

Courtagen's nucSEEK®



Mitochondrial
Disease

Novel Disease Associations and Novel Disease-Associated Genes Elucidated Among MitoCarta Gene Sequencing in 182 Probands for Mitochondrial Disease

Introduction:

Mitochondrial disorders present diagnostic challenges due to phenotypic and genotypic heterogeneity. Looking beyond the mitochondrial genome, nuclear mitochondrial genes continue to surface as major causes of mitochondrial disease. In this study, we describe our experience sequencing ~1,200 nuclear mitochondrial genes in 182 unrelated patients referred for DNA analysis based on clinical suspicion of mitochondrial disease.

Methods:

nucSEEK®: Comprehensive Sequence Analysis of Nuclear Mitochondrial Genes

Courtagen Life Sciences has developed nucSEEK®, a Next Generation DNA sequencing assay that provides diagnostic sequence information for 1,200 nuclear-encoded genes for proteins that localize to the mitochondria. The panel includes the 1,034 gene MitoCarta, as well as ~100 additional phenocopy, peroxisomal and cytosolic “metabolic” genes. DNA was extracted from saliva samples and sequenced using the Illumina MiSEQ. The test sensitivity for nucSEEK® is 99% with a specificity of 99.99%. All variants of clinical interest were verified by Sanger sequencing.

Utility of nucSEEK® Comprehensive Sequence Analysis

Courtagen's nucSEEK® offers the most comprehensive gene panel for accurate detection of variants in the entire nuclear, mitochondrial exome.

Since one of the great benefits of genetic testing is identifying conditions which are treatable, we highlight patient examples for both known and novel genotype-phenotype correlations. In the cases presented, medical management was altered and positive outcomes achieved based on nucSEEK® testing results.

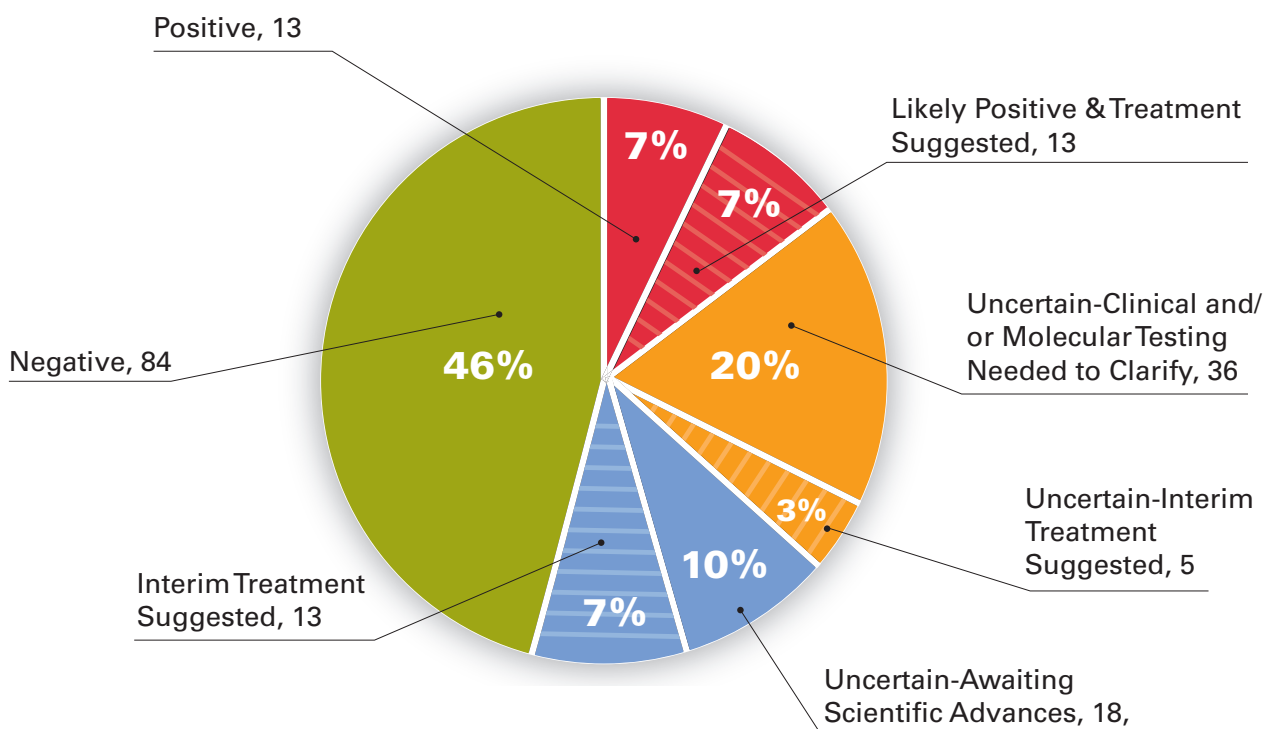
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Results

Nuclear mitochondrial testing resulted in a highly-probable diagnosis in 26 cases (18% of cases where work-up is completed), half of which suggested specific therapies, and many of which were anecdotally successful. Figure 1 displays the results of the 182 patients tested.

FIGURE 1: Results of 182 nucSEEK® Tests



Description of Data Categories:

- **Positive and Likely Positive:** Variants identified are associated with or are predicted to be associated with disease. The variant correlates well with the phenotype presented.
- **Uncertain:** Clinical and/or Molecular testing needed for clarification. Variants identified are of uncertain significance. Further testing is needed to confirm if the variant is likely disease associated/causal.
- **Uncertain:** Awaiting scientific advances.
- **Negative:** No variants identified of suspected disease association.
- **Treatment Options Suggested:** Hatch marks within each category indicate the proportion of patients for whom management changes, such as folinic acid, fasting avoidance, or valproate therapy, were suggested based on testing results.

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Among the 182 probands, both established and potentially novel genotype-phenotype correlations were identified. Mutations in fourteen genes with established associations to disease include:

<i>ACAD9</i>	<i>MFN2</i>	<i>SCN2A</i>
<i>ALDH5A1</i>	<i>NDUFA1</i>	<i>SCN4A</i>
<i>ATP7B</i>	<i>NRXN1</i>	<i>TPH2</i>
<i>COQ2</i>	<i>PNKD</i>	<i>UBE3A</i>
<i>KIF1B</i>	<i>RRM2B</i>	

Novel Associations

Table 1: Novel Associations Identified, 2 Mutations per Gene

<i>Gene</i>	<i>Gene Name</i>	<i>Associated Phenotype</i>
<i>ACSM3</i>	Acyl-CoA synthetase medium-chain family member 3	Severe irritability, hypersensitivity, growth issue, PANS, and immunodeficiency
<i>ALDH1L2</i>	Aldehyde dehydrogenase 1 family, member L2	Tic disorder and OCD
<i>ATAD3B</i>	ATPase family, AAA domain containing 3B	Severe autism
<i>IARS2</i>	Isoleucyl-tRNA synthetase 2	Infantile-lethal Leigh syndrome
<i>SHMT1</i>	Serine hydroxymethyl transferase 1	Loss of milestones, tics and jerks, and progressive regression

Table 2: Novel Associations Identified, 1 Mutation per Gene

<i>Gene</i>	<i>Gene Name</i>	<i>Associated Phenotype</i>
<i>ABCB6</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 6 (10 pts/270)	Pediatric Autoimmune Neurological Syndrome (PANS)
<i>CHAT</i>	Choline O-acetyltransferase (4 patients)	Episode encephalopathy and adverse reactions to anti-cholinergic drugs
<i>FPGS</i>	Folypolyglutamate synthetase enzyme (8 patients/270)	Pediatric Autoimmune Neurological Syndrome (PANS)
<i>LETM1</i>	Leucine zipper-EF-hand containing transmembrane protein 1 (1 patient)	Neuromuscular disease
<i>TRAP1</i>	TNF receptor-associated protein 1 (10 patients/270)	Loss of milestones, tics and jerks, and progressive regression
<i>WFS1</i>	Wolfram syndrome 1 (4 patients)	Autistic spectrum disorders

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Case Reports

Novel Conditions in which Management was Altered

TRAP1: A 3 year old girl presented with multiple manifestations of functional disease, including GI dysmotility requiring TPN, chronic fatigue, and chronic pain in many locations. Sequencing revealed a predicted deleterious variant, I235V, in the TNF receptor-associated protein 1 (*TRAP1*), a mitochondrial chaperone involved in antioxidant defense. This patient is one of 12 cases identified by Courtagen to date who have previously unidentified disease associated with mutations in the *TRAP1* ATPase domain, all of which have a triad of dysmotility, pain and fatigue, with normal intelligence. Chronic pain improved greatly on antioxidant therapy.

CHAT: Multiple cases with mutations in the choline O-acetyltransferase gene (*CHAT*) gene improved substantially with acetylcholine esterase inhibitor therapy.

IARS2: A couple who had lost a child to Leigh disease requested prenatal testing. Two predicted deleterious mutations in the isoleucyl-tRNA synthetase gene (*IARS2* W607X, E708K) were identified in the deceased child, one inherited from each parent. Although no defects have been reported in *IARS2*, other tRNA synthetase genes are implicated in Leigh disease. The couple underwent prenatal testing, revealing the fetus to be heterozygous for *IARS2* and thus predicted to be healthy.

Known Conditions in which Management was Altered

SHMT1: A 4 year old female presented with ataxia and developmental regression. Sequencing revealed the presence of a novel homozygous predicted deleterious variant, E344Q, in the serine hydroxymethyltransferase 1 (*SHMT1*) gene, within the folate metabolism pathway. Treatment with folinic acid and glycine resulted in improvement of her gross motor, fine motor and expressive language skills.

COQ2: A newborn girl presented with severe dilated cardiomyopathy and hypotonia. Sequencing identified a homozygous variant, V393A, in the *COQ2* gene. While she was empirically started on CoQ10 by her physician early on, this diagnosis resulted in a many-fold increase of the dosage. Her cardiac function has improved substantially, and she no longer requires transplantation.

CHRNA4: A 14 year old male presented with abnormal movements that were described as tics. Testing revealed a predicted deleterious mutation, E92Q, in the nicotinic cholinergic receptor (*CHRNA4*). Treatment was altered to address seizures, resulting in substantial clinical improvement.

PNKD: A 6 year old boy was referred for intermittent ataxia, diarrhea, exercise intolerance and speech articulation difficulties. Sequencing identified the predicted deleterious mutation G89R in the paroxysmal nonkinesigenic dyskinesia (*PNKD*) gene, associated with the rare AD movement disorder. On retrospective inquiry, he and his mother were noted to have dyskinesia. Counseling regarding the benign nature of this condition and disease triggers was helpful to the family, and the boy's manifestations have improved. This mutation differs from the two classic mutations previously reported, and with a phenotype dominated with ataxia, not dyskinesia or dystonia, this family represents an undescribed variant of the disorder. An unrelated case of a novel *PNKD* mutation with ataxia and dystonia was also identified by our laboratory.