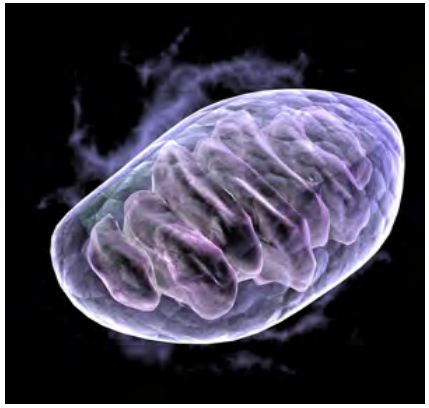
Courtagen DIAGNOSTICS LABORATORY

Children's Hospital LOS ANGELES We Treat Kids Better

USC UNIVERSITY OF SOUTHERN CALIFORNIA

Interpreting Genetic Testing



Richard G. Boles, M.D.
Medical Director, Courtagen Life Sciences, Inc.
Associate Professor of Pediatrics, Keck School of Medicine at USC
Division of Medical Genetics, Children's Hospital Los Angeles

September 13, 2013

Disclosure: I wear many hats.

Medical Director of Courtagen Life Sciences Inc.

- Test development
- Test interpretation
- Marketing

Researcher with NIH and foundation funding

- Studying sequence variation that predispose towards functional disease
- Treatment protocols

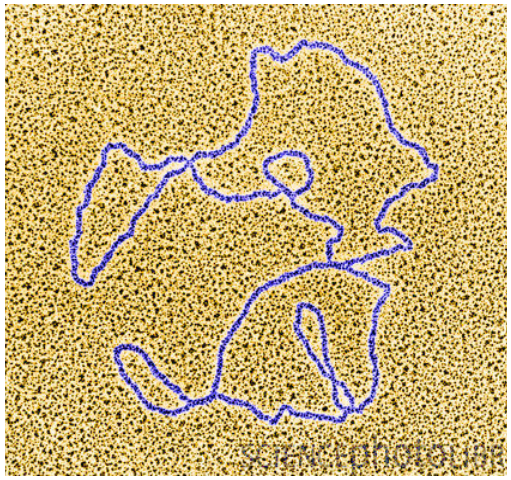
Clinician treating patients

- Functional disease (CVS, autism, etc.)
- Genetic/metabolic disorders
- General pediatrics

Mitochondrial Genetics

The Basics

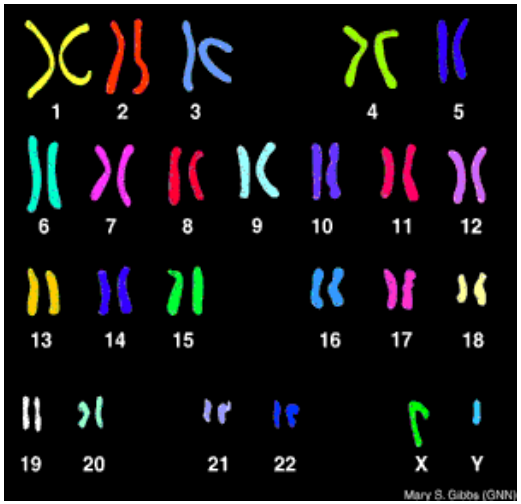
Mitochondrial DNA



37 genes

- Maternal inheritance

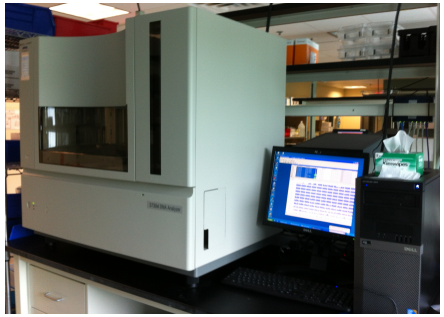
Nuclear DNA

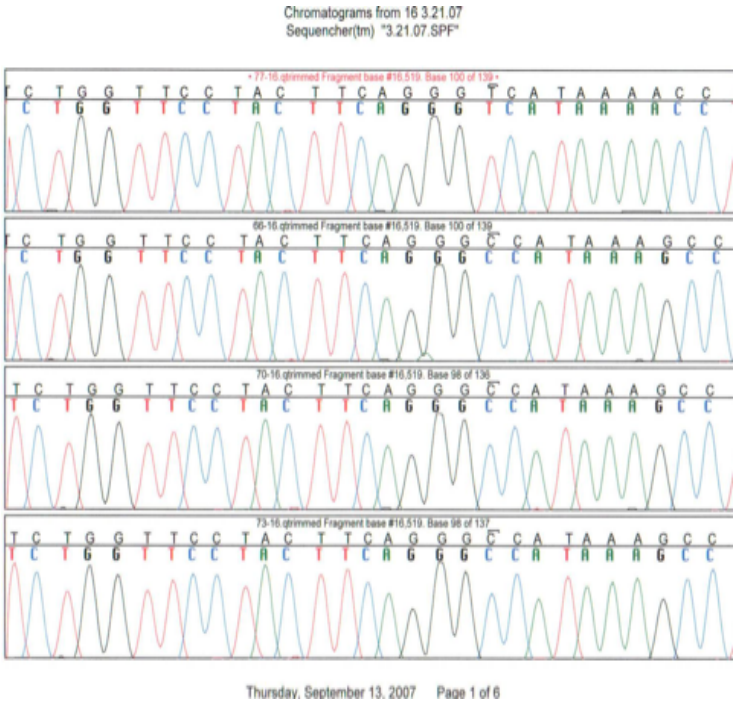


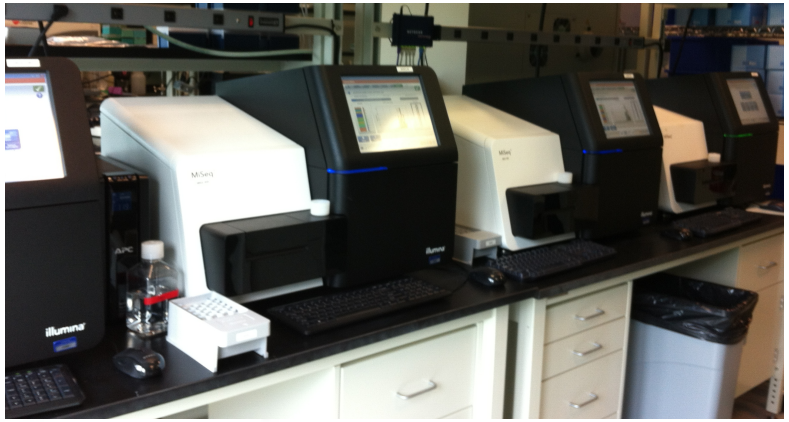
1,013 genes

- Autosomal recessive
- Autosomal dominant
- X-linked

DNA Sequencing: Sanger (3730XL) and Next Generation (MiSeq)





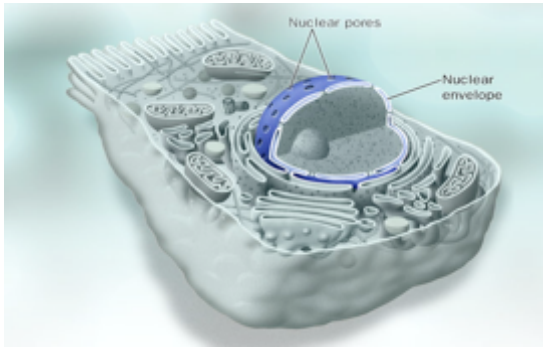


Courtagen DIAGNOSTICS LABORATORY

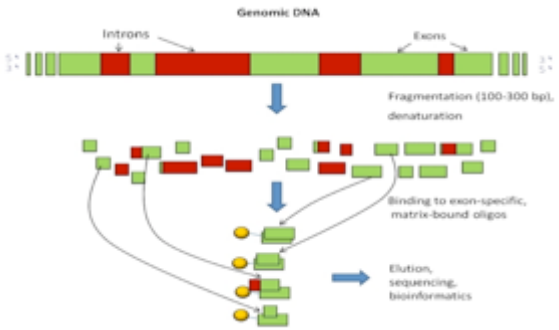
Novel Assay Design

- Uses a combination of PCR and Probe based approaches to selectively enrich pseudogene free DNA
- 2 x 250bp reads assists in reducing mis-mapping to pseudogenes
- Average variant depth of coverage greater than 200X
- Sequence data analyzed using Courtagen's proprietary bioinformatics analysis program (Ziphyr®)
- Non-synonymous variants reviewed and scored for correlation with the clinical phenotype of each patient.
- Variants suspected to be related to disease are confirmed by Sanger sequencing

Pseudogene depletion step



On target reads



.

Turnaround = Weeks not Months

Why order a Genetic Test?

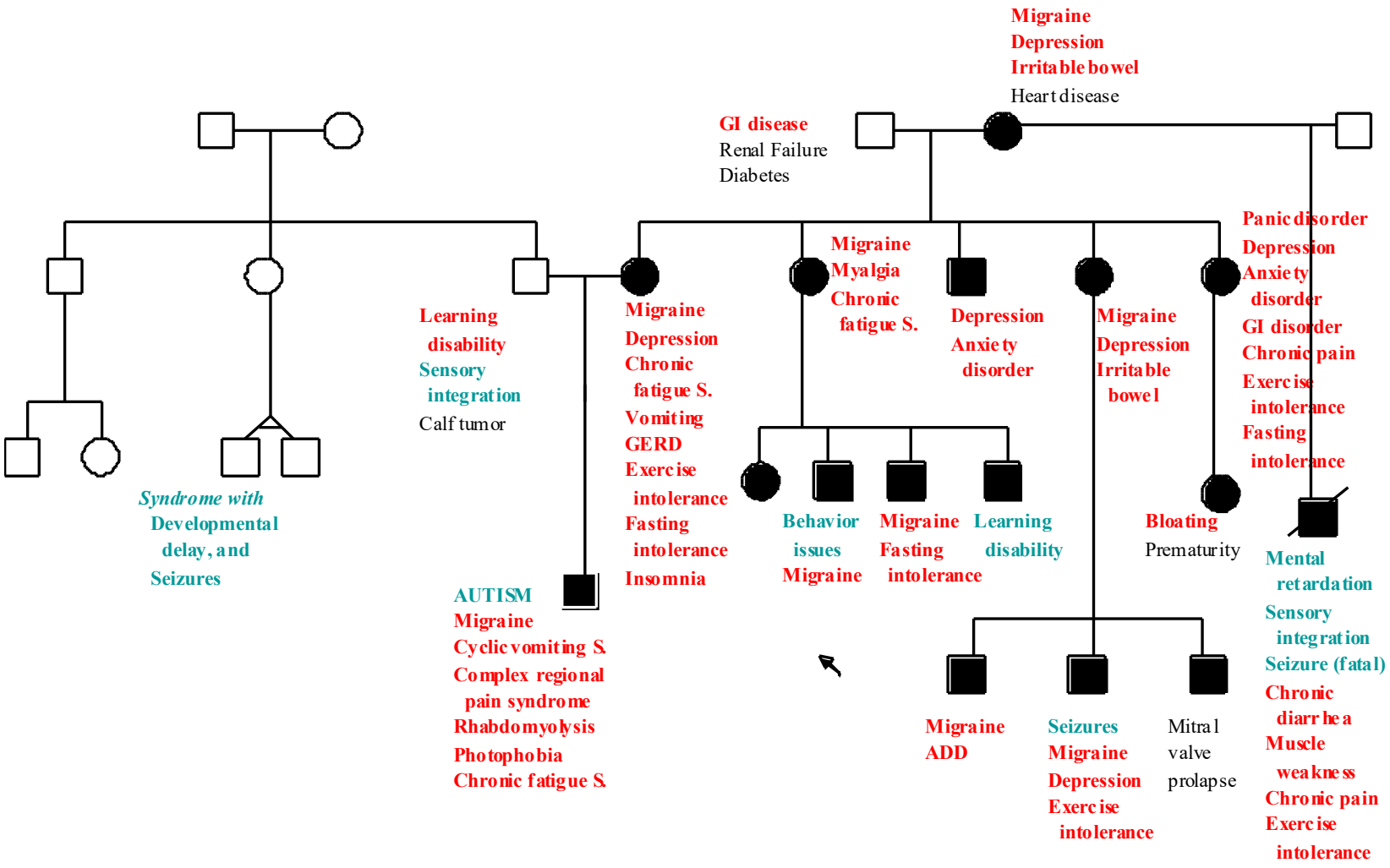
- Establish/prove an exact diagnosis
  - Justify existing mitochondrial treatments/precautions
  - Limit further diagnostic testing
  - Finally, an answer
- Determine the mode of inheritance
- Help guide therapy
  - Which cofactors are likely to work?
  - Suggest new/different therapies
- An investment in further knowledge
  - Delayed diagnoses/recommendations

Case Report

Clinical History

- Autism – early infancy
  - Lost language skills acquired at 18 months.
  - Diagnosed with “autism” at age 2 yrs
- Cyclic vomiting syndrome – age 6 years
  - Episodes of nausea, vomiting and lethargy lasting from a few days to a week or more
- Rhabdomyolysis – age 11 years
  - Hospitalized twice, maximum CK = 100K; precipitated by anesthesia (dental) and influenza B
- Complex regional pain syndrome – age 12 yrs
  - Episodes in which right foot becomes cold, purple, tender, allodynia, unable to bear weight; wheelchair bound for months
- Other chronic intermittent symptoms
  - Headache, muscle pain, constipation, photophobia, ptosis, tics, hours-long episodes of hiccups.
- Severe exercise intolerance

Pedigree



Ins512C, statistically associated with CVS with O.R. = 5

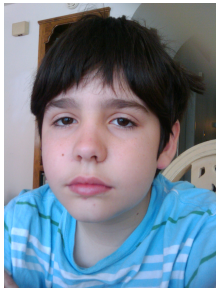
Genomics

- NextGen sequencing of the 1,100 gene MitoCarta - 200-fold
  - L340F change in the *CHAT* gene encoding choline O-acetyltransferase gene, the enzyme catalyzes the synthesis of acetylcholine from choline and acetyl-CoA in cholinergic neurons
  - Variant is highly conserved, predicted as deleterious by MutationTaster, SIFT, and PolyPhen2. Prevalence: 1 in 200

- Good genotype-phenotype match
  - Cognitive decline is a prominent factor in this patient's disease
  - Poorly tolerates anticholinergic medications
  - One *CHAT* mutation was found in another patient with a similar phenotype of pseudoobstruction, POTS, chronic fatigue, dysautonomia, autistic spectrum disorder, and exaggerated anticholinergic effects to medications

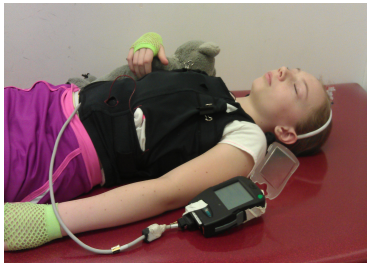
Courtagen DIAGNOSTICS LABORATORY

Haploinsufficiency as a Novel Treatable Disorder



- Four cases, each with apparent maternal inheritance, biochemical data demonstrating mitochondrial dysfunction, and typical symptomatology of neurological disease, pain, fatigue, and GI dysmotility.

- Additional manifestations are distinct:
  - Episodic mental status changes w/o known triggers
  - POTS/dysautonomia
  - Severe reactions to anticholinergic medications



- Parasympathetic deficiency by LifeShirt (- 3 to 4 SD)

- Anecdotal, yet dramatic, improvement with donepezil (Aricept)

- Digenic: mtDNA + *CHAT* “polymorphism”

nucSEEK™ Case Report

Courtagen DIAGNOSTICS LABORATORY

- 17-year-old female. Presents with “functional” disease:
  - Chronic pain: migraine, myalgia, joints, abdomen (“functional”)
  - Depression, anxiety, and panic
  - Chronic fatigue syndrome
  - Tinnitus, POTS, other dysautonomic symptoms

- Apparent maternal inheritance:
  - Brother is the proband, presented at age 8 years with ketotic hypoglycemia, chronic pain “cramps”, and tinnitus.
  - Mother with migraine, fibromyalgia, depression, parasthesia
  - Functional symptoms is maternal aunt, uncle, and grandmother

- Biochemical testing: minimal due to HMO, anion gap metabolic acidosis/ketosis, high urine carnitine and blood C8

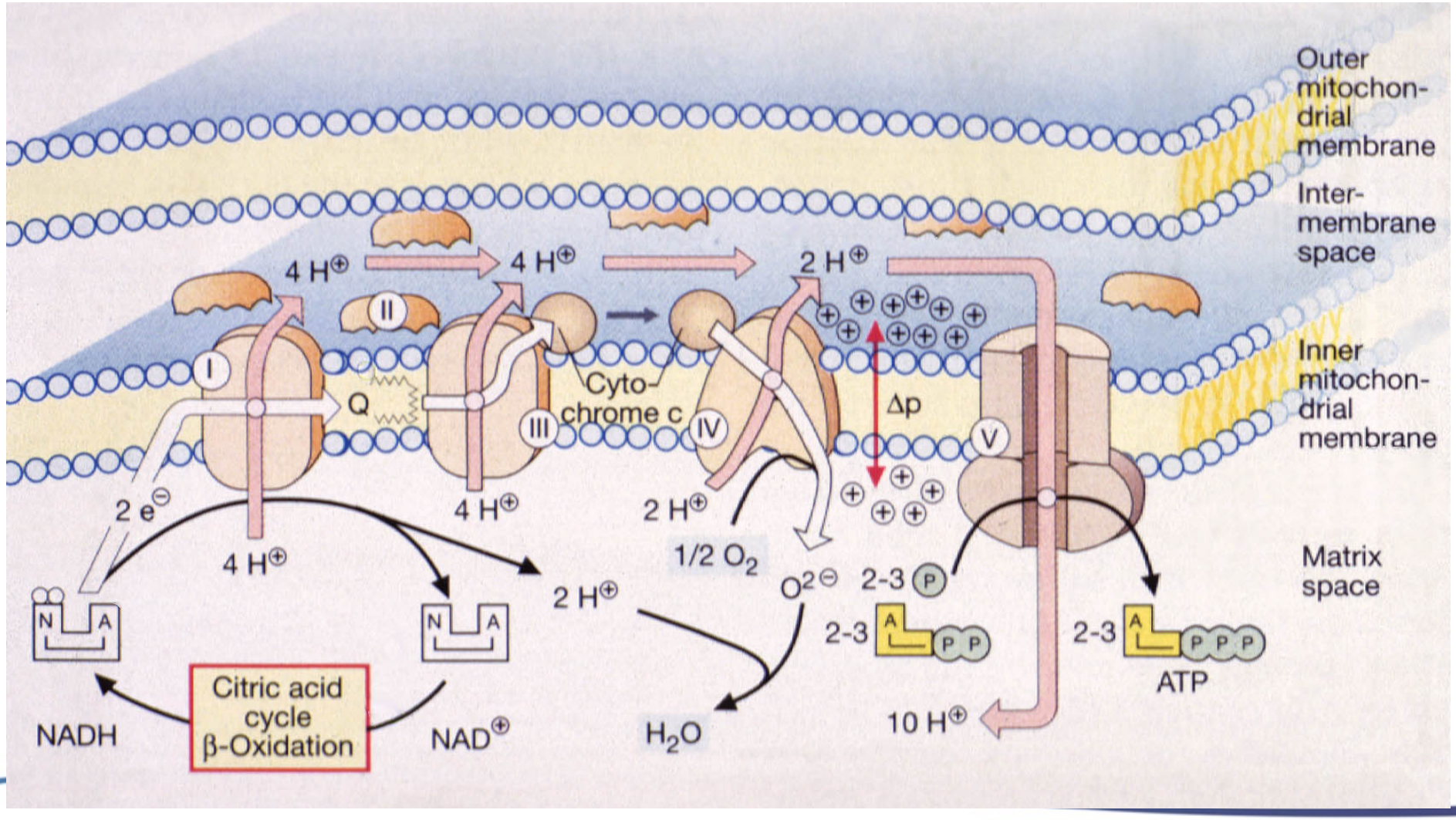
- Substantial improvement on 600 mg BID of coenzyme Q10, B100, and sertraline (Zoloft)

H599R in the *SCN4A* Gene

- The variant found in this patient is predicted to be deleterious
  - very-highly evolutionarily conserved
  - per computer algorithms
  - rare, present in 1 out of 670 people.
- Encodes the alpha subunit of the voltage-gated, type IV sodium channel, which is expressed in skeletal muscle.
- Along with other proteins in its category, these proteins are responsible for the generation and propagation of action potentials in neurons and muscle.
- Mutations in this gene have been linked to hyperkalemic periodic paralysis (type 2), myasthenic syndrome (acetazolamide-responsive), myotonia congenita atypical (acetazolamide-responsive) and paramyotonia congenita
- All are autosomal dominant conditions.
- Phenotypic variability is considerable. Some patients manifest as muscle stiffness or cramps.
- On retrospect, the patient, her two siblings and their mother suffer from muscle cramps, especially her brother, and especially in the toes.
- Acetazolamide treatment is generally effective in this condition. In this patient:
  - reduced pain in abdomen and joints
  - Less depression and anxiety
- Her mother carries the mutation by Sanger; testing in other relatives is pending.

How Do We Get an Exact Diagnosis?

Electron Transport Chain



Metabolic Pathways

Courtagen
DIAGNOSTICS LABORATORY

Genotype-Phenotype Correlation in Mitochondrial Disease

- A single condition can be caused by mutation in a large number, perhaps hundreds, of genes.

- A single family segregating an identified mutation can present with very-different disease manifestations in each affected relative.

What genotype-phenotype correlation!

crazy20nancy20straight20jacket.jpg

Molecular (DNA) Diagnosis

Courtagen DIAGNOSTICS LABORATORY

- Mitochondrial DNA (mtDNA)
  - Standard mtDNA analysis
    - PCR for common point mutations (3243A>G, 8344A>G, 8993T>G or C)
    - PCR or Southern blotting for large rearrangements
  - Full mtDNA sequencing
- Nuclear DNA testing
  - Single gene (MNGIE)
  - Small Panel (few-several genes: e.g. COX deficiency, mtDNA depletion,)
  - Mito-exome (1,100 genes)
  - Exome (22,000 genes)
  - Genome (all of the DNA)

Our Approach – Sequence 'Em All!

Courtagen DIAGNOSTICS LABORATORY

mtSEEK™

NextGen sequencing of the 37 mtDNA genes and control region

nucSEEK™

NextGen sequencing of the 1,197 nuclear-coded genes involved in energy metabolism

- MitoCarta (1013 genes)
- Peroxisomal genes
- Cytosolic "metabolic" genes
- Phenocopies

Bioinformatics – Simplifying the Data

Courtagen DIAGNOSTICS LABORATORY

- Sequences are compared to the human genome reference sequence.
- Each sequence will produce about 3,000 variants

- The data are filtered for:
  - coverage (how many times each base was measured) - removing variants that have not been sequenced enough times to provide confidence in the result
  - variants located in the intron regions
  - variants that are synonymous – change a nucleotide, but do not result in an amino acid change

  producing about 300 variants per nucSEEK sequence

- Final filter: all common variants present in 1% or greater of the population are removed – leaving about 30 variants per sequence that may have some association with disease.

Bioinformatics – Interpreting the Data

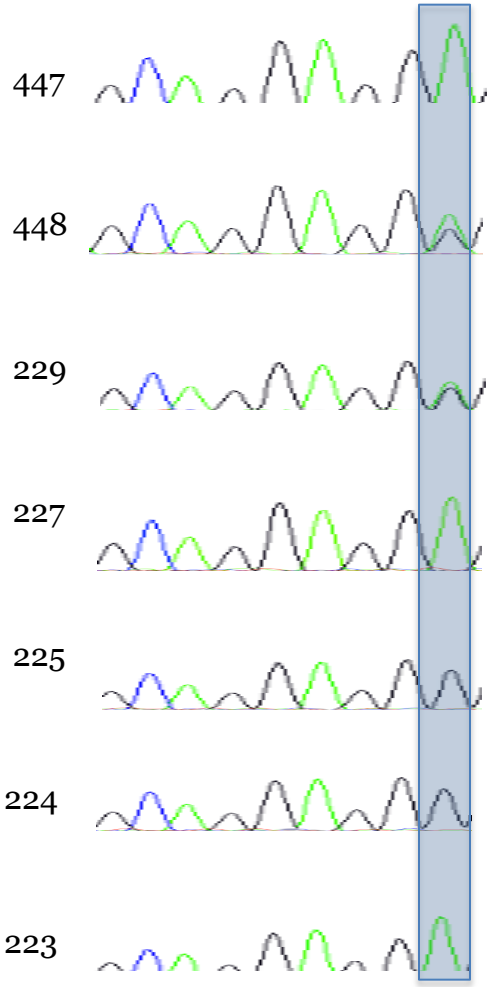
Courtagen DIAGNOSTICS LABORATORY

- Sequence variants are evaluated for predicted pathology = the likelihood that a variant adversely affects protein function:
  - prevalence – how frequent the variant is in humans
  - conservation – how common mutation is in other species
  - protein function – predicted by 3 computer algorithms

- Suspected mode of inheritance: dominant, recessive, unknown versus the number of probable mutations found.

- The patient's phenotype – clinical manifestations.
- Any laboratory or other data provided.

Sanger Confirmation



Mother – wild type

Father – het variant

Het variant – matches nucSEEK

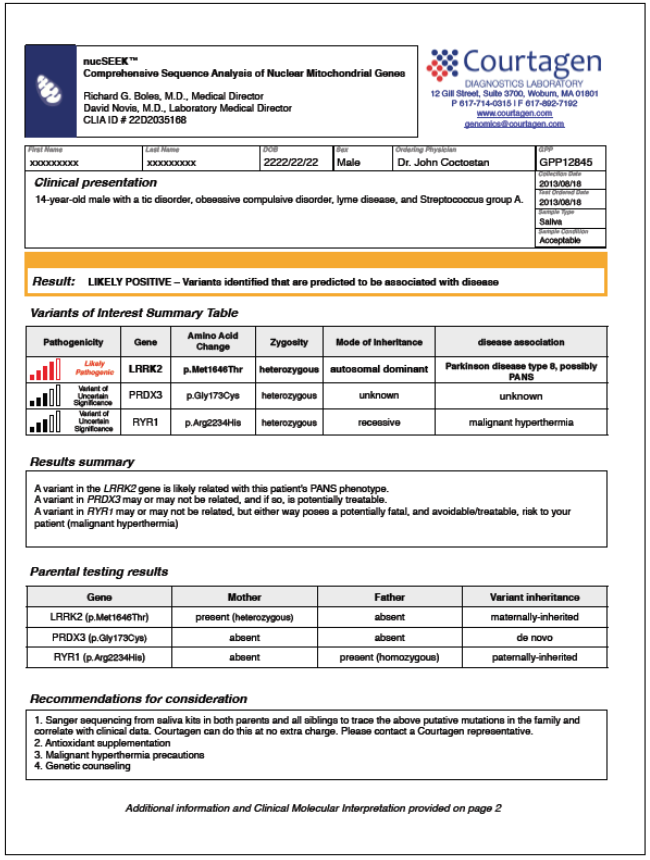
Wild type– matches nucSEEK

Homozygous variant – matches nucSEEK

Homozygous variant – matches nucSEEK

Wild type– matches nucSEEK

Sample Report



nucSEEK™
Comprehensive Sequence Analysis of Nuclear Mitochondrial Genes

Richard G. Boles, M.D., Medical Director
David Novis, M.D., Laboratory Medical Director
CLIA ID # 22D2035168

Courtagen DIAGNOSTICS LABORATORY
12 Gill Street, Suite 3700, Woburn, MA 01801
P 617-714-0315 I F 617-892-7192
www.courtagen.com
genomics@courtagen.com

First Name	Last Name	DOB	Sex	Ordering Physician	GPP
xxxxxxxxx	xxxxxxxxx	2222/22/22	Male	Dr. John Coctostan	GPP12845

Collection Date: 2013/08/18
Test Ordered Date: 2013/08/18
Sample Type: Saliva
Sample Condition: Acceptable

Clinical presentation

14-year-old male with a tic disorder, obsessive compulsive disorder, lyme disease, and Streptococcus group A.

Result: **LIKELY POSITIVE – Variants identified that are predicted to be associated with disease**

Variants of Interest Summary Table

Pathogenicity	Gene	Amino Acid Change	Zygosity	Mode of Inheritance	disease association
Likely Pathogenic	LRRK2	p.Met1646Thr	heterozygous	autosomal dominant	Parkinson disease type 8, possibly PANS
Variant of Uncertain Significance	PRDX3	p.Gly173Cys	heterozygous	unknown	unknown
Variant of Uncertain Significance	RYR1	p.Arg2234His	heterozygous	recessive	malignant hyperthermia

Results summary

A variant in the *LRRK2* gene is likely related with this patient's PANS phenotype.
A variant in *PRDX3* may or may not be related, and if so, is potentially treatable.
A variant in *RYR1* may or may not be related, but either way poses a potentially fatal, and avoidable/treatable, risk to your patient (malignant hyperthermia)

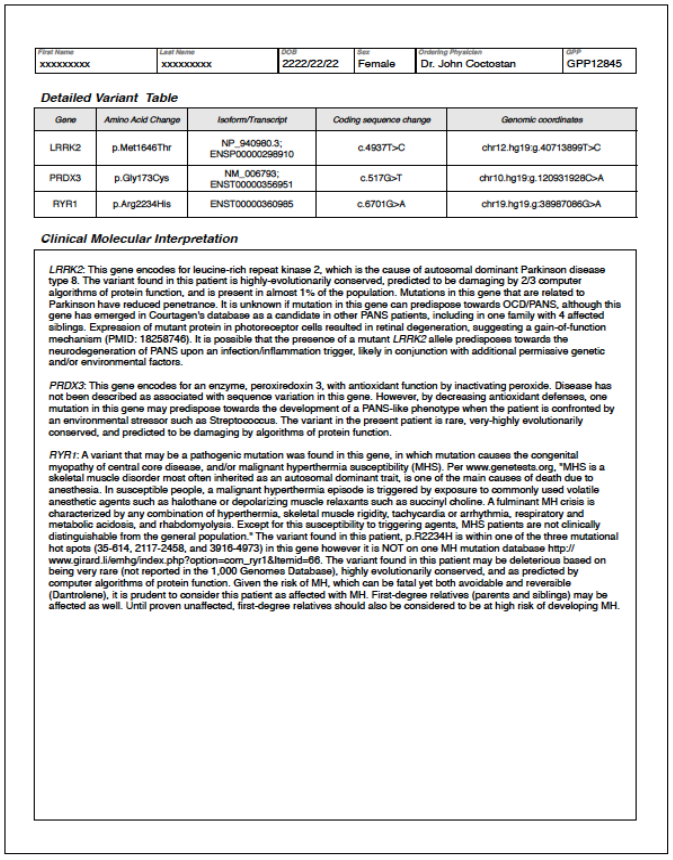
Parental testing results

Gene	Mother	Father	Variant inheritance
LRRK2 (p.Met1646Thr)	present (heterozygous)	absent	maternally-inherited
PRDX3 (p.Gly173Cys)	absent	absent	de novo
RYR1 (p.Arg2234His)	absent	present (homozygous)	paternally-inherited

Recommendations for consideration

1. Sanger sequencing from saliva kits in both parents and all siblings to trace the above putative mutations in the family and correlate with clinical data. Courtagen can do this at no extra charge. Please contact a Courtagen representative.
2. Antioxidant supplementation
3. Malignant hyperthermia precautions
4. Genetic counseling

Additional information and Clinical Molecular Interpretation provided on page 2



First Name	Last Name	DOB	Sex	Ordering Physician	GPP
xxxxxxxxx	xxxxxxxxx	2222/22/22	Female	Dr. John Coctostan	GPP12845

Detailed Variant Table

Gene	Amino Acid Change	Isoform/Transcript	Coding sequence change	Genomic coordinates
LRRK2	p.Met1646Thr	NP_940980.3; ENSP00000298910	c.4937T>C	chr12.hg19:g.40713899T>C
PRDX3	p.Gly173Cys	NM_006793; ENST00000356951	c.517G>T	chr10.hg19:g.12093192BC>A
RYR1	p.Arg2234His	ENST00000360985	c.6701G>A	chr19.hg19:g:38987086G>A

Clinical Molecular Interpretation

LRRK2: This gene encodes for leucine-rich repeat kinase 2, which is the cause of autosomal dominant Parkinson disease type 8. The variant found in this patient is highly-evolutionarily conserved, predicted to be damaging by 2/3 computer algorithms of protein function, and is present in almost 1% of the population. Mutations in this gene that are related to Parkinson have reduced penetrance. It is unknown if mutation in this gene can predispose towards OCD/PANS, although this gene has emerged in Courtagen's database as a candidate in other PANS patients, including in one family with 4 affected siblings. Expression of mutant protein in photoreceptor cells resulted in retinal degeneration, suggesting a gain-of-function mechanism (PMID: 18258746). It is possible that the presence of a mutant *LRRK2* allele predisposes towards the neurodegeneration of PANS upon an infection/inflammation trigger, likely in conjunction with additional permissive genetic and/or environmental factors.

PRDX3: This gene encodes for an enzyme, peroxiredoxin 3, with antioxidant function by inactivating peroxide. Disease has not been described as associated with sequence variation in this gene. However, by decreasing antioxidant defenses, one mutation in this gene may predispose towards the development of a PANS-like phenotype when the patient is confronted by an environmental stressor such as Streptococcus. The variant in the present patient is rare, very-highly evolutionarily conserved, and predicted to be damaging by algorithms of protein function.

RYR1: A variant that may be a pathogenic mutation was found in this gene, in which mutation causes the congenital myopathy of central core disease, and/or malignant hyperthermia susceptibility (MHS). Per www.genetests.org, "MHS is a skeletal muscle disorder most often inherited as an autosomal dominant trait, is one of the main causes of death due to anesthesia. In susceptible people, a malignant hyperthermia episode is triggered by exposure to commonly used volatile anesthetic agents such as halothane or depolarizing muscle relaxants such as succinyl choline. A fulminant MH crisis is characterized by any combination of hyperthermia, skeletal muscle rigidity, tachycardia or arrhythmia, respiratory and metabolic acidosis, and rhabdomyolysis. Except for this susceptibility to triggering agents, MHS patients are not clinically distinguishable from the general population." The variant found in this patient, p.R2234H is within one of the three mutational hot spots (35-614, 2117-2458, and 3916-4973) in this gene however it is NOT on one MH mutation database http://www.girard.li/emhg/index.php?option=com_ryr1&themid=66. The variant found in this patient may be deleterious based on being very rare (not reported in the 1,000 Genomes Database), highly evolutionarily conserved, and as predicted by computer algorithms of protein function. Given the risk of MH, which can be fatal yet both avoidable and reversible (Dantrolene), it is prudent to consider this patient as affected with MH. First-degree relatives (parents and siblings) may be affected as well. Until proven unaffected, first-degree relatives should also be considered to be at high risk of developing MH.

Report Classifications

Negative: No variants were detected that are suspected to be associated with the patient's disease.

Likely Negative: One or more variants were identified that are likely not associated with the patient's disease. However, one or more variants were identified for which further testing may be indicated. Parental testing, clinical correlation, or further biochemical, imaging, or other tests may alter the test interpretation.

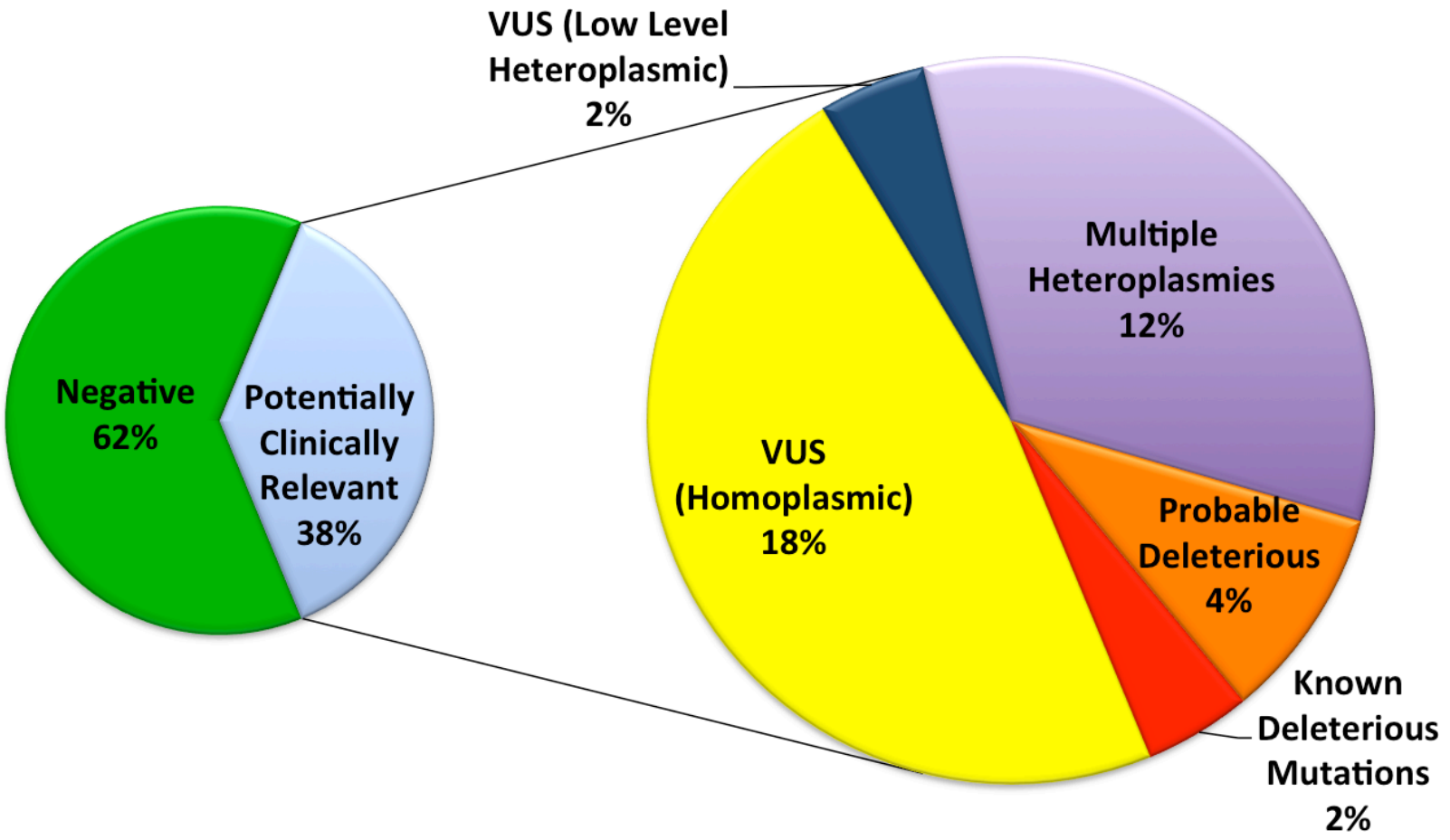
Uncertain. Variant of Uncertain Significance (VUS): One or more variants were detected for which clinical significance is uncertain. Parental testing, clinical correlation, or further imaging, biochemical, or other tests are likely indicated.

Likely positive: One or more variants were found that are likely associated with the patient's disease. While these variants have not previously been reported in the scientific literature as being associated with disease, based on our current interpretation algorithm, these variants are predicted to be damaging to protein structure and function. Parental testing, clinical correlation, or further imaging, biochemical, or other tests are likely indicated.

Positive: One or more genetic variants were identified that have been previously associated with disease or are very likely pathogenic based on their predicted effect on protein structure.

mtSEEK™ Results on Sequencing Entire mtDNA (112 Probands)

Courtagen DIAGNOSTICS LABORATORY

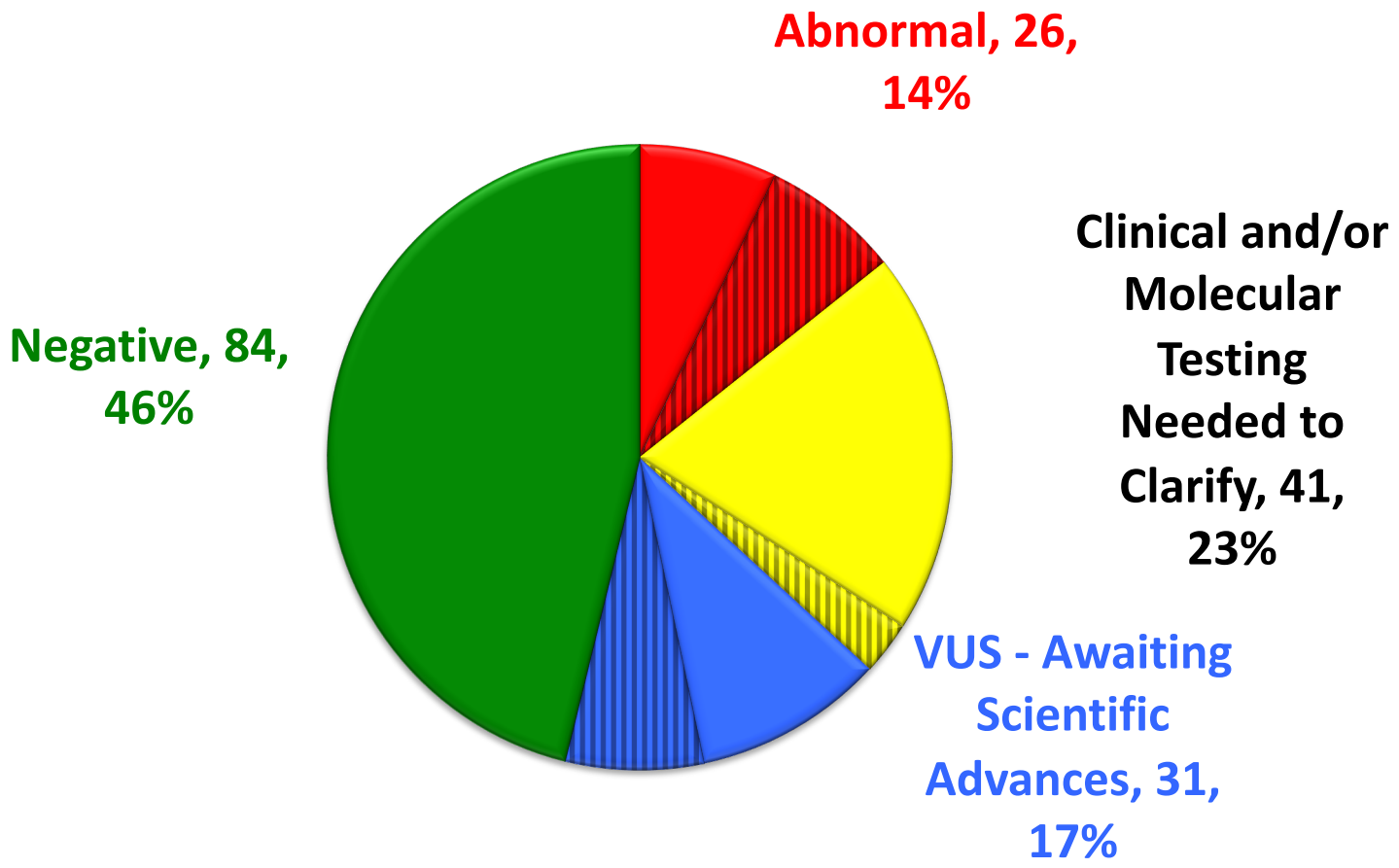


mtSEEK™ (112 Probands) Multiple Heteroplasmy

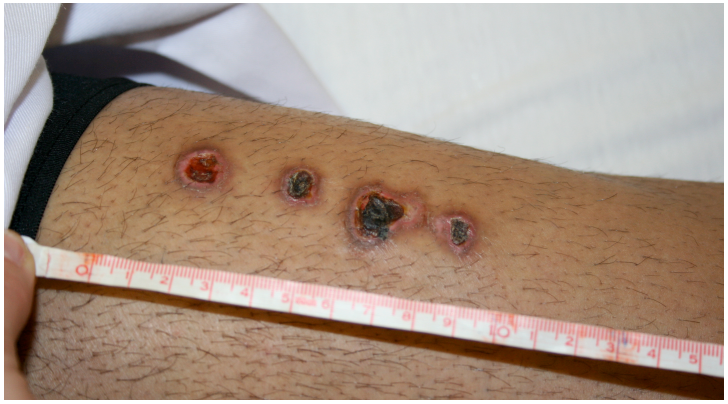
Courtagen DIAGNOSTICS LABORATORY

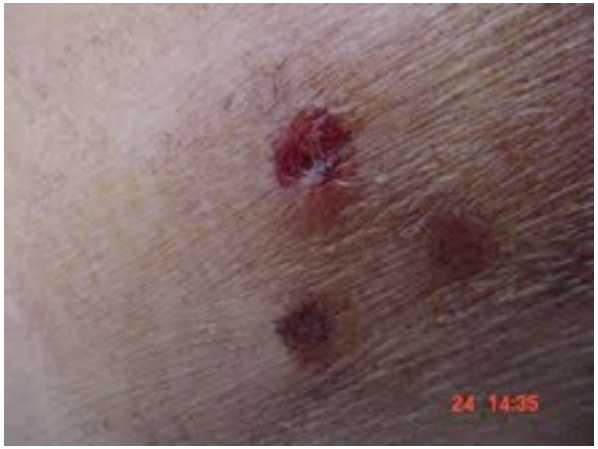
	Clinical Samples (n=112)		Negative Controls (n=38)
0 heteroplasmies	50%		68%
1 heteroplasmy	34%		32%
2 heteroplasmies	12%	(*P* = 0.007)	0
3 heteroplasmies	4%		0

nucSEEK™ Results (182 Probands)



Who Is Really “Normal”?





One Negative Control Subject had an Abnormal Interpretation:

- *CPOX:* coproporphyrinogen oxidase
- Sixth enzyme of the heme biosynthetic pathway
- Cause of hereditary coproporphyria (HCP)
- Autosomal dominant
- Hepatic, neurological and psychiatric disease, like acute intermittent porphyria PLUS skin rash
- not reported in the 1,000 Genomes Database), high evolutionary conservation (at least as distant as zebrafish), and 3/3 computer algorithms of protein function.

Case Report, 3-year-old girl

- GI dysmotility – on full TPN cannot tolerate any enteral intake, including jejunal drips
- Chronic pain – severe leg pain and headache
- Chronic fatigue – sleeping 22 hours a day
- Hypoglyemia, even on 24-hour drip feedings
- Episodic right arm limpness
- Anemia – received multiple blood transfusions
- Dysautonomia: tachycardia, temperature instability, hypoxia, neurogenic bladder
- Rotenone-sensitive NADH-cytochrome c reductase deficiency = 7%.

nucSEEK Case Report 2

- *TRAP1*: A 3-year-old girl presented with multiple manifestations of functional disease, including gastrointestinal 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.

- *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.

Courtagen DIAGNOSTICS LABORATORY

- *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 the dosage being increased many fold.

- Her cardiac function has improved substantially, and no longer requires transplantation.

Courtagen DIAGNOSTICS LABORATORY

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

Courtagen DIAGNOSTICS LABORATORY

- *IARS2*: A couple who had lost a child to Leigh disease were expecting and requested prenatal testing.

- Two deleterious-predicted mutations in *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's disease.

- The couple underwent prenatal testing, revealing the fetus to be heterozygous for *IARS2* and thus predicted to be healthy.

nucSEEK™ Case Report 7

Courtagen DIAGNOSTICS LABORATORY

- *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 2 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 identified by our laboratory as well.

Known Conditions Identified

- *ACAD9:* acyl-CoA dehydrogenase family, member 9
- *ALDH5A1:* aldehyde dehydrogenase 5 family, member A1 (succinic semialdehyde dehydrogenase)
- *ATP7B:* ATPase, Cu++ transporting, beta polypeptide (Wilson)
- *CLCN2:* chloride channel, voltage-sensitive 2
- *COQ2:* coenzyme Q2 homolog, prenyltransferase
- *GARS*: glycyl-tRNA synthetase
- *KIF1B:* kinesin family member 1B
- *MFN2:* mitofusin 2 (2 families)
- *NDUFA1:* NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1
- *NRXN1:* neurexin 1 (2 families)
- *PNKD:* paroxysmal nonkinesigenic dyskinesia (2 families)
- *RRM2B:* ribonucleotide reductase M2 B
- *SCN1A:* sodium channel, voltage-gated, type I, alpha subunit
- *SCN2A:* sodium channel, voltage-gated, type II, alpha subunit
- *SCN4A:* The gene encodes the alpha subunit of the voltage-gated, type IV sodium channel
- *SPAST:* Spastin
- *SPTLC2:* serine palmitoyltransferase, long chain base subunit 2 (hereditary sensory neuropathy type 1C)
- *TPH2:* tryptophan hydroxylase 2
- *UBE3A:* ubiquitin protein ligase E3A (Angelman)

> 1 Conserved Variants in a Folate-Related Gene

- *GLDC*, *MTRR*, *SHMT2*: Developmental delay, hypotonia, and skeletal muscle weakness
- *ALDH1L2*, *GLDC*: Encephalopathy (seizure disorder, mental retardation, and cerebral palsy), optic atrophy, hearing loss, GI dysmotility and dysautonomia
- *GLDC*, *SHMT1*: Autism
- *MTHFD2*, *MTRR*: Sudden-onset OCD, motor tics, and IgA deficiency
- *ALDH1L1*, *ALDH1L2*: Severe irritability, hypersensitivity, growth issue, twin also affected, severe PANS, immunodeficiency
- *ALDH1L1*, *GLDC*: Autism, macrocephaly, and PANS
- *FPGS*, *MTHFD2L*: Myopathy with muscle biopsy suggestive of mitochondrial myopathy
- *ALDH1L1*, *FPGS*: Tics, OCD
- *ALDH1L1*, *ALDH1L2*, *FPGS* (x2): Severe primordial growth retardation, in-utero stroke, and functional disease
- *ALDH1L1*, *MTHFD1L*: Seizures, hypotonia, large bowel dysmotility and optic neuropathy
- *GLDC*, *SLC25A32*: Multiple functional/dysautonomic symptomatology, including chronic pain and post-prandial nausea
- *ALDH1L1*, *MTHFD1L*: Cyclic vomiting
- *ALDH1L2*, *GLDC*, *MTHFS*: Obsessive compulsive disorder and tic disorder
- *MTHFD1L*, *SHMT1*: Tic disorder and obsessive compulsive disorder


STAR WARS
16+
10240
1559
7
12

Case Report – Dylan, age 6

PLCG2

- Angioedema
- Chronic pain
- Pancytopenia
- Chronic fatigue
- Immunodeficiency
- Renal disease (stones and acidosis)
- Severe GI dysmotility requiring TPN
- Multiple endocrinopathies, incl diabetes
- Muscle biopsy c/w mitochondrial disease.
- Exome sequencing at UCLA: *PLCG2* mutation causing phospholipase C, gamma 2 deficiency, a disorder of inflammation.

Panel versus Exome?

Courtagen DIAGNOSTICS LABORATORY

PANEL

- Looks at many genes
- Misses the dx if you are wrong
- Many VUS
- Occasional incidentals
- Expert interpretation
- Data to mine later
- Family secrets unsafe

EXOME

- Looks at “all” genes
- Misses very little, but will you notice it?
- Buried in VUS
- Many incidentals
- Less-than-expert
- Tremendous data to mine
- Family secrets revealed

Panel versus Exome?

Courtagen DIAGNOSTICS LABORATORY

PANEL

- Looks at many genes
- Misses the dx if you are wrong
- Many VUS
- Occasional incidentals
- Expert interpretation
- Data to mine later
- Family secrets unsafe

You think you know.

EXOME

- Looks at “all” genes
- Misses very little, but will you notice it?
- Buried in VUS
- Many incidentals
- Less-than-expert
- Tremendous data to mine
- Family secrets revealed

You haven’t a clue.

Potentially Unwanted Findings

Courtagen DIAGNOSTICS LABORATORY

- "Incidental findings" = risk for unrelated disease: cancer, neurodegeneration, sudden cardiac death, drug interactions, anesthesia complications, others

- Variants of Unclear Significance (VUS)

Common in Mito-exome

Very common in exome

Potentially Unwanted Findings

Courtagen DIAGNOSTICS LABORATORY

- "Incidental findings" = risk for unrelated disease: cancer, neurodegeneration, sudden cardiac death, drug interactions, anesthesia complications, others

- Variants of Unclear Significance (VUS)

  Common in Mito-exome

  Very common in exome

- Risks of disclosure of genetic information
- Family secrets
  - Consanguinity
  - Non-paternity

I never know when its going to come back
This fatigue is an internal attack
It so easily cripples me
Only no one can see

Its so hard when you easily tire
And everyone around you thinks your lazy and a liar
They cant see so they don't know
I know in my heart its real though

Its a relief to get the answer and know you're not crazy
You can finally prove you're not just lazy
Its still not easy and never will be
But maybe some day the world will see

"Functional" Disorders List:

Courtagen DIAGNOSTICS LABORATORY

• Autistic spectrum disorders	Social and language oddities, and Restricted interests
• Chronic fatigue syndrome	Post-exertional fatigue for > 1 day, and Unrefreshed sleep
• Complex regional pain syndrome	Out-of-proportion pain following tissue damage in severity and duration, and Autonomic signs
• Cyclic vomiting syndrome	Severe nausea, vomiting, and lethargy, and Much reduced between cycles
• Fibromyalgia	Widespread pain and Allodynia (painful response to pressure)
• Irritable bowel syndrome	Abdominal discomfort less with BM, and Constipation, diarrhea, or alternating
• Migraine	Headache with nausea and photophobia, or Transient sensory loss or gain

Why Courtagen?

Courtagen DIAGNOSTICS LABORATORY

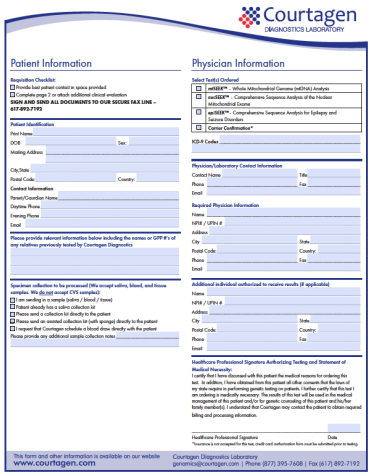
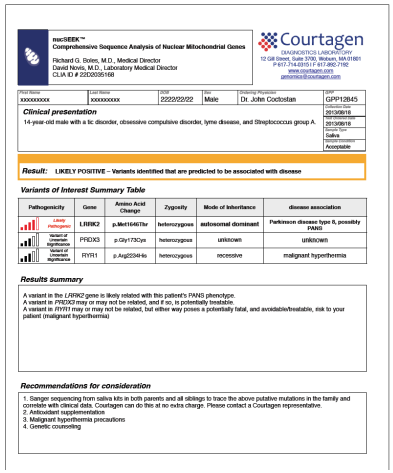
- Specificity: 99.99% Sensitivity: 99%
- Deeper investigation into sequence variants
- Far less filtering (only >3%, synonymous, non-coding or splice)
- Special commitment towards functional disease
- High sample volume with these disorders for comparison
- Ongoing data mining
- Interpretation, recommendations, treatment suggestions
- Turn-around in weeks, not months or years
- Courtagen handles obtaining authorization
- Aggressive financial assistance program
- Less “incidental findings”

Mito-Exome Limitations

Courtagen DIAGNOSTICS LABORATORY

- ~150 mitochondrial genes are yet to be discovered
- Phenocopies: 200 does not cover all
- 98% coverage is still not 100%
- Promoter and other regulatory mutations
- BioInformatic/interpretation is not perfect
- Some genes might have unrecognized dominant mutations
- Some patients have polygenic disease

Ordering a Test from Courtagen

Step 1	Step 2	Step 3	Step 4
Physicians order genetic testing Doctors fill out Courtagen's test request form and submit by fax or online Courtagen verifies Patient Insurance Benefits Courtagen Care Financial Program available for qualified patients	**Saliva** collection kit is shipped to the patient or provided in the doctor office Sample is collected and sent directly to Courtagen Physician signature and Patient Consent signature required	DNA is process using the most advanced laboratory methods in Next Generation Sequencing Results are analyzed by Courtagen's experienced bioinformatics team and analysis pipeline Clinical interpretation by our clinical genomics team, led by Dr. Richard Boles	Report is posted to Courtagen's online portal for your physician Genetic Counselors available to address questions
			

What Do I Need To Get Started?

1. An order for the test from any physician
   (e.g. “nucSEEK on Juan Garcia”).

2. Authorization from your insurance company, followed by a financial survey completed by the family.

3. Clinical information, either a sub-specialist physician note and/or completed checklist.

4. A collection kit will be sent by mail for the saliva sample. Results are not affected by diet, treatment, or time.

What Test?

Courtagen DIAGNOSTICS LABORATORY

- mtSEEK™
  - Entire mitochondrial DNA
  - Maternal inheritance
- nucSEEK™
  - All 1,100 nuclear-encoded mitochondrial genes + phenocopies
  - Consanguinity
- epiSEEK™
  - All 327 known genes that result in seizures
  - Seizures are a prominent aspect of the phenotype
- autSEEK™ *COMING SOON*
  - All known genes that result in autistic spectrum disorders
  - ASD is a prominent aspect of the phenotype

NextGen in Clinical Practice
Is the Future Here?

Courtagen DIAGNOSTICS LABORATORY

SUCCESSFUL NOW

- Practical
- Non-invasive
- Rapid
- Replaces previous testing
- Cost-effective
- Covered by PPOs
- High positive rate
- Often leads to therapy
- Occasional incidentals
- Data to mine later

REMAINING OBSTACLES

- Lack of understanding
  - public
  - clinicians
  - genetic specialists
  - payers
- Costs are still substantial, but decreasing
- Occasional incidentals
- Unreasonable expectations

"Any sufficiently advanced technology is indistinguishable from magic."

Clarke's Third Law

In children, mitochondrial disease is more common than cancer and muscular dystrophy, combined!

Who to Contact?

John Linnan

john.linnan@courtagen.com

(603) 560-1320

Courtagen
DIAGNOSTICS LABORATORY

Thank You