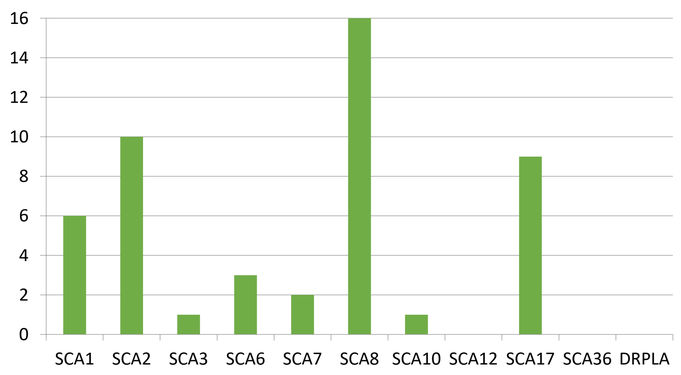
SPINOCEREBELLAR ATAXIAS (SCA)

- A group of autosomal dominant ataxias characterized by degenerative changes in the cerebellum
- Lead to slowly progressive incoordination of gait, and poor coordination of hands, speech, and eye movements
- Age of symptom onset can vary dramatically
- Expansion size often increases in length in subsequent generations - Anticipation
- Prevalence of SCAs is estimated to be 1-5 in 100,000
- Most common SCAs worldwide are SCA1, SCA2, SCA3, SCA6, & SCA7
  - Together these make up about **60%** of the reported dominant cases of ataxia

ADDITION OF SCA REPEAT EXPANSION TESTING CAN INCREASE SENSITIVITY OF ATAXIA NGS PANEL

- Performed SCA repeat expansion testing on 503 patient samples submitted for Ataxia/Episodic Ataxia NGS Panel
- Tested for 11 different SCAs: SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, SCA36, and DRPLA
- Identified 48 patient samples that contained a pathogenic expansion

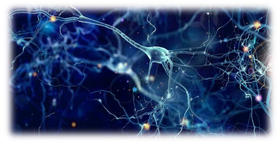
Distribution of SCAs identified in 503 patients



- Patients with SCA8 expansion contained higher penetrance alleles (>80 repeats). Patients may develop SCA8
- Patients with SCA17 expansions contained reduced penetrance alleles (41 to 43 repeats). Patients may develop SCA17

AMYOTROPHIC LATERAL SCLEROSIS (ALS) / FRONTOTEMPORAL DEMENTIA (FTD)

- Complex neurodegenerative disorders with a considerable clinical and pathological overlap
  - **ALS** - Progressive disease that affects motor neurons in the brain and spinal cord causing atrophy that leads to muscle weakness, loss of muscle mass, and inability to control movement.
  - **FTD** - Progressive changes in behavior, executive dysfunction, and/or language impairment
- Pathogenic GGGGCC hexanucleotide repeat expansion in C9ORF72 is the most common cause of both familial and sporadic ALS and FTD
  - ALS: 34% of familial and 5.9% of sporadic
  - FTD: 26% of familial and 5.1% of sporadic
- Autosomal dominant inheritance



ADDITION OF C9ORF72 REPEAT EXPANSION TESTING CAN INCREASE THE SENSITIVITY OF ALS NGS PANEL

- Tested 126 patient samples submitted for ALS NGS panel for C9ORF72 repeat expansion

Type of Report	Number	Percentage
Positive by sequencing	11	8.7
Indeterminate by sequencing	34	27.0
Negative by sequencing	81	64.3
Positive for C9ORF72 expansion	13	10.3

- 10 Negative and 3 Indeterminate reports changed to positive due to repeat expansion testing

CONCLUSION

Increase in Sensitivity of NGS Panels Due to Inclusion of Repeat Expansion Testing

Percent increase in causative variants identified by adding repeat expansion analysis to NGS panels

Test Type	Diagnostic Increase
Ataxia Panel with SCA Repeat Expansion Panel	**9.5%**
Amyotrophic Lateral Sclerosis with C9orf72 Repeat Expansion Testing	**10.3%**

REFERENCES

1. Sandford E, Burmeister M. Genes and genetic testing in hereditary ataxias. Genes (Basel). 2014 5:586-603.
2. van Blitterswijk M, DeJesus-Hernandez M, Rademakers R. How do C9ORF72 repeat expansions cause amyotrophic lateral sclerosis and frontotemporal dementia: can we learn from other noncoding repeat expansion disorders? Curr Opin Neurol. 2012, 25:689-700.