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Neurogenetic Answers™

Mitochondrial genome sequencing in phenotype-based panels and exome sequencing increases test sensitivity

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Introduction

Next-generation sequencing (NGS) allows rapid variant analysis for identifying disease causing variants. Exclusion of mitochondrial sequencing analysis in NGS disease screening panels limits their power by ignoring the potential of pathogenic mitochondrial variants. Initially thought to be rare, mitochondrial genetic diseases represent important and common sources of disorders. Recent genetic epidemiological studies quantifying the most common pathogenic mtDNA variants have shown the incidence of clinical mitochondrial diseases is about 1 in 5000[1]. Additionally, a survey of newborn cord bloods revealed that 1 in 200 infants harbored common pathogenic mtDNA variants[2,3]. Coupled with variant assessment and curation database software, the addition of mtDNA sequencing to phenotype-based panels and exomes increases the ability to discover variants of interest that aid in the determination of patient treatment. Upon including mtDNA sequencing with the panels, we have positively identified pathogenic or potentially pathogenic variants in patient samples having no definitively pathogenic nuclear genome variants. The effect of this inclusion 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 test instead of two provides added value for both clinicians and their patients.

Methodology

- **MITOCHONDRIAL DNA SINGLE NUCLEOTIDE POLYMORPHISM, SMALL INDEL SEQUENCING, AND DELETION ASSESSMENT**

  Next-generation sequencing of a long-range mtDNA PCR product of approximately 16,476 base pairs is performed on the Illumina® MiSeq™ instrument. This region encompasses all known pathogenic variants in the mtDNA. Deletion assessment is performed based on identification of regions flanking a deletion (LUMPY[4]).

- **RESULTS INTERPRETATION**

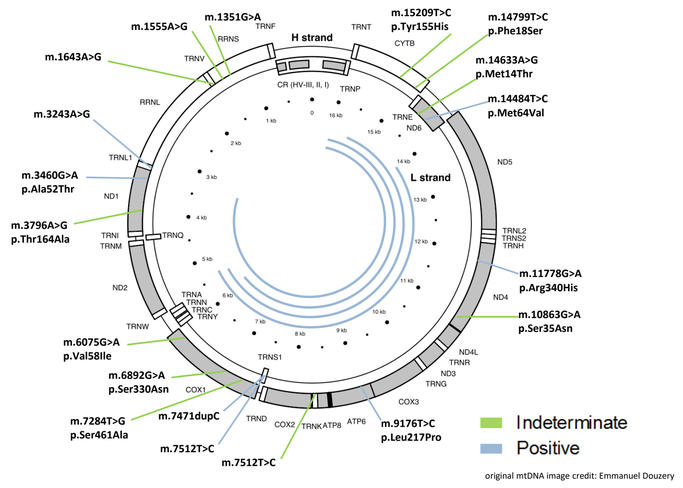
  Interpretation of PATHOGENIC and LIKELY PATHOGENIC variants, as well as Variants of Uncertain Significance (VUS) with pathogenic predictions or possible disease association related to the patient's phenotype are determined using online database resources (e.g., ClinVar[5], OMIM[6], MitoMap[7]) and our in-house Genome MaNaGer® variant curation database software.

- **PANELS WITH mtDNA ANALYSIS INCLUDED**

  The data reported were collected from the following NGS panels which include mtDNA sequencing and deletion analysis:

Panel Code	Panel Name	# Nuclear Genes
NGS301	Comprehensive Cellular Energetics Defects	343
NGS302	Carbohydrate Metabolism Deficiency	47
NGS303	Lipid Metabolism Deficiency	46
NGS304	Pyruvate Metabolism Disorders	14
NGS305	PDH/Tricarboxylic Acid Cycle (TCA) Defects	23
NGS306	Oxidative Phosphorylation (OXPHOS) Defects	193
NGS324	Ataxia/Episodic Ataxia Disorders	362
NGS330	Comprehensive Muscular Dystrophy/Myopathy	460
NGS337	Spastic Paraplegia	130
NGS345	Charcot Marie Tooth Disease	55
NGS345A	AXONAL Charcot Marie Tooth Disease	55
NGS345D	DEMYELINATING Charcot Marie Tooth Disease	55
NGS351	Leigh Disease	74
NGS352	Comprehensive Ophthalmoplegia Syndromes	55
NGS353	Cellular Energetics Ophthalmoplegia Syndromes	22
NGS355	Cytochrome C Oxidase Deficiency	44
NGS358	Comprehensive Dystonia	115
NGS361	OXPHOS Defect Dystonia	22
NGS363	Comprehensive Cardiomyopathy	129
NGS372	Comprehensive Leukodystrophy/Leukoencephalopathy	122
NGS374	Mitochondrial Leukodystrophy/Leukoencephalopathy	44
NGS383	Comprehensive Metabolic Disease Hepatomegaly	79
NGS385	Comprehensive Epilepsy	554
NGS387	Comprehensive Neuronal Migration Disorders	182
NGS389	Mitochondrial Neuronal Migration Disorders	11
NGS408	Ataxia/Episodic Ataxia Disorders with HTT Repeat Expansion	362
NGS409	Comprehensive Dystonia with HTT Repeat Expansion	115
NGS417	Ataxia/Episodic Ataxia Disorders with SCA & HTT Repeat Expansion	362
NGS419	Ataxia/Episodic Ataxia Disorders with FRDA Repeat Expansion	362
NGS420	Ataxia/Episodic Ataxia Disorders with SCA & FRDA Repeat Expansion	362
NGS429	Familial Hemiplegic Migraine	5
NGS430	Stroke	29
NGS431	Ataxia/Episodic Ataxia Disorders with SCA Repeat Expansion	431
WES001	MNG Exome™ Trio Sequencing	Exome
WES003	MNG Exome™ Proband Only Sequencing	Exome

FIG 2: Reported mtDNA variants



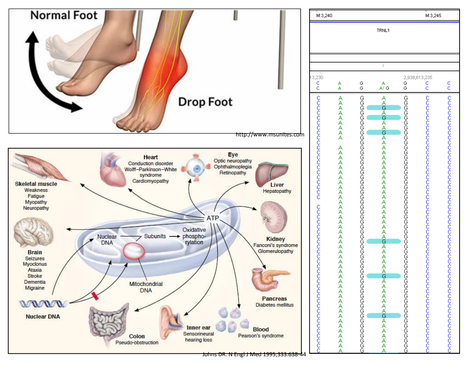
original mtDNA image credit: Emmanuel Douzery

Variants shown were responsible for either Indeterminate reports or Positive reports of NGS panels with mtDNA sequencing included. Inner curved lines represent approximate lengths and locations of reported deletions.

FIG 3: MELAS detection in exome

Clinical Information

- 14 year-old male
- Progressive gait abnormality of unknown etiology
- Proximal limb weakness and areflexia
- Bilateral foot drops



Leukocyte sample from exome patient determined to be at 11% heteroplasmy for the m.3243A>G MELAS variant (raw data on left). Clinician stated discovery of MELAS was unexpected but was a fitting diagnosis for the clinical presentation of the patient (clinical information above).

Standard exomes excluding mtDNA sequencing would not have provided diagnostic evidence for cause of disease in this case.

TABLE 1: Reported Variant Details

A

Reported Variant (Positive Reports)	Panel Description	Variant Info with Heteroplasmy Percentage (DNA from leukocytes unless specified)	Patient Phenotype from Clinical	Age	Sex
mtDNA deletion (m.3273-16072)	**NGS352** Comprehensive Ophthalmoplegia Syndromes	mtDNA deletion, in 34% of the mitochondria. (frozen muscle)	Ptosis, imbalance, muscle weakness, numbness to lower legs and hands, diabetes mellitus, obesity.	62	M
mtDNA deletion (m.5841-16070)	**NGS352** Comprehensive Ophthalmoplegia Syndromes	mtDNA deletion, in 9% of the mitochondria. (frozen muscle)	No clinical information was provided.	64	M
mtDNA deletion (m.6340-13994)	**NGS352** Comprehensive Ophthalmoplegia Syndromes	mtDNA deletion, in 10% of the mitochondria. (frozen muscle)	CPEO, ptosis, fatigue, limb weakness, migraines, cardiomegaly, right sided heart failure, lipomas, short stature, depression, cerebral atrophy, low CPK.	56	F
mtDNA deletion (m.6469-15591)	**NGS301** Comprehensive Cellular Energetics Defects	mtDNA deletion, in 65% of the mitochondria.	Mitochondrial myopathy.	10	M
MT-TL1 m.3243A>G	**NGS330** Comprehensive Muscular Dystrophy/Myopathy	MELAS @ 2.7%	Proximal lower extremity weakness, normal CPK.	12	M
MT-TL1 m.3243A>G	**NGS330** Comprehensive Muscular Dystrophy/Myopathy	MELAS @ 1.4%	Myopathy.	88	F
MT-TL1 m.3243A>G	**NGS385** Comprehensive Epilepsy	MELAS @ 17.4%	No clinical information was provided.	1	M
MT-TL1 m.3243A>G	**NGS372** Comprehensive Leukodystrophy/Leukoencephalopathy	MELAS @ 32%	No clinical information was provided.	53	M
MT-TL1 m.3243A>G	**WES001** MNG Exome™ Trio	MELAS @ 11%	Progressive gait abnormality of unknown etiology, limb weakness, areflexia, bilateral foot drops.	14	M
MT-ND1 m.3460G>A Ala52Thr	**NGS324** Ataxia/Episodic Ataxia Disorders	LHON @ 16%	Hereditary ataxia	3	M
MT-TS1 m.7471dupC	**NGS385** Comprehensive Epilepsy	PEM/AMDF/Motor neuron disease-like @ 25%	Focal seizures, EMG/NCV show generalized spikes, 1-6x focal seizures per day, eye deviation to left side, negative family history.	2	M
MT-TS1 m.7512T>C	**NGS385** Comprehensive Epilepsy	MELAS/MERFF overlap @ 8.1%	Tonic clonic seizures, stroke, EEG shows focal slowing, family history of epilepsy.	13	M
MT-ATP6 m.9176T>C Leu217Pro	**NGS324** Ataxia/Episodic Ataxia Disorders	Leigh / Bilateral striatal necrosis @ 96% (extracted DNA, tissue not specified)	Autosomal recessive spastic ataxia.	51	M
MT-ND4 m.11778G>A Arg340His	**NGS372** Comprehensive Leukodystrophy/Leukoencephalopathy	LHON - homoplasmic	Leukoencephalopathy and neonatal onset seizures of uncertain etiology, controlled with medication.	1	M
MT-ND6 m.14484T>C Met64Val	**NGS324** Ataxia/Episodic Ataxia Disorders	LHON - homoplasmic	No clinical information was provided.	2	M

B

Reported Variants (Indeterminate Reports)	Panel Description	Variant Info with Heteroplasmy Percentage (DNA from leukocytes unless specified)	Patient Phenotype from Clinical	Age	Sex
MT-RNR1 1351G>A	**NGS324** Ataxia/Episodic Ataxia Disorders	VUS @ 83.5% . Variants in this gene have been seen in cases of deafness, cardiomyopathy.	No clinical information was provided.	84	F
MT-RNR1 1555A>G	**NGS372** Comprehensive Leukodystrophy/Leukoencephalopathy	Homoplasmic. Variant is known to cause deafness due to use of aminoglycoside antibiotics.	Degenerative disease of nervous system, unspecified, no deafness reported.	5	M
MT-TV m.1643A>G	**NGS385** Comprehensive Epilepsy	VUS, homoplasmic. Variant seen in patient with symptoms of epileptic status with myoclonic jerks, but mother was 60% heteroplasmic.	No clinical information was provided.	9	M
MT-ND1 m.3796A>G Thr164Ala	**NGS385** Comprehensive Epilepsy	VUS, homoplasmic. Reported in publication of patient with adult-onset dystonia.	Localization-related idiopathic epilepsy and epileptic syndromes with seizures of localized onset, not intractable, without status epilepticus, EEG shows left centrotemporal sharp waves, tonic clonic epilepsy, maternal great uncle with Lennox-Gastaut Syndrome, mother has seizures, father hydrocephalus, healthy brother.	8	F
MT-CO1 m.6075G>A Val58Ile	**NGS330** Comprehensive Muscular Dystrophy/Myopathy	VUS, homoplasmic in leukocytes. Variants in this gene have been seen in cases of cytochrome C oxidase deficiency, sideroplastic anemia, optic atrophy, deafness, and colorectal cancer.	No clinical information was provided.	28	M
MT-CO1 m.6892G>A Ser330Asn	**NGS358** Comprehensive Dystonia	VUS, homoplasmic in DNA sample . Variants in this gene have been seen in cases of cytochrome C oxidase deficiency, sideroplastic anemia, optic atrophy, deafness, and colorectal cancer. (extracted DNA, tissue not specified)	Intermittent jerky movements of the right upper limb and neck, movements are episodic, quick and jerky, brain MRI shows diffuse increase in the thickness of the posterior body and the splenium of the corpus callosum, no jaw tensing, no head or hand tremors, parents are distantly related, 6 healthy siblings.	13	M
MT-CO1 m.7284T>G Ser461Ala	**NGS345A** AXONAL Charcot Marie Tooth Disease	VUS, homoplasmic in leukocytes. Variants in this gene have been seen in cases of cytochrome C oxidase deficiency, sideroplastic anemia, optic atrophy, deafness, and colorectal cancer.	Idiopathic progressive neuropathy.	52	M
MT-TK m.8296A>G	**NGS372** Comprehensive Leukodystrophy/Leukoencephalopathy	VUS, homoplasmic. Variant seen in patients with MELAS, optic atrophy, bilateral striatal necrosis, epilepsy, cardiomyopathy, stroke-like episodes, and diabetes. Cybrid studies show variant may cause defects in oxidative phosphorylation.	Spastic diplegic cerebral palsy, congenital hypotonia, intellectual disability, staring spells, normal EEG, MRI during infancy showed abnormal white matter changes not present in recent MRI, hypotonia in upper extremities, obstructive sleep apnea, normal microarray.	12	M
MT-ND4 m.10863G>A Ser35Asn	**NGS372** Comprehensive Leukodystrophy/Leukoencephalopathy	VUS, homoplasmic. Variants in this gene have been seen in cases of optic atrophy, MELAS, dystonia, complex 1 deficiency.	Atypical febrile seizures, mild speech delay, abnormal brain MRI, T2 flair signal in the centrum semiovale and periventricular white matter and the splenium of the corpus collosum with enlarged perivascular spaces predominantly in white matter.	5	F
MT-ND6 m.14633A>G Met14Thr	**NGS385** Comprehensive Epilepsy	VUS, homoplasmic. Variants in this gene have been seen in cases of optic atrophy, MELAS, dystonia, complex 1 deficiency, bilateral striatal necrosis, oncocytoma, Parkinson's.	No clinical information was provided.	3	M
MT-CYB m.14799T>C Phe18Ser	**NGS372** Comprehensive Leukodystrophy/Leukoencephalopathy	VUS @ 13% . Variants in this gene have been seen in cases of optic atrophy, exercise intolerance, encephalomyopathy, multisystem disorder, septooptic dysplasia, obesity, and parkinsonism/MELAS overlap syndrome.	Tonic clonic seizures, developmental delay, ocular apraxia, parents and sibling are healthy, negative family history.	5	M
MT-CYB m.15209T>C Tyr155His	**NGS324** Ataxia/Episodic Ataxia Disorders	VUS, homoplasmic. Previously seen in a patient with Prader-Willi with multisystem involvement (brain atrophy, motor axonal neuropathy, hyponatremia, frequent infections, lactic acidosis).	Lack of coordination, unspecified ataxia.	85	F

(Highlighted variants: NOT present in MitoMap)

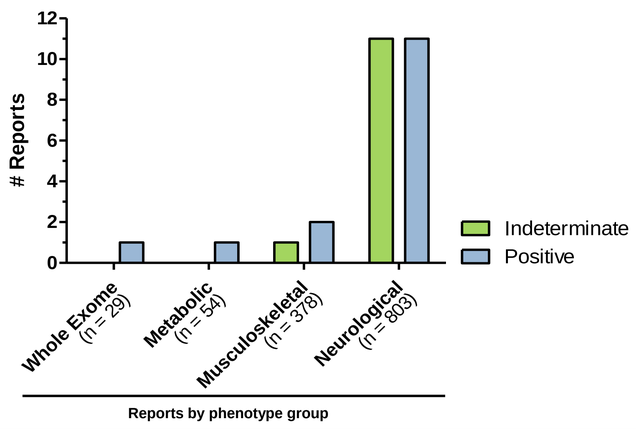
FIG 1: Number of reported mtDNA variants

A

	Data Range: Oct 2016-Aug 2017	Totals
I.	# NGS Panel Tests with mtDNA	1265
	% All NGS Panel Indeterminate Tests due to mtDNA	12/1265 = 0.9%
	% All NGS Panel Positive Tests due to mtDNA	15/1265 = 1.2%
II.	# Indeterminate NGS Panel Tests with mtDNA	733
	% NGS Panel Indeterminate Tests due to mtDNA	12/733 = 1.6%
III.	# Positive NGS Panel Tests with mtDNA	232
	% NGS Panel Positive Tests due to mtDNA	15/232 = 6.5%

I. Indeterminate and Positive reports due to mtDNA variants out of ALL mtDNA-included NGS panels reported.
II. Indeterminate reports due to mtDNA variants out of ALL Indeterminate mtDNA-included NGS panels reported.
III. Positive reports due to mtDNA variants out of ALL Positive mtDNA-included NGS panels reported.

B



Results

- mtDNA variants were responsible for 6.5% of all positive reports in panels screening for both nuclear and mitochondrial genes **(Figure 1A)**. Additionally, neurological phenotype-based panels were the group with the highest number of mtDNA variants of interest **(Figure 1B)**.
- Reported mtDNA variants were detected throughout the mitochondrial chromosome in protein-coding, tRNA, and rRNA genes. Large mitochondrial deletions were also detected. **(Figure 2 and Table 1)**.
- Inclusion of mtDNA in exome sequencing allowed for identification of the m.3243A>G MELAS variant in a patient whose symptoms were not initially suspected to be of mitochondrial origin **(Figure 3)**.

Conclusions

- Mitochondrial dysfunction is important in pathogenesis and clinical manifestation of disease[8,9] making mtDNA analysis both a logical and practical addition for sequencing panels.
- Benefits to including mtDNA analysis not only manifest in the identification of variants of interest that could direct patient care, but also in relation to the cost, time, and interpretation that would be required for ordering and receiving separate results for individual nuclear and mitochondrial sequencing panels. This is especially important in cases where the rapid decline of a patient is of concern.
- Our data demonstrate these benefits, through identification of pathogenic variants in patients that would have gone undetected in nuclear gene only testing. Increased sensitivity to identify variants of interest, combined with the efficiency of ordering a single test, provides added value and increases the potential of proper clinical diagnoses.

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