DYSAUTONOMIA INTERNATIONAL

AWARENESS ADVOCACY ADVANCEMENT

Mitochondria and Dysautonomia

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Pasadena, California

Dysautonomia International; 18-July, 2015
Herndon, Virginia

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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

Courtagen LIFE SCIENCES, INC.

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

Children's Hospital LOS ANGELES We Treat Kids Better

USC UNIVERSITY OF SOUTHERN CALIFORNIA

- Clinician treating patients
  - Interest in functional disease (CVS, autism)
  - Geneticist/pediatrician 20 years at CHLA/USC
  - In private practice since 2014

Mitochondrial and Molecular Medicine

Richard G. Boles, M.D.
Medical Genetics
Pasadena, California

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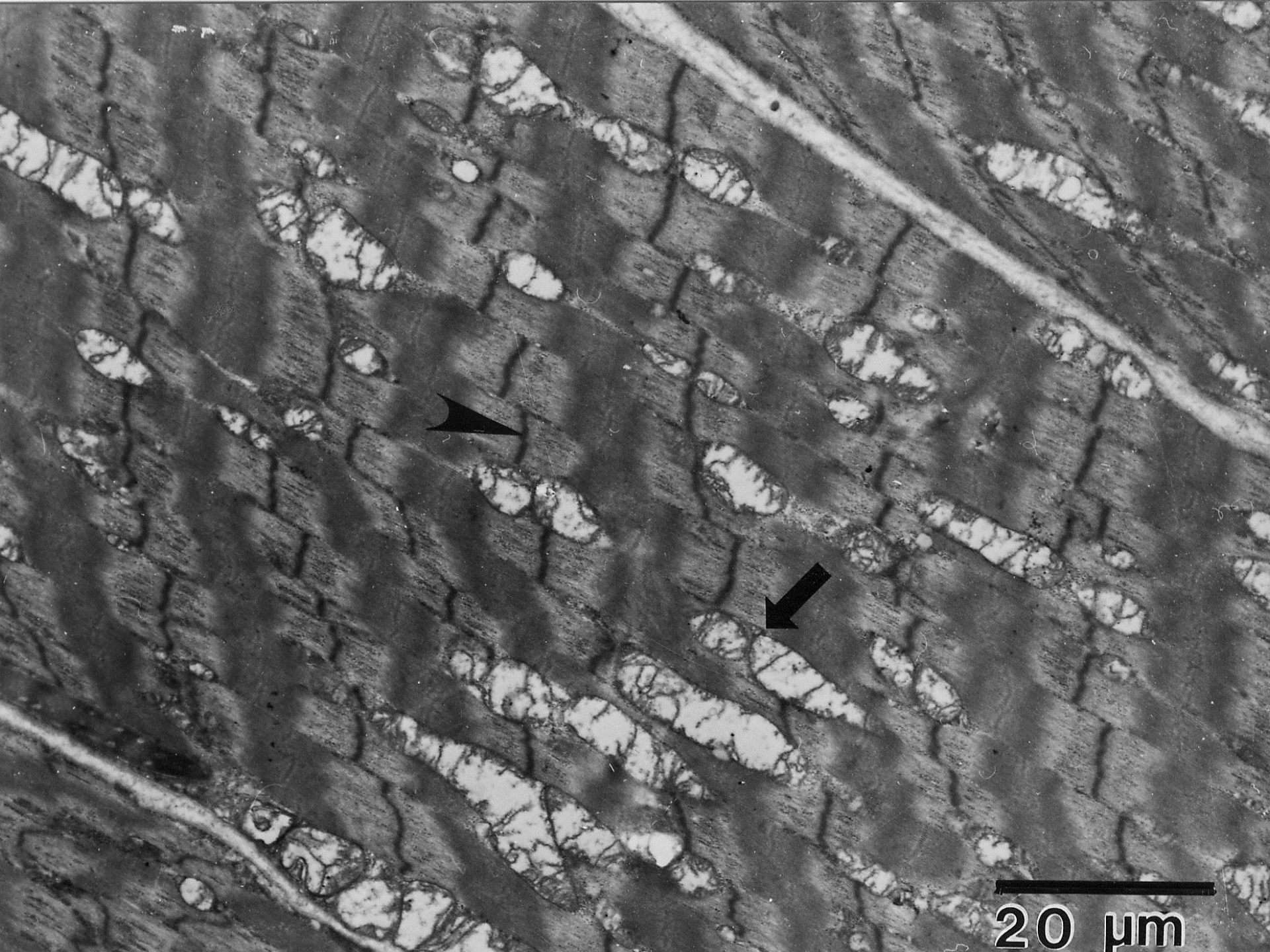
Disclosure: Off-label Indications

There are no approved treatments for mitochondrial disease.

Everything is “off label”


20 µm

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Payton, 15-year-old

- 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

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TRAP1-Related Disease (T1ReD) *Mitochondrion*, 2015

- 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.

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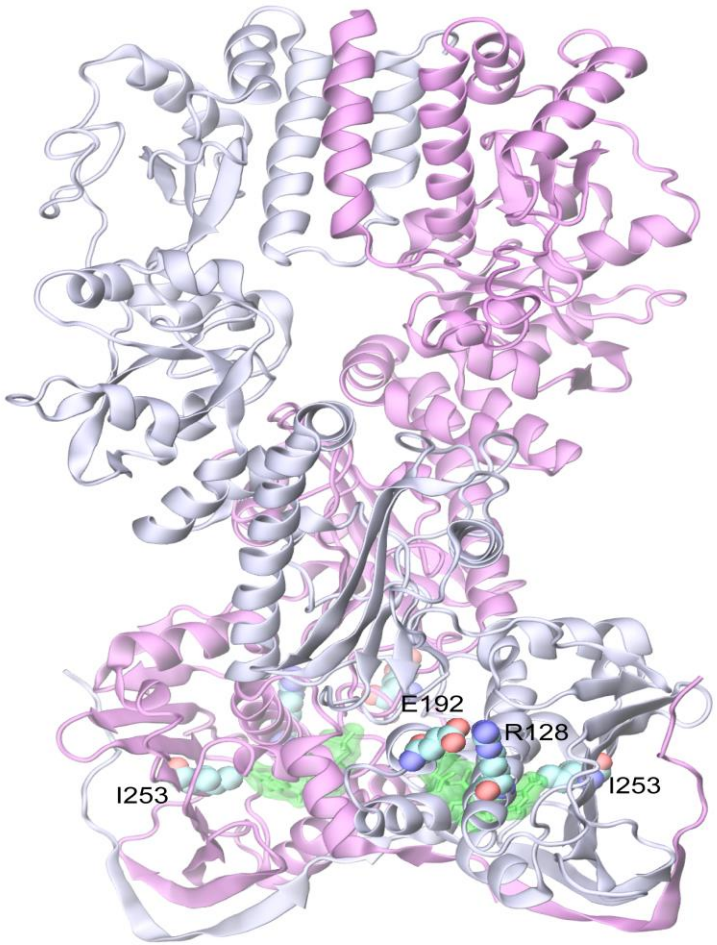
Molecular structure of TRAP1

TRAP1-Related Disease (T1ReD)



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.

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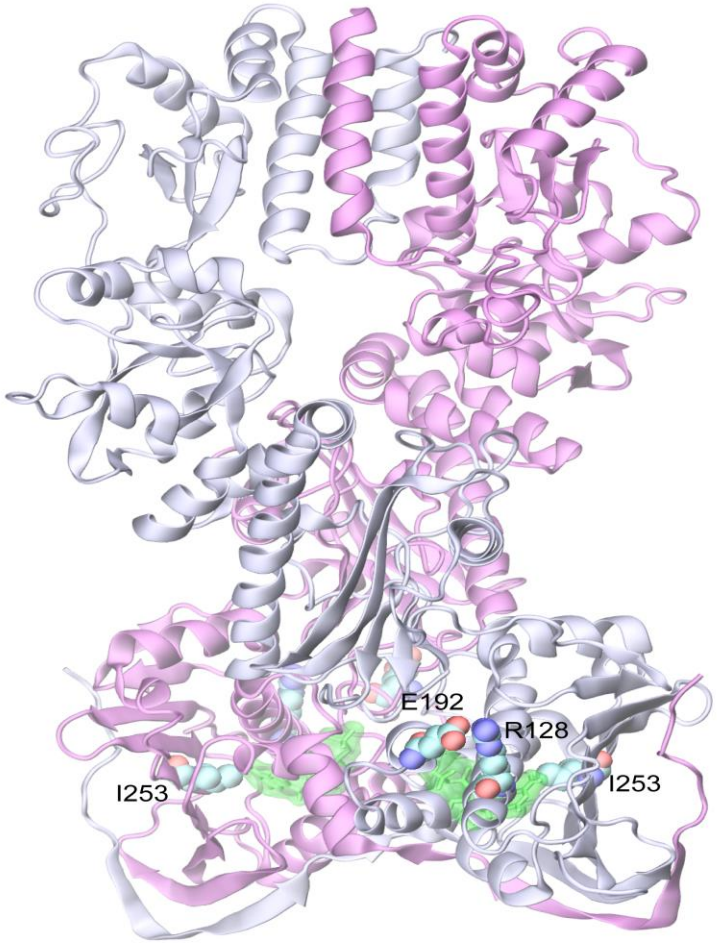
Molecular structure of TRAP1

TRAP1-Related Disease (T1ReD)



- 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.

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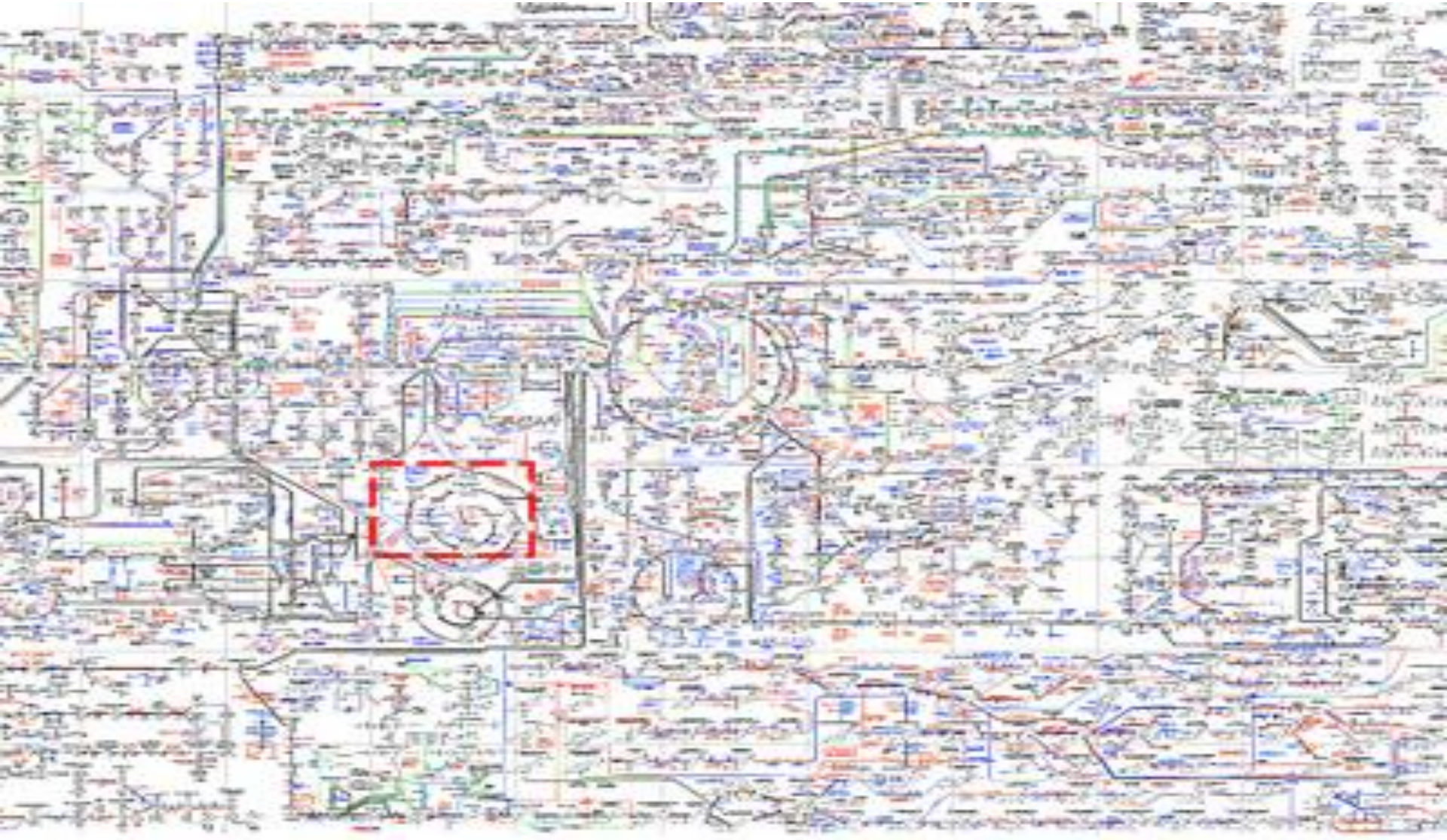
Metabolic Pathways



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"Any sufficiently advanced technology is indistinguishable from magic."

Clarke's Third Law

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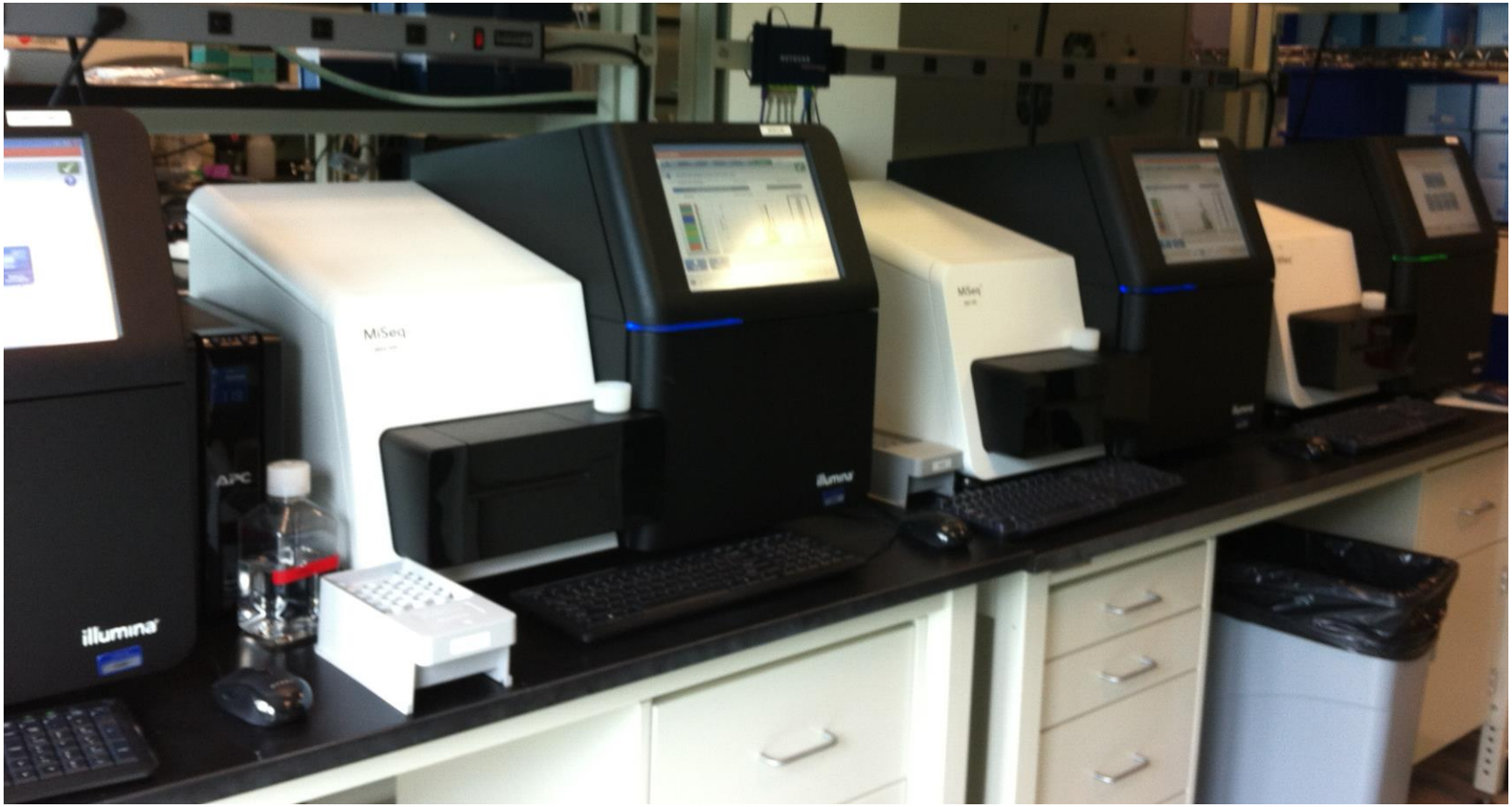
Next Generation Sequencing
Illumina MiSeq



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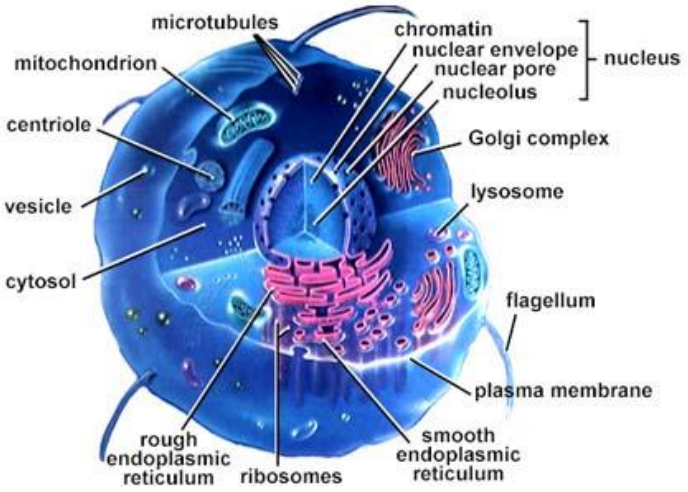
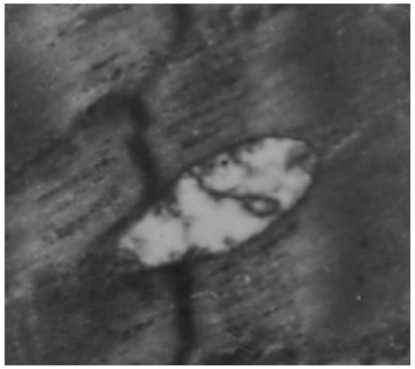
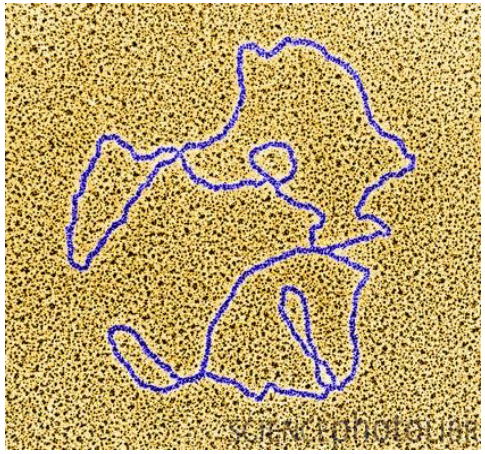
Mitochondrial Genetics
The Basics





Mitochondrial DNA

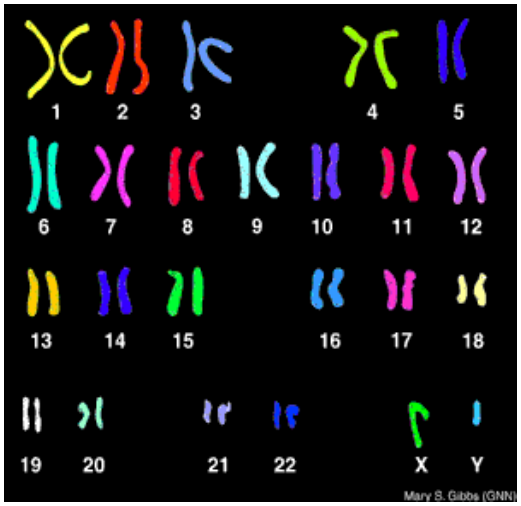


37 genes

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

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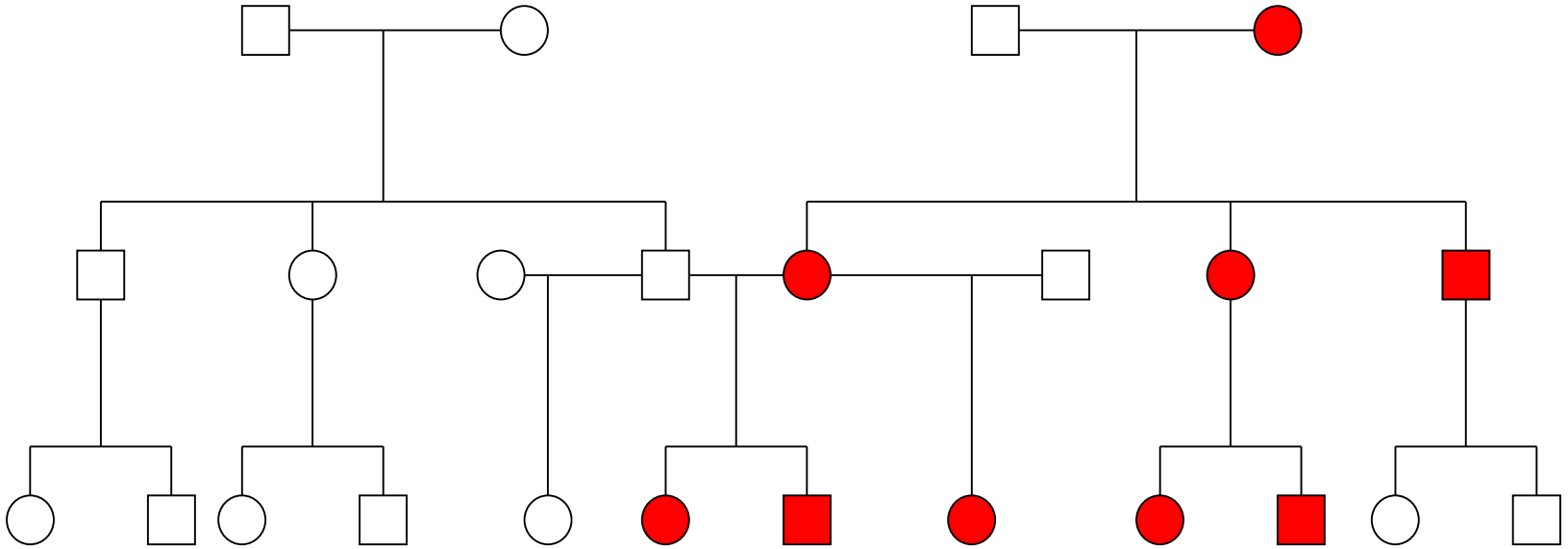
AWARENESS ADVOCACY ADVANCEMENT

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.

UNITED MITOCHONDRIAL DISEASE FOUNDATION®
HOPE. ENERGY. LIFE.

Mitochondrial Medicine
The Spectrum of Mito

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
- Gastroesophogeal 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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AWARENESS ADVOCACY ADVANCEMENT

Dysautonomia In Mitochondrial Disease

- Accommodative failure
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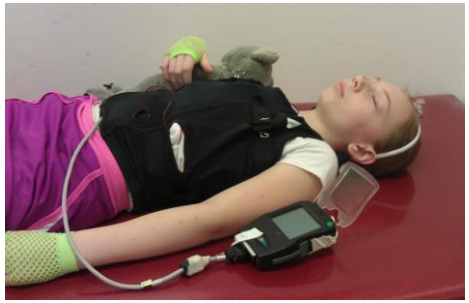

Most common is parasympathetic failure

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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

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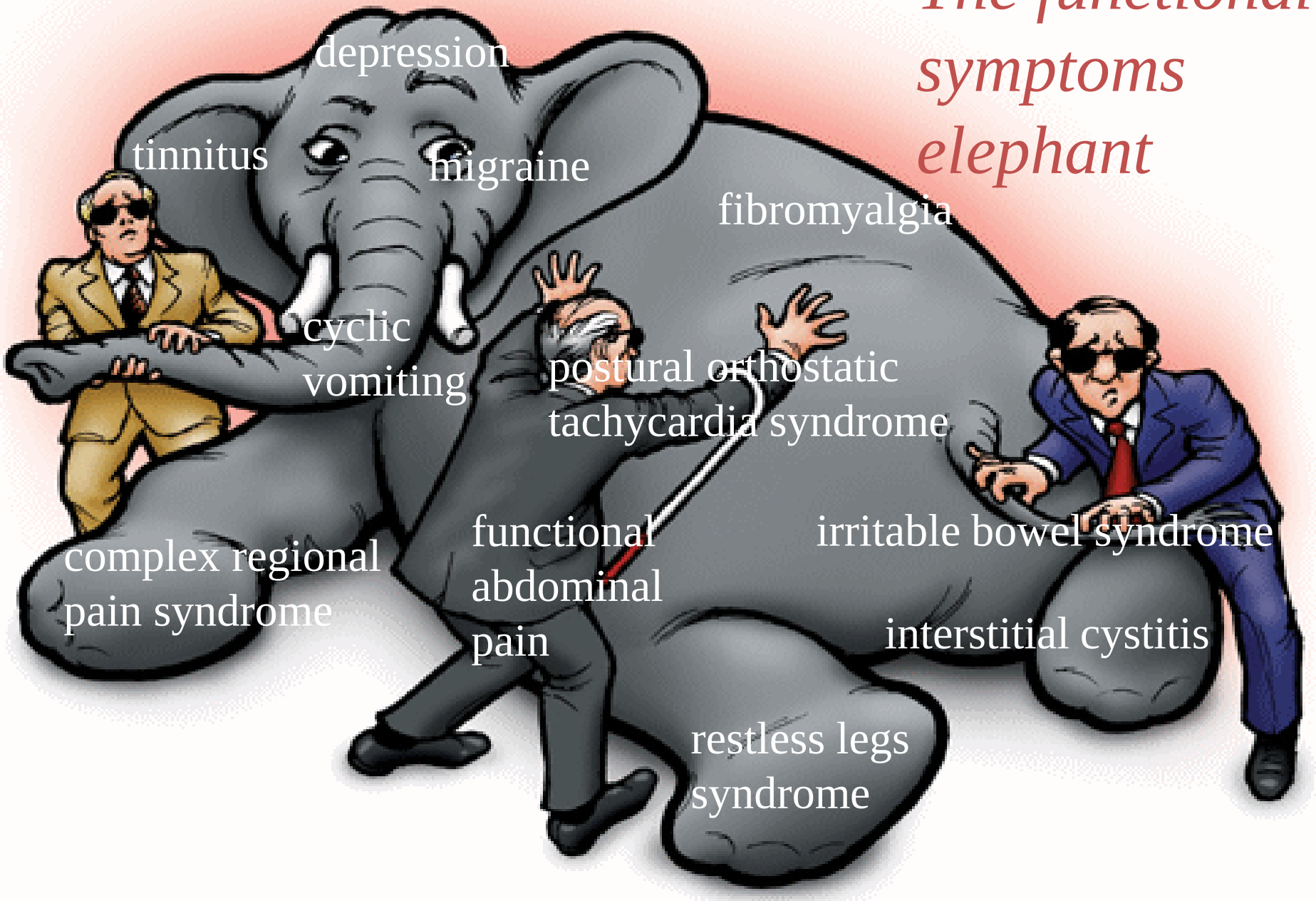
Comorbidity: Functional Conditions Are Often Found Together

- 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.

Gardner
Boles 2006

The functional symptoms elephant



The elephant is lying down due to chronic fatigue

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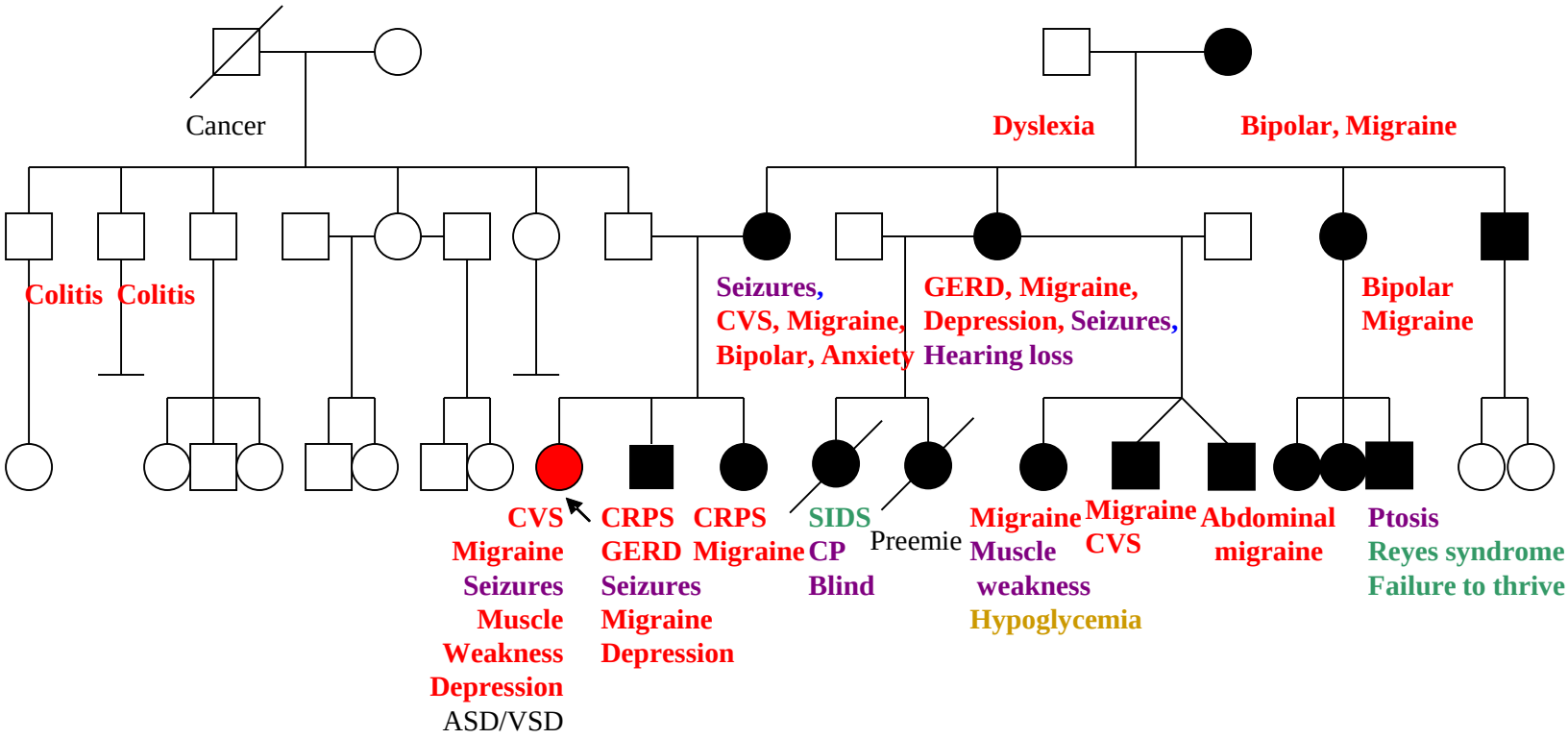
AWARENESS ADVOCACY ADVANCEMENT

Maternal Inheritance of Functional Disorders - 1



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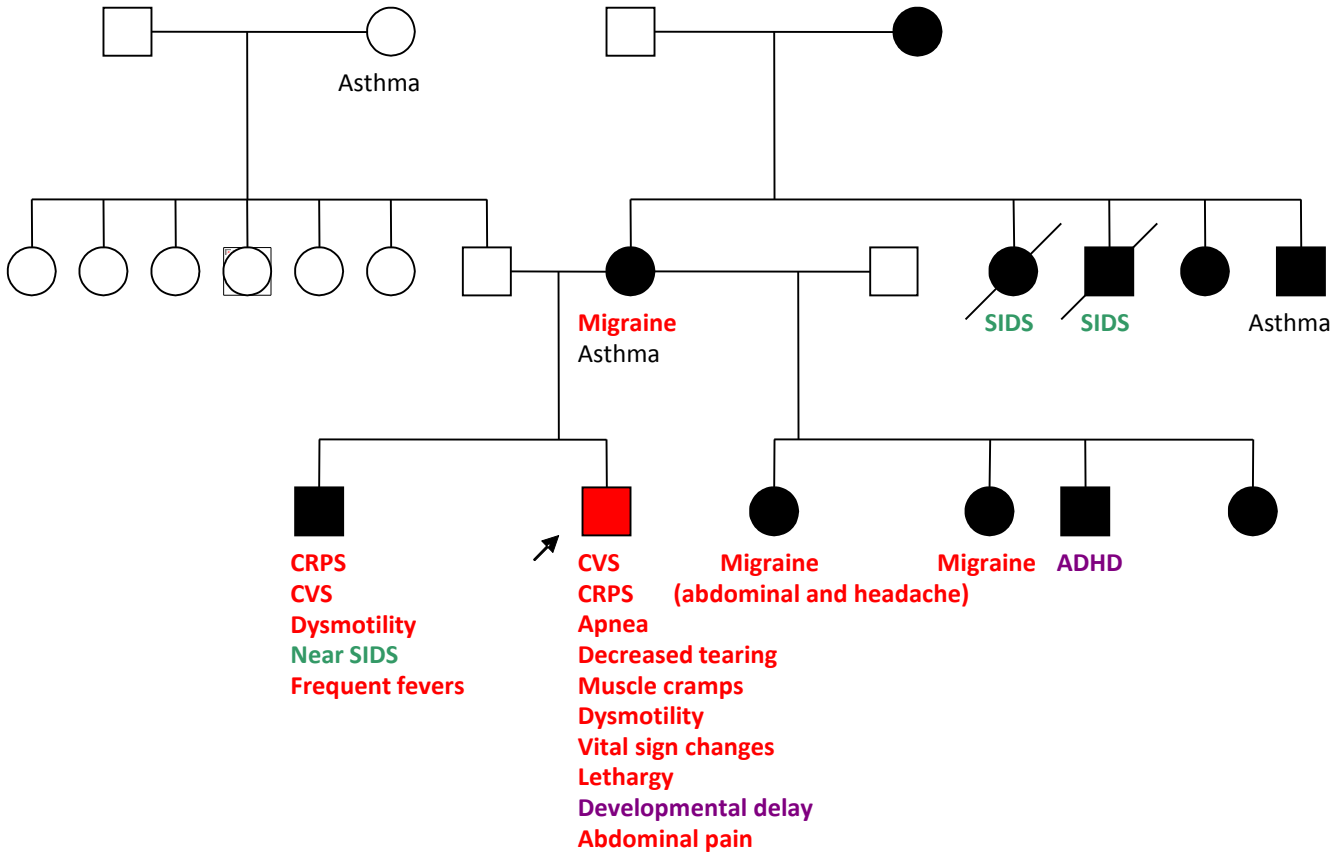
AWARENESS ADVOCACY ADVANCEMENT

Maternal Inheritance of Functional Disorders - 2



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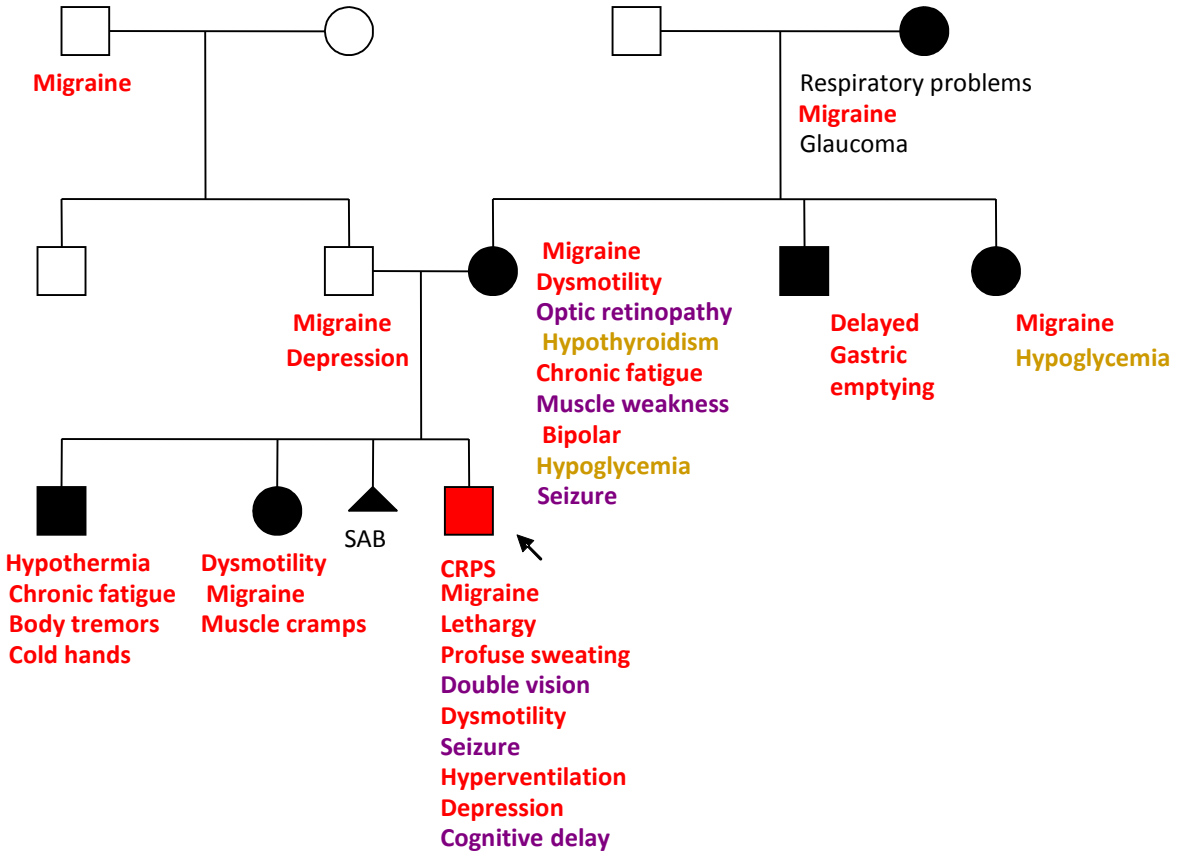
AWARENESS ADVOCACY ADVANCEMENT

Maternal Inheritance of Functional Disorders - 3



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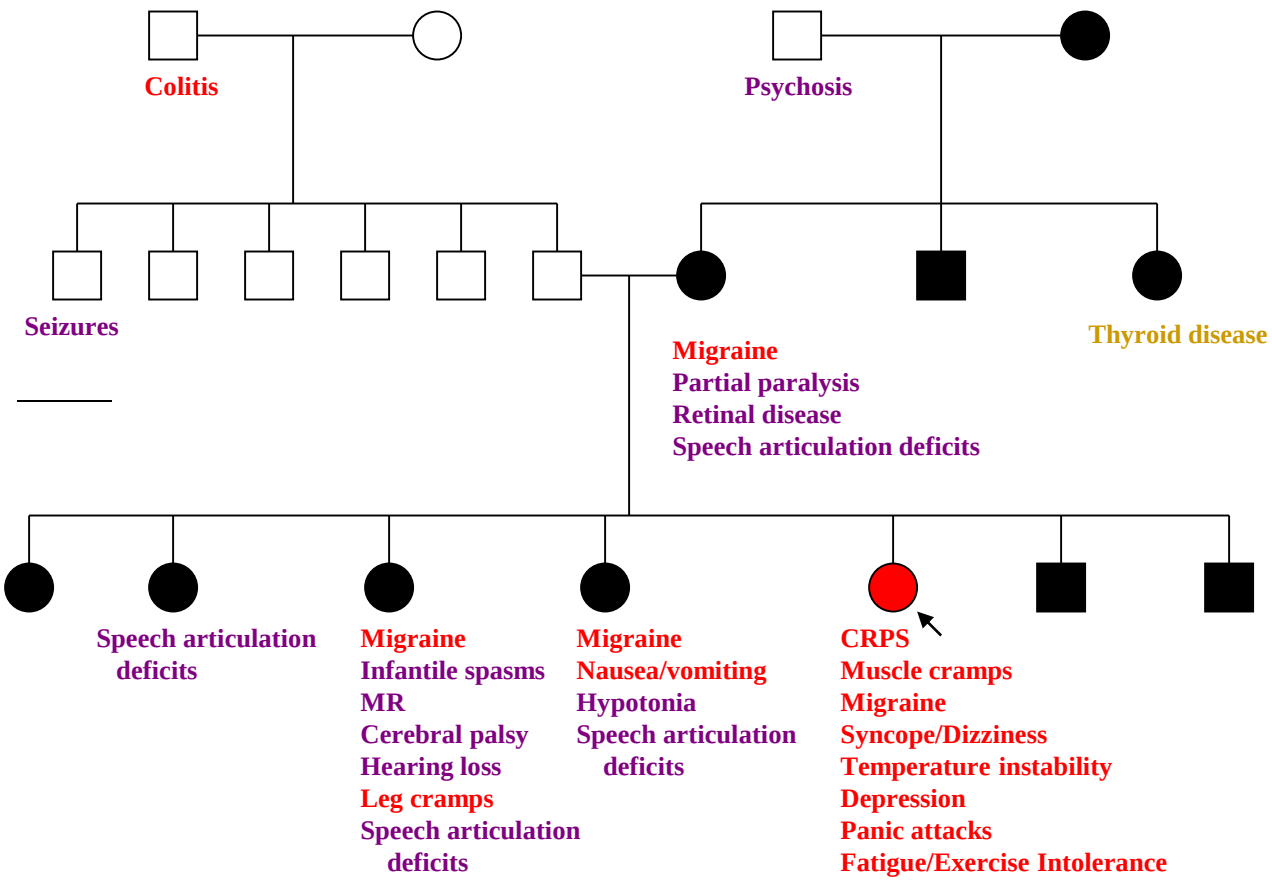
AWARENESS ADVOCACY ADVANCEMENT

Maternal Inheritance of Functional Disorders - 4



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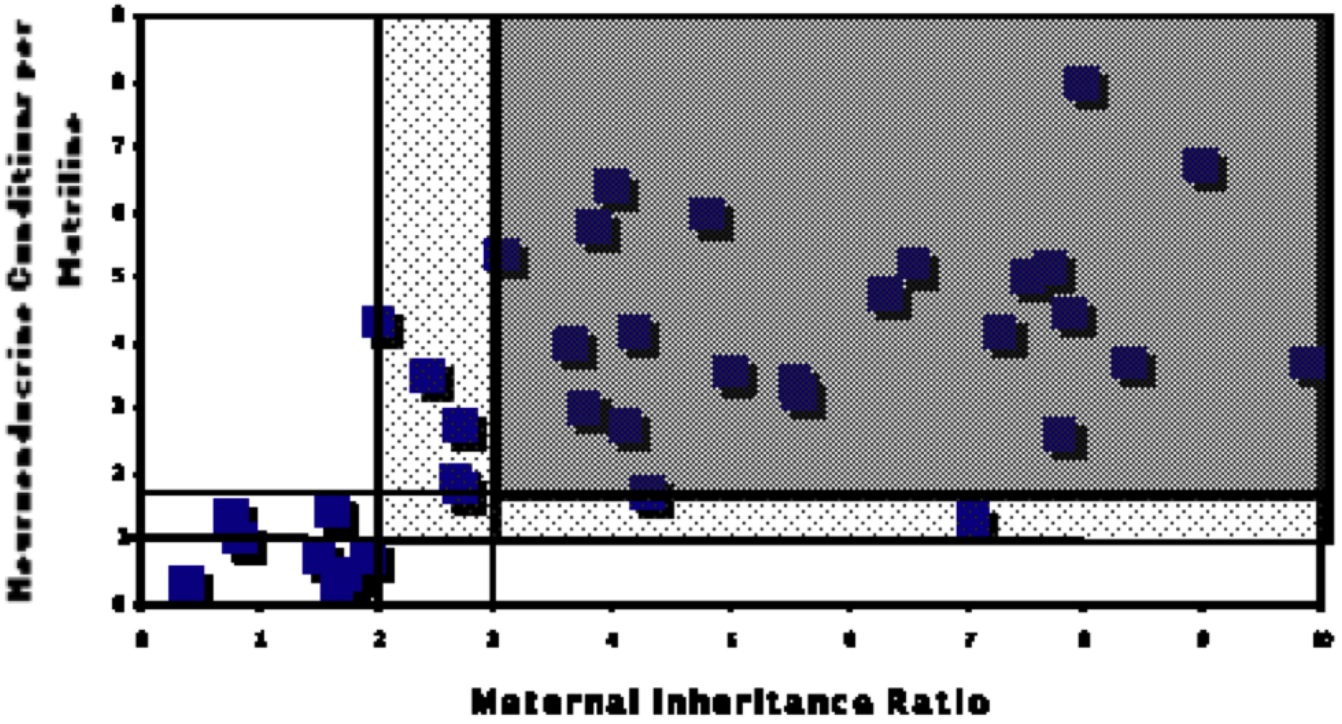
AWARENESS ADVOCACY ADVANCEMENT

Quantitative Pedigree Analysis In Cyclic Vomiting Syndrome



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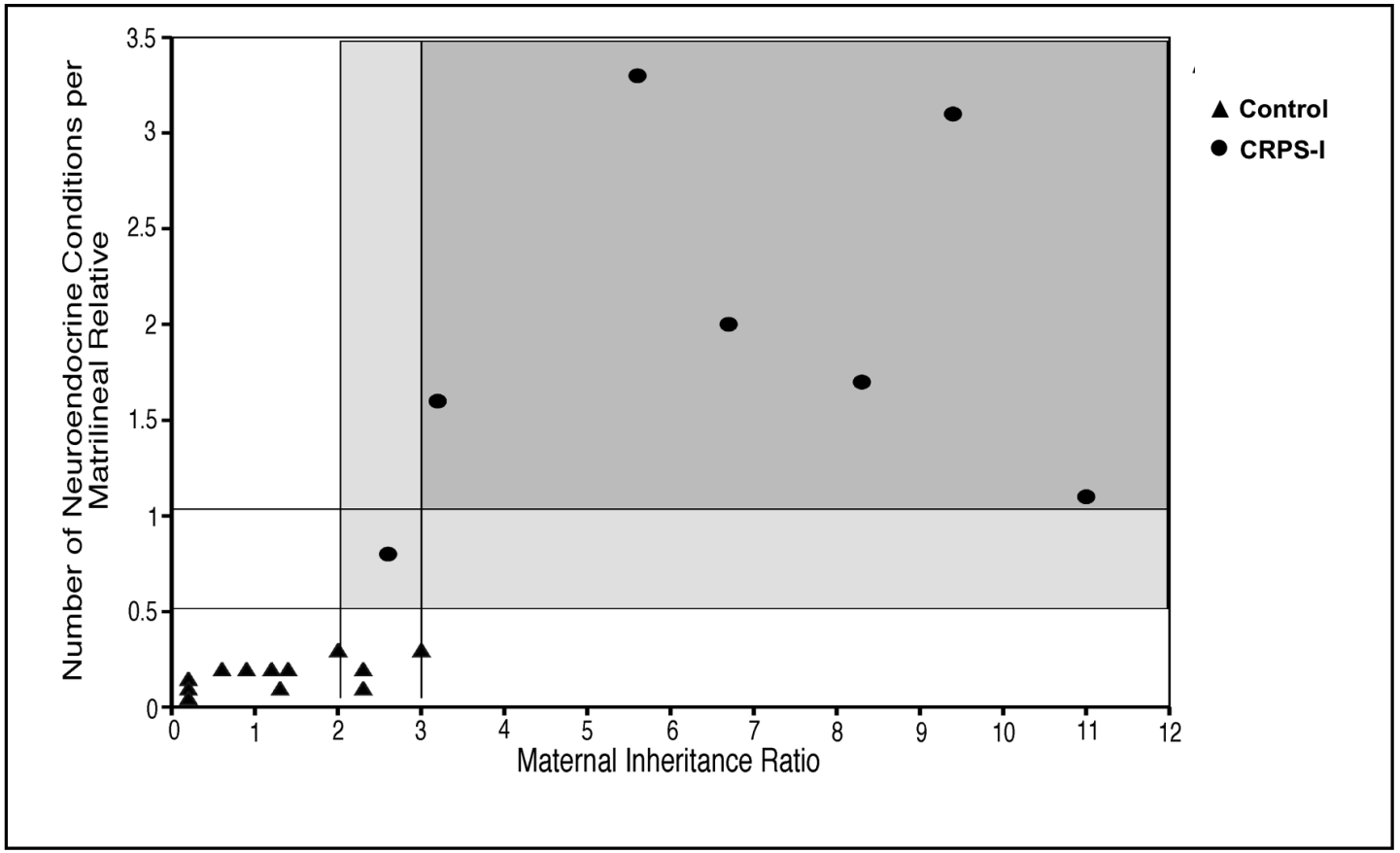
Quantitative Pedigree Analysis In Complex Regional Pain Syndrome

Figure 2: Labeling of pedigrees as "probable maternal inheritance," "probable non-maternal inheritance," or "indeterminate."



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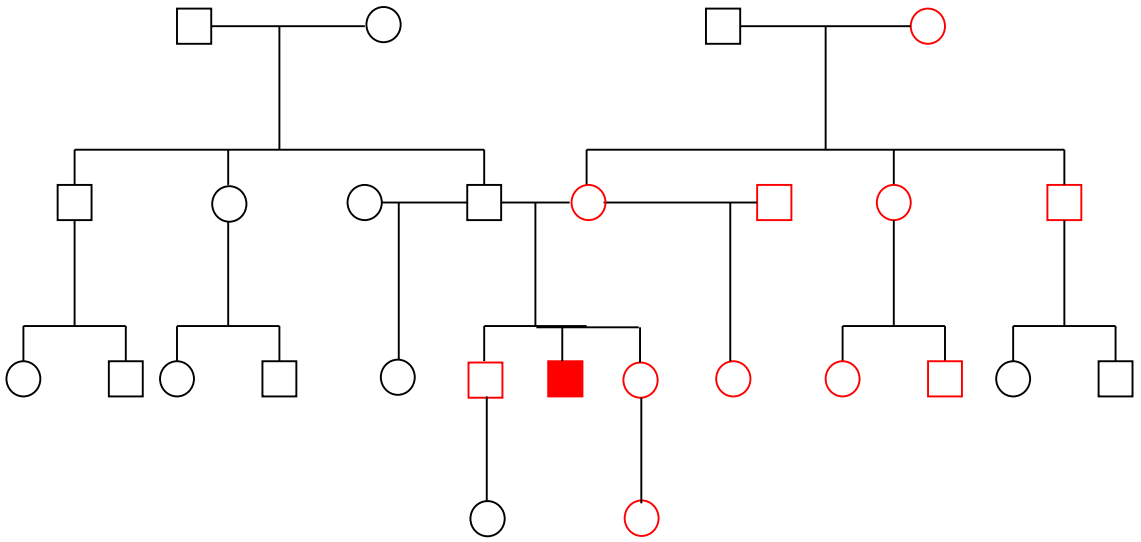
AWARENESS ADVOCACY ADVANCEMENT

Maternal Inheritance in Major Depressive Disorder Bergemann and Boles, 2010

- 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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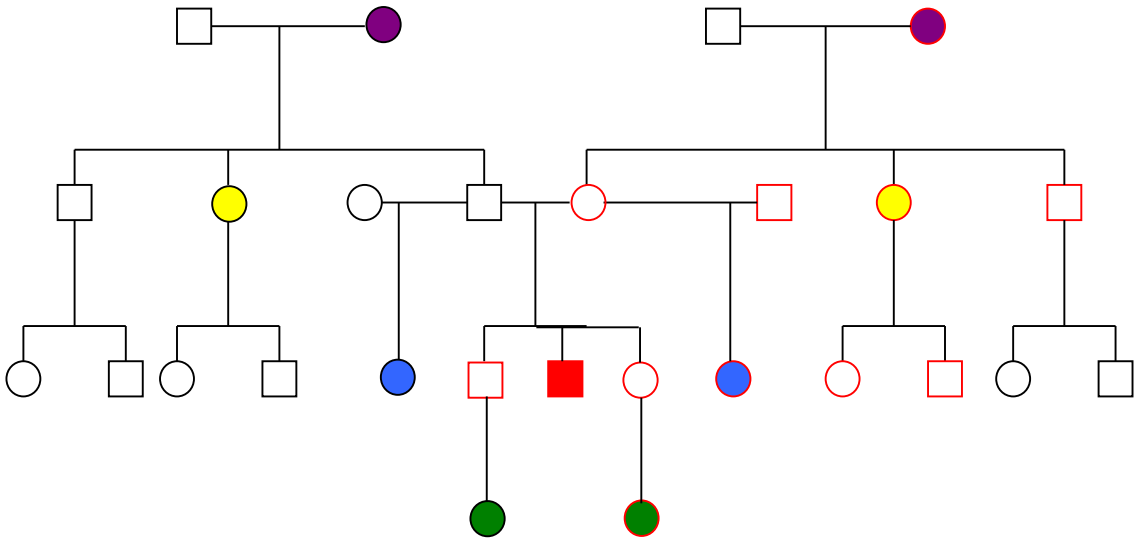
- Analyzed for 5 matrilineal/non-matrilineal pairs controlled for sex, age and autosomal gene contribution
- Matrilineal relatives (with the same mtDNA sequence as the proband) were significantly more likely to suffer from a mood disorder than were non-matrilineal relatives (with another mtDNA sequence); OR 2.0, 95% CI: 1.5-2.6, $P = 3 \times 10^{-6}$.



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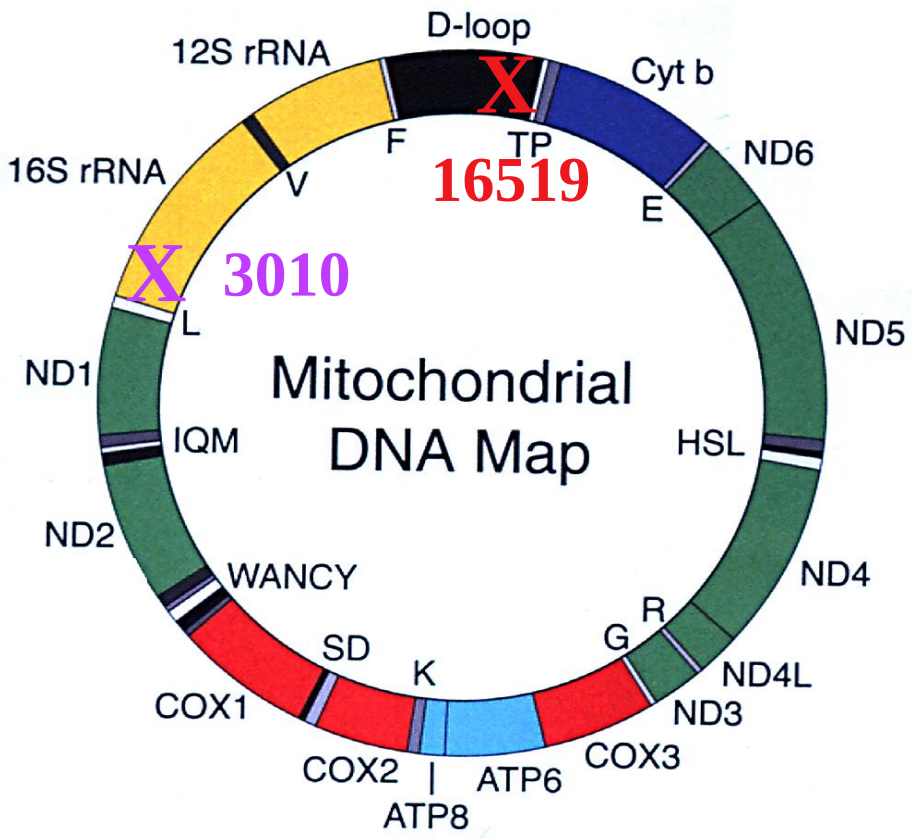
Functional Disorder-Associated mtDNA Polymorphisms



16519 C>T

mtDNA control region

~25% of population

3010 G>A

16S-ribosomal RNA gene

~30% of population

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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

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AWARENESS ADVOCACY ADVANCEMENT

Cyclic Vomiting and Migraine Prevalence of Two mtDNA Common Variants in Haplogroup H Individuals With Functional Disorders

	Cyclic Vomit Syndr.	Odds Ratio (95% C.I.)	Migraine w/o Aura	Odds Ratio (95% C.I.)	Ctrl
16519T	21/30 70%	6.2 (2.7-14)	58/112 52%	3.6 (2.2-5.9)	63/231 27%
3010A	9/30 30%	N/A	37/112 33%	N/A	143/444 32%
3010A among pts with 16519T	6/24 29%	**17** (2-156)	15/58 26%	**15** (1.9-117)	1/63 1.6%

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AWARENESS ADVOCACY ADVANCEMENT

Chronic Fatigue Syndrome The 3010A mtDNA Variant Predicts a Several-fold Increase in Functional Symptoms.

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

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AWARENESS ADVOCACY ADVANCEMENT

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.

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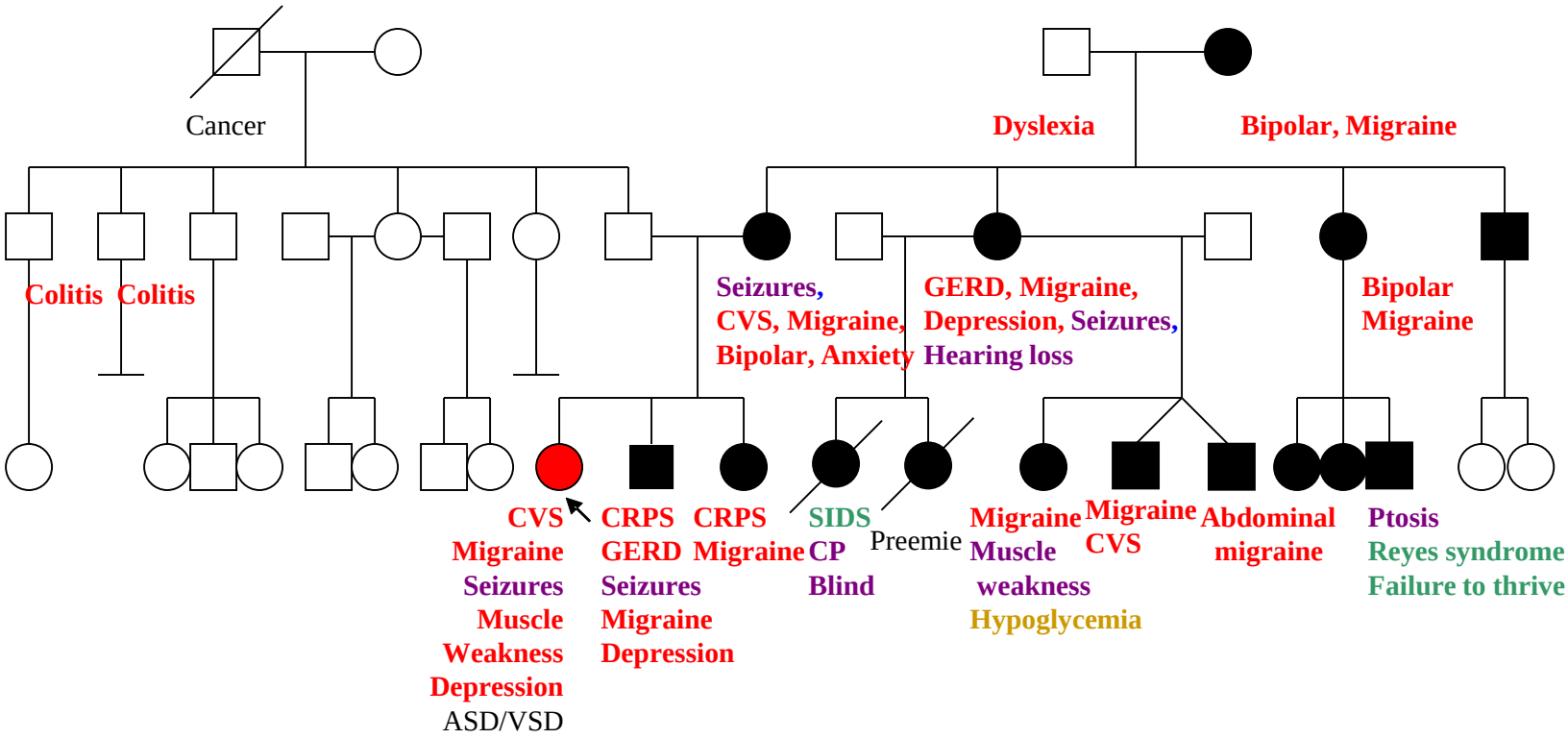
Maternal Inheritance of Functional Disorders - 1



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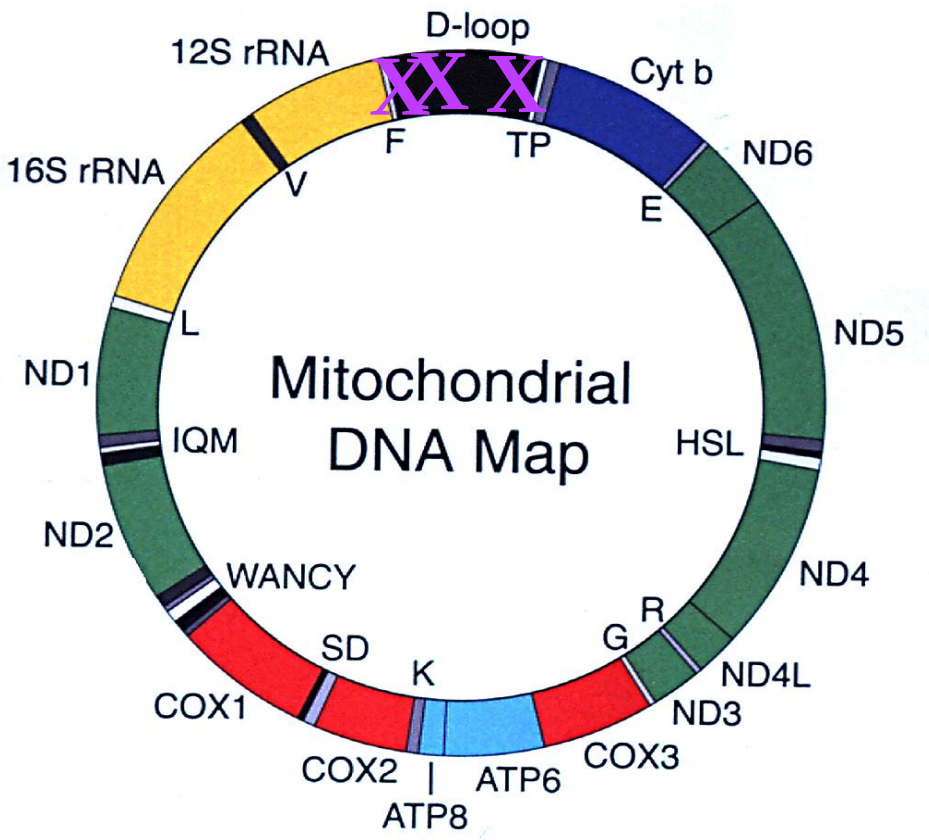
Thomas' mtDNA



Three different length heteroplasmic variants

mtDNA control region – area involved in replication and transcription of mtDNA

Thomas, age 22 years
POTS

- 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.

DYSAUTONOMIA INTERNATIONAL

AWARENESS ADVOCACY ADVANCEMENT

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.

DYSAUTONOMIA INTERNATIONAL

AWARENESS ADVOCACY ADVANCEMENT

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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.

RYR2

Neurogastroenterology and Motility, 2015

- 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)

When to Suspect Mitochondrial Disease?

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)