Mitochondria and Dysautonomia

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Dysautonomia International; 18-July, 2015
Herndon, Virginia
Disclosure:
Dr. Boles wears many hats

Dr. Boles is a consultant for Courtagen, which provides diagnostic testing.

- **Medical Director of Courtagen Life Sciences Inc.**
  - Test development
  - Test interpretation
  - Marketing

- **Researcher with prior NIH and foundation funding**
  - Studying sequence variation that predispose towards functional disease
  - Treatment protocols

- **Clinician treating patients**
  - Interest in functional disease (CVS, autism)
  - Geneticist/pediatrician 20 years at CHLA/USC
  - In private practice since 2014
There are no approved treatments for mitochondrial disease.

Everything is “off label”
Presented to my clinic at age 11 years.
Cyclic vomiting syndrome from ages 1-10 years, with 2-day episodes twice a month of nausea, vomiting and lethargy.
Episodes had morphed into daily migraine.
Chronic pain throughout her body.
Chronic fatigue syndrome = chief complaint.
Substantial bowel dysmotility/IBS
   Multiple admissions for bowel clean-outs.
Excellent student
Pedigree: probable maternal inheritance
NextGen sequencing at age 14 years revealed the p.Ile253Val variant in the TRAP1 gene.

TRAP1 encodes a mitochondrial chaperone involved in antioxidant defense.

This patient is one of 26 unrelated cases identified by Courtagen to date who have previously unidentified disease associated with mutations in the ATPase domain.

The common feature recognized at present is chronic pain, fatigue and GI dysmotility.

Tachycardia/palpitations and dizziness may also be common.

That variant comes from Payton’s father, who himself has frequent pain, fatigue and diarrhea.

In these patients, chronic pain and fatigue improved greatly on aggressive antioxidant therapy.

On aggressive antioxidant therapy, all manifestations of disease in Payton were substantially improved. Issues remaining included chronic abdominal pain and moderate fatigue. She became functional in life, but still on a shortened school schedule.
1. An ATPase domain hydrolyze the energy-rich triphosphate bond of ATP to convert into mechanical work of folding proteins.

2. The two homodimers of TRAP1 are shown in grey and pink.

2. ATP bound in its pocket is shown in green, in each dimer.

3. The “common mutation” p.Ile253Val is labeled in each dimer.

4. The “salt bridge” mutations, R128H (p.Arg128His) and E192K (p.Glu192Lys), are labeled in one dimer.

5. These 3 variants have odds ratios of about 6 for both chronic pain and GI dysmotility.

6. Together, about 2 percent of people with European heritage have one of these variants.
Can we design a therapy that blocks ATP entrance into mutant TRAP1, but not normal TRAP1?

Computer modeling was performed based on the human TRAP1 crystal structure by Jeffrey Skolnick at the Georgia Institute of Technology.

The result suggested that one drug, granisetron, binds far more strongly to the valine-253 mutant protein than to the isoleucine-253.

Granisetron was tried in Payton, with near-"miraculous" results in terms of the resolution of most signs and symptoms of disease.
Metabolic Pathways
“Any sufficiently advanced technology is indistinguishable from magic.”

Clarke’s Third Law
Next Generation Sequencing
Illumina MiSeq
Mitochondrial Genetics
The Basics

Mitochondrial DNA
- 37 genes
- 16,000 base pairs
- Maternal inheritance

Nuclear DNA
- ~22,000 genes
- 3,000,000,000 base pairs
- 1,013 genes encode mitochondrial proteins
- Autosomal recessive
- Autosomal dominant
- X-linked
Maternal Inheritance

mtDNA is inherited exclusively from the mother. There is no recombination.

Thus, all relatives with red symbols have exactly the same mtDNA sequence, in the absence of a new mutation.
### Brain
- Developmental delays
- Dementia
- Neuro-psychiatric disturbances
- Migraines
- Autistic Features
- Mental retardation
- Seizures
- Atypical cerebral palsy
- Strokes

### Nerves
- Weakness (may be intermittent)
- Absent reflexes
- Fainting
- Neuropathic pain
- Dysautonomia - temperature instability

### Muscles
- Weakness
- Cramping

### Gastrointestinal problems
- Dysmotility
- Irritable bowel syndrome
- Hypotonia
- Muscle pain
- Gastroesophageal reflux
- Diarrhea or constipation
- Pseudo-obstruction

### Kidneys
- Renal tubular acidosis or wasting

### Heart
- Cardiac conduction defects (heart blocks)
- Cardiomyopathy

### Liver
- Hypoglycemia (low blood sugar)
- Liver failure

### Ears & Eyes
- Visual loss and blindness
- Ptosis
- Ophthalmoplegia
- Optic atrophy
- Hearing loss and deafness
- Acquired strabismus
- Retinitis pigmentosa

### Pancreas & other glands
- Diabetes and exocrine pancreatic failure (inability to make digestive enzymes)
- Parathyroid failure (low calcium)

### Systemic
- Failure to gain weight
- Fatigue
- Unexplained vomiting
- Short stature
- Respiratory problems
Dysautonomia In Mitochondrial Disease

- Accommodative failure
- Photophobia
- Hyper/hypothermia
- Hyper/hypoventilation
- Tachy/bradycardia
- Hyper/hypotension
- Palpitations
- POTS/syncope
- Complex regional pain syndrome
- Dysphagia
- GI dysmotility
- Constipation/diarrhea
- Urinary obstruction
- Urinary urgency
- Sexual dysfunction
- Abnormal sweating
- Pregnancy-related conditions
- SIDS
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- Abnormal sweating
- Pregnancy-related conditions
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Most common is parasympathetic failure
20 “Functional” Disorders:

- Attention deficit hyperactivity disorder
- Anxiety disorder
- Autistic spectrum disorders
- Chronic fatigue syndrome
- Complex regional pain syndrome
- Cyclic vomiting syndrome
- Depression (MDD)
- Fibromyalgia
- Functional abdominal pain
- Interstitial cystitis
- Insomnia (chronic, severe)
- Irritable bowel syndrome
- Migraine
- Panic disorder
- Post-traumatic stress disorder
- Postural orthostatic tachycardia syndrome
- Restless legs syndrome
- Temporomandibular disorder
- Tinnitus
- Vulvovaginitis syndrome
44% of patients with interstitial cystitis also have symptoms suggestive of irritable bowel syndrome (IBS) (v. 12% of controls).

59% of patients with cyclic vomiting syndrome met the standardized questionnaire criteria for a generalized anxiety disorder.

67% of migraineurs fulfilled criteria for chronic fatigue syndrome.

75% of patients with cyclic vomiting syndrome are projected to develop migraine by age 18.

20% to 80% of patients with temporomandibular disorders suffer from additional chronic pain disorders such as headache, low back pain, fibromyalgia, and irritable bowel syndrome.
The elephant is lying down due to chronic fatigue.
Maternal Inheritance of Functional Disorders - 1

Cancer

Colitis
Colitis

Seizures, CVS, Migraine, Depression, Seizures, Hearing loss

Dyslexia

Bipolar, Migraine

GERD, Migraine, Bipolar, Anxiety

Ptosis

Reyes syndrome

Failure to thrive

Preemie

CRPS
CRPS

SIDS

CP

Blind

Hypoglycemia

Muscle weakness

Abdominal migraine

Muscle

ASD/VSD

Depression

Seizures

Migraine

Muscle weakness

Depression

Seizures

Migraine

Colitis

Cancer
Maternal Inheritance of Functional Disorders - 2
Quantitative Pedigree Analysis In Cyclic Vomiting Syndrome
Figure 2: Labeling of pedigrees as “probable maternal inheritance,” “probable non-maternal inheritance,” or “indeterminate.”
672 pedigrees from the Genetics of Recurrent Early-Onset Depression project

Analyzed for 5 matrilineal/non-matrilineal pairs controlled for sex, age and autosomal gene contribution
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Analyzed for 5 matrilineal/non-matrilineal pairs controlled for sex, age and autosomal gene contribution

Matrilineal relatives (with the same mtDNA sequence as the proband) were significantly more likely to suffer from a mood disorder than were non-matrilineal relatives (with another mtDNA sequence); OR 2.0, 95% CI: 1.5-2.6, $P = 3 \times 10^{-6}$. 

[Diagram showing a family tree with symbols indicating different family members and their conditions. Red indicates those with mood disorders, and other symbols represent various family members with different statuses.]
Functional Disorder-Associated mtDNA Polymorphisms

16519 C>T
mtDNA control region
~25% of population

3010 G>A
16S-ribosomal RNA gene
~30% of population
Do Maternally Inherited mtDNA polymorphisms constitute a “Unified Theory” of Functional Disease?

- **16519T** is statistically associated with:
  - Migraine headache (*odds ratio* 4)
  - Cyclic vomiting syndrome (*odds ratio* 6)
  - Chronic fatigue syndrome (*odds ratio* 2)
  - Complex regional pain syndrome (*odds ratio* 2)
  - Irritable bowel syndrome with maternal inheritance (*odds ratio* 6)
  - Atypical autism (*odds ratio* 2.5)
  - SIDS subset with low hepatic glucose

- **3010A** is statistically associated with:
  - Migraine headache in patients with 16519T (*odds ratio* 15)
  - Cyclic vomiting syndrome in patients with 16519T (*odds ratio* 17)
  - Constipation-type irritable bowel syndrome
  - Non-specific abdominal pain (*odds ratio* 3)
  - Functional co-morbidity in chronic fatigue syndrome (*OR* 4-6)
  - SIDS (common glucose-normal type) (*odds ratio* 3)

- **3010G** is statistically associated with:
  - Atypical autism (*odds ratio* 3)
  - GI co-morbidity in major depressive disorder
  - Total functional symptomatology in high school students
Cyclic Vomiting and Migraine Prevalence of Two mtDNA Common Variants in Haplogroup H Individuals With Functional Disorders

<table>
<thead>
<tr>
<th></th>
<th>Cyclic Vomit Syndr.</th>
<th>Odds Ratio (95% C.I.)</th>
<th>Migraine w/o Aura</th>
<th>Odds Ratio (95% C.I.)</th>
<th>Ctrl</th>
</tr>
</thead>
<tbody>
<tr>
<td>16519T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21/30</td>
<td>6.2 (2.7-14)</td>
<td>58/112</td>
<td>3.6 (2.2-5.9)</td>
<td>63/231 27%</td>
</tr>
<tr>
<td></td>
<td>70%</td>
<td></td>
<td>52%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3010A</td>
<td>9/30</td>
<td>N/A</td>
<td>37/112</td>
<td>N/A</td>
<td>143/444 32%</td>
</tr>
<tr>
<td></td>
<td>30%</td>
<td></td>
<td>33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3010A among pts with 16519T</td>
<td>6/24</td>
<td>17 (2-156)</td>
<td>15/58</td>
<td>15 (1.9-117)</td>
<td>1/63 1.6%</td>
</tr>
<tr>
<td></td>
<td>29%</td>
<td></td>
<td>26%</td>
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</table>
Chronic Fatigue Syndrome
The 3010A mtDNA Variant Predicts a Several-fold Increase in Functional Symptoms.

<table>
<thead>
<tr>
<th></th>
<th>Headache</th>
<th>Fainting or Dizziness</th>
<th>Muscle Pain</th>
<th>Muscle Weakness</th>
<th>Sleep Problems</th>
<th>Numbness or Tingling</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3010A</strong></td>
<td>14/21</td>
<td>11/21</td>
<td>19/21</td>
<td>17/21</td>
<td>19/22</td>
<td>12/21</td>
</tr>
<tr>
<td></td>
<td>67%</td>
<td>52%</td>
<td>90%</td>
<td>81%</td>
<td>86%</td>
<td>57%</td>
</tr>
<tr>
<td><strong>3010G</strong></td>
<td>8/25</td>
<td>5/28</td>
<td>16/28</td>
<td>17/28</td>
<td>13/27</td>
<td>6/24</td>
</tr>
<tr>
<td></td>
<td>32%</td>
<td>18%</td>
<td>57%</td>
<td>61%</td>
<td>48%</td>
<td>25%</td>
</tr>
<tr>
<td><strong>Chi Square</strong></td>
<td>P = 0.04</td>
<td>P = 0.02</td>
<td>P = 0.03</td>
<td>P = 0.22</td>
<td>P = 0.01</td>
<td>P = 0.06</td>
</tr>
<tr>
<td><strong>Odds Ratio (95% C.I.)</strong></td>
<td>4.0 (1.1-18)</td>
<td>4.7 (1.2-23)</td>
<td>5.9 (1.2-54)</td>
<td>NA</td>
<td>6.0 (1.4-38)</td>
<td>3.7 (0.95-18)</td>
</tr>
<tr>
<td><strong>T-test</strong></td>
<td>P = 0.004</td>
<td>P = 0.06</td>
<td>P = 0.005</td>
<td>P = 0.03</td>
<td>P = 0.046</td>
<td>P = 0.03</td>
</tr>
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</table>
Thomas, age 22 years
POTS

- Presented as the lesser-affected brother of a girl with multi-system presumed “mitochondrial disease”.
- Had mild “functional” symptoms only in first decade, such as occasional pain, fatigue and dyautonomia.
- Episode of complex regional pain syndrome following removal of benign tumor on back.
- In early adolescence, developed episodes of POTS/pre-syncope that were dramatic, occurred with little warning, often in school.
- Episodes appeared like grand-mal seizures, paramedics called to school often.
- Episodes became frequent, sometimes followed by severe dysautonomia failure that required ICU admissions for up to a few weeks.
- Effectively disabled by his condition.
- Asked me for medical clearance to go SCUBA diving with his high-school class from a remote base on Catalina Island.
Maternal Inheritance of Functional Disorders - 1
Three different length heteroplasmic variants mtDNA control region – area involved in replication and transcription of mtDNA
• Placed on L-arginine supplementation, which dramatically improved his POTS to about one episode a year.
• L-arginine is an amino acid, part of natural protein. It is involved with nitric oxide synthesis, which dilates blood vessels. It is very effective in preventing stroke in MELAS.
• He DID go SCUBA diving with his class!
• On sequencing of nuclear-encoded mitochondrial proteins he was found to have a mutation in the TRAP1 gene, p.Tyr229*
• His affected sisters and affected mother have the same mutation.
• Doing very well at present, essentially normal other than chronic fatigue (sleeps 10-11 hours at night) and some pain.
Karl, age 27 years
Abdominal migraine

- Presented with cyclic episodes of abdominal pain, nausea, vomiting and pallor.
- Episodes became very frequent and coalesced to near-continuous.
- Status-post cholecystectomy and appendectomy
- On narcotics, fully disabled, and labeled as a drug addict
- Other issues: migraine headaches, fatigue, GERD, anxiety
- Seen in my clinic at age 23 and placed on amitriptyline, coenzyme Q10 and L-carnitine. Initial success with only rare episodes.
- Stopped treatment, and at age 26 was refractory to above therapy, including episodes every 4 to 7 days for several hours; again disabled. Had 10-15 ER visits in 5 months.
- Family history is negative.
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• Stopped treatment, and at age 26 was refractory to above therapy, including episodes every 4 to 7 days for several hours; again disabled. Had 10-15 ER visits in 5 months.
• nucSEEK sequencing revealed 3 known mutations in the RYR2 gene.
• The patient was place on propranolol.
• Dramatic improvement with the resolution of episodes.
• Ryanodine receptor 2
• Encodes a stress-induced calcium channel across the endoplasmic reticulum
• Links with VDAC on the outer mitochondrial membrane to link ER directly with mitochondria
• Dominant mutations are associated with adrenergic-triggered arrhythmia (often fatal) and right-sided cardiomyopathy
• Channel also present in neurons
• Highly-conserved variants are associated with cyclic vomiting
• Have “functional triad” as well – common in CVS
• All are VERY nervous people, with stress-triggered disease
• Disease responds favorably to beta blockade (propranolol)
Suspect mitochondrial/metabolic disease if there are two or more of the following “Red Flags”: 

- Autistic spectrum disorder/pervasive developmental disorder
- Loss of milestones/regression
- Movement disorder (including ataxia, dystonia, chorea, tics)
- Stroke or stroke-like episodes
- Myopathy, especially ocular or cardiac
- Chronic bowel dysmotility (especially if severe or at more than one level)
- Cyclic vomiting
- Dysautonomia (including POTS, frequent tachycardia, unexplained fevers)
- Chronic pain condition (including migraine, myalgia)
- Chronic fatigue
- Mood disorders
- Waxing and waning clinical course (including altered mental status or psychosis)
- Hypoglycemia
- Metabolic acidosis (either renal tubular loss and/or anion gap)
- Elevated liver transaminases (including only trace elevated, if frequent)