Extensive DNA Sequencing in Cyclic Vomiting and Chronic Fatigue: Implication for Genetic Testing and Personalized Treatment Options

MitoAction Expert Series
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Look here for the “take-home message” for each slide.

CASE REPORT 1

- 13-year-old female
- Presented with developmental regression in the first grade, recovered
  - Given autism diagnosis
  - Auditory and visual processing delays dx at age 7 years
- At age 12 years with a respiratory virus:
  - Lost all academic skills within one day.
  - Regressed behavior, often “almost catatonic”
  - Seizure-like events: always preceded by headache and muscle weakness (L>R), often with ataxia, tachycardia, nausea, pallor, urinary incontinence, clammy
  - EEG never shown seizure activity.
- Functional disease:
  - Status migrainosus
  - Chronic pain: spine>throat>neck
  - Chronic fatigue
  - POTS
- Frequent infections, dx CVID – treated with IVIG, high-dose steroids

This is a case of autistic regression with multiple chronic functional disorders.

CASE REPORT 1

- Diagnosis: Hemiplegic migraine/episodic ataxia due to calcium channelopathy

- Prolonged episodes of headache, confusion, ataxia, and other neurological signs and symptoms, including asymmetrical weakness, is consistent with and highly suggestive of hemiplegic migraine.

- Hemiplegic migraine is generally due to mutations in cation transporters.
  - Cations are positively-charged salts (e.g., Na^{+}, K^{+}, Ca^{+2}, Mg^{+2})

- She has variants that is likely disease related in all three of these genes:

• CACNA1A	p.His2225dup	Calcium channel
• CACNA1S	p.Pro1839Ser	Calcium channel
• ATP1A2	p.Arg564Gln	Sodium-potassium ion pump

Each of these variants are rare and highly conserved, and thus likely to affect protein function.

Variants in cation transporter genes are common causes of paroxysmal disease.

CASE REPORT 1

Follow-Up:

- Interventions (multiple natural supplements, one drug):
  - Mitochondrial cocktail *(leaky channels deplete ATP to pump ions back)*
  - KCl *(exchanges for Ca^{+2})* and acetazolamide *(cation targeted)*
- Dramatic return of cognitive functioning:
  - 2/2020: Academics tested at kindergarten in reading and 1st grade in math.
  - 2/2021: Same test – reading 8th grade (her grade) and math 7th grade.
- Regressed behavior has resolved, and behavior is now age appropriate.
- Ataxia has resolved; POTS and anxiety improved.
- Chronic pain and fatigue remain and are presently being addressed.

Variants in cation transporter genes are treatable causes of paroxysmal disease.

LEARNING OBJECTIVES

Learn that:

1. ***Diagnosis:*** Whole genome sequencing (WGS) can provide precise diagnostic information for most people with chronic functional symptomatology such as pain, fatigue, and nausea/vomiting; also, autism.
   a. The majority of precise diagnoses are NOT on the laboratory report and require additional review by an expert.
   b. Trio sequencing (including parents) is needed for neurodevelopmental disorders such as autism to find *de novo* mutations.
2. ***Treatment:*** Many of the known pathways in which genetic variants predispose towards autism, cyclic vomiting, & ME/CFS are treatable.
   a. WGS can provide individualized treatment options for most people.
   b. Cation transport & energy metabolism are the main common pathways.
   c. There are many good treatments for both cation transport & energy metabolism disorders, most of which are dietary supplements.
   d. Combination products are available that cover all of the main treatable pathways.

Modern genomics is a powerful tool to treat disease!

DISCLOSURE: DR. BOLES WEARS MANY HATS

- Clinician treating patients
  - Primary interests in neurodevelopmental (e.g., autism) and functional (e.g., CVS) disorders.
  - Past: Geneticist/pediatrician 20 years at CHLA/USC
  - Present: Molecular & Mitochondrial Medicine (drboles@molecularmito.com)
- Chief Medical & Scientific Officer of NeuroNeeds LLC
  - Present: The company that produces EnergyNeeds® (https://neuroneeds.com)
- Medical Director for DNA sequencing companies
  - Past: 5 years at Courtagen Life Sciences; 6 months at Lineagen
  - Present: Free agent, ordering from only the best sequencing company (Variantyx)
- Expert witness in legal cases
  - Present: Medical child abuse, child neglect, and med malpractice cases
- Researcher with prior NIH and foundation funding
  - Past: USC faculty for 20 years
  - Present: Studying DNA sequence variation that predisposes towards neurodevelopmental and functional disorders

This talk is from the point-of-view of a treating physician/vendor/genomicist/scientist.

DNA SEQUENCING HAS ADVANCED RAPIDLY

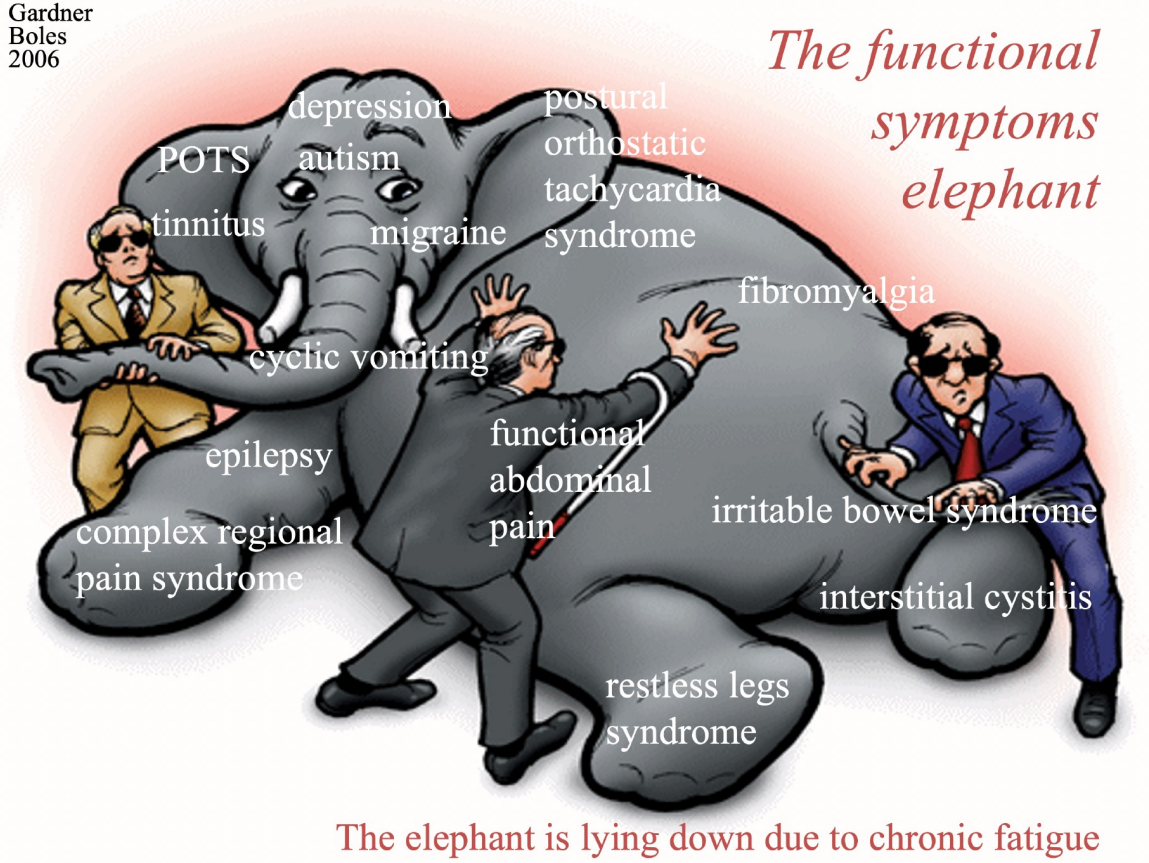
1. Amount of DNA sequenced
2. Sequencing accuracy
3. Computing power
4. Interpretation computer algorithms
5. Cases reported
6. Our understanding of the biology

The result has made it difficult to determine clinical impact, which rapidly improves over time.

This talk will demonstrate the current use of whole genome sequencing (WGS) in people with common conditions (CVS, ME/CFS, autism) in a non-academic practice.

HiSeq 4000, about the size of 2 refrigerators and priced at $900,000, can sequence a human genome in about 6 hours.

Very high-tech medicine is available for clinical care today!

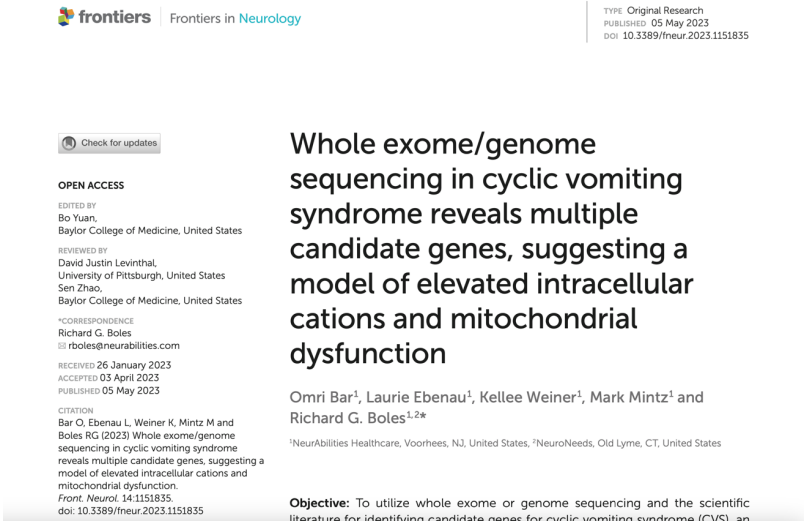


- There is substantial co-morbidity among the functional, neurodevelopmental, & neuropsychiatric disorders.
  - Within individuals
  - Within families
- These conditions are by far the major drivers of disability in developed countries.

Functional disease is common yet underfunded; the largest "elephant in the room".

CYCLIC VOMITING SYNDROME (CVS)

- Episodes of nausea & vomiting
- Episodes are abrupt, distinct, stereotypical, & multiple
- None to substantially less nausea & vomiting between episodes



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Whole exome/genome sequencing in cyclic vomiting syndrome reveals multiple candidate genes, suggesting a model of elevated intracellular cations and mitochondrial dysfunction

Omri Bar[1], Laurie Ebenau[1], Kellee Weiner[1], Mark Mintz[1] and Richard G. Boles[1,2]*

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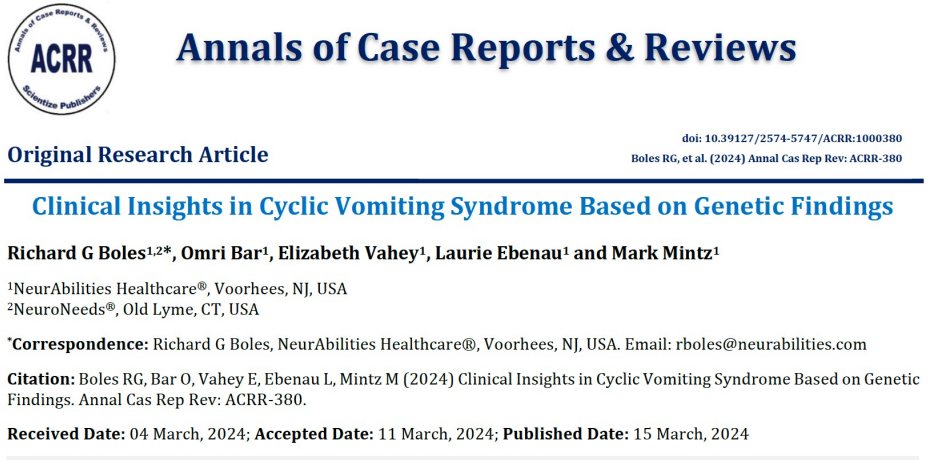
Objective: To utilize whole exome or genome sequencing and the scientific literature for identifying candidate genes for cyclic vomiting syndrome (CVS), an



Annals of Case Reports & Reviews

Original Research Article

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Clinical Insights in Cyclic Vomiting Syndrome Based on Genetic Findings

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CVS is clinically described; two recent papers reveal its causation.

CYCLIC VOMITING SYNDROME (CVS)

Table 2. Clinical Manifestations Between Vomiting Episodes [1].

Pain (60/80, 75%)	Headache: 51 (migraine 39/51), Joints: 22, Abdomen: 20, Extremities: 20, Neck/back: 18, Muscles: 10, Throat/lymph nodes: 7, Chest: 7, Pelvic: 3[b]
Gastrointestinal (58/80, 72.5%)	Bowel[c]: 42, Nausea: 30, GERD: 10, Other: 20[b]
Dysautonomia (57/80, 71.25%)	Temperature intolerance[d]: 35, POTS-like manifestations[e]: 32, Unexplained fevers: 5, Other: 8[b]
Allergy & Immunity (33/80, 41.25%)	Immunodeficiency[f]: 16, Pruritis: 15, Rash: 12 (urticaria 5/12), Asthma: 9, MCAS: 4, Other: 3[b]
Sleep (45/80, 56%)	Parasomnia: 28, Insomnia: 21, Hypersomnia: 3, Other: 1[b]
Psychological (54/80, 68%)	Anxiety: 51, Depression: 22 (suicide attempt 2/22), Mood instability: 3, Other: 5[b]
Neurological[g] (47/80, 68%)	Muscular: 18 (hypotonia: 8, muscle weakness: 5, other: 5[c]), Tinnitus: 13, Abnormal movements: 14 (tics: 7, tremors: 5, other: 4[c]), Sensory: 13 (photophobia: 8, aversions: 1, other: 2), Ophthalmological: 8, Paresthesia: 7, Seizures: 7, Ataxia: 7, Neuropathy: 5, Periodic paralysis: 5, Other: 17[b]
Neurodevelopmental (31/80, 40%)	ADHD: 14, ASD: 10, Learning disability: 8, Intellectual disability: 7, Other: 4[b]
Fatigue (56/80, 70%)	
Urinary (20/80, 25%)	Frequency: 11, Hesitancy: 4, Enuresis: 4, Dysuria: 3, Other: 8b
Connective (9/80, 11%)	Hypermobility: 6, Ehlers-Danlos syndrome: 3
Endocrine (10/80, 12.5%)	Thyroid: 5, Other: 5
Other (21/80, 26.25%)	Heme: 8, Syndromic: 3, Cardiac: 3, Other: 7b

Boles et al, 2024

- CVS sufferers have many functional co-morbidities, especially chronic:
  - Fatigue
  - Pain
  - GI dysmotility/nausea
  - Dysautonomia
  - Anxiety
  - Neurodevelopmental disease
- The same applies to their close relatives.
- This indicates shared genetic factors

CVS, pain, fatigue, and autism share genetic components.

Table 3. Candidate Genes at Least Likely to Be Related to CVS Expression and Function

Gene	Points by Our Study	Points by Literature	Composite	Expression, with Emphasis per Our Model	Protein Function
ATP1A2	1	21		Muscle, brain, and glia (mainly astrocytes)	Maintains the electrochemical gradients of Na and K ions across the plasma membrane; requires ATP
ATP1A3	0	26		Most prominently in GABA interneurons; in vagal afferents	Maintains the electrochemical gradients of Na and K ions across the plasma membrane; requires ATP
CACNA1A	19	14		In most CNS synapses including the NTS; also in vagal afferents	Transmembrane pore-forming subunit of a voltage-gated calcium channel
CACNA1S	17	0		Skeletal muscle (e.g., diaphragm)	Transmembrane pore-forming subunit of a voltage-gated calcium channel
CHAMP1	1	6		Ubiquitous (essentially uniform in all cell types)	Enables chromosome segregation. Associated with expression of genes involving cations and neurotransmitter transport
GFAP	3	18		Glial cells only	Intermediate filaments in glia. Related to regulation of voltage-gated Na+, K+ and Ca2+ channels
HMBS	0	6		Ubiquitous	Intermediate step in heme production, which is necessary for cytochrome production in the mitochondrial respiratory chain
MEFV	12	2		Ubiquitous, yet higher in blood cells	Modulates innate immunity. Promotes inflammasome formation and maturation of IL-beta, which can induce TNF signaling
OTC	0	54		Ubiquitous, yet higher in liver	Mitochondrial matrix enzyme involved in the urea cycle to detoxify ammonia into urea for excretion
POGZ	7	10		Ubiquitous	Regulates mitotic progression. Interacts with CHAMP1 and thus may regulate cation transport
POLG	9	2		Ubiquitous, highest in muscle & nerve	Replication and proof-reading of mitochondrial DNA
PPM1D	3	8		Ubiquitous, including nervous tissue	Regulates the DNA damage response through p53 & ATM. ATM affects mitochondrial homeostasis & modulates mitophagy.
RYR2	15	4		Highest in heart and nerve, including brain and vagus	Stress-activated calcium channel on the endoplasmic reticulum
SCN4A	20	0		Skeletal muscle (e.g., diaphragm), also in brain	Transmembrane pore-forming subunit of a voltage-gated sodium channel
SCN9A	8	0		Ubiquitous, high in sensory neurons (e.g., in viscera, vagus)	Transmembrane pore-forming subunit of a voltage-gated sodium channel
SCN10A	9	0		Ubiquitous, including in sensory neurons (e.g., vagus)	Transmembrane pore-forming subunit of a voltage-gated sodium channel
SLC2A1	0	11		Brain astrocytes, placenta, and erythrocytes	Major glucose transporter across the blood-brain barrier
TNFRSF1A	10	0		Ubiquitous including brain, vagus, and glia	Binds TNF-alpha, important in inflammation; involved in mitophagy and glial/neuronal excitation; binds TRAP1
TRAP1	14	2		Mitochondria; ubiquitous, including brain, vagus, and glia	Mitochondrial chaperone protein induced in times of oxidative stress; involved in mitophagy
TRPA1	7	0		Ubiquitous, highest in gut, also present in nerve (e.g., vagus)	Non-specific cation channel involved in perception of pain, cold, itch, sound, and oxygen concentration
TUBB3	0	9		Highest in brain, present in vagus	A beta tubulin protein that forms microtubules in neurons
mtDNA	-	-		Ubiquitous, highest in nerve and muscle	Encodes subunits of the respiratory chain

Cation transporter

Indirectly associated with cation transport

Energy metabolism

Indirectly associated with energy metabolism

Auto-inflammation; indirectly associated with energy metabolism

Highly likely to be a CVS gene

Likely to be a CVS gene

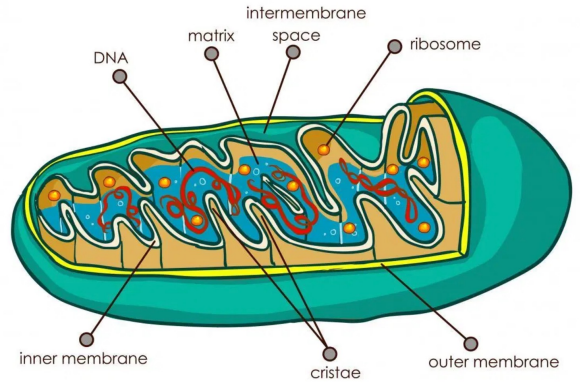
Possibly a CVS Gene

Boles et al, 2024

Cation transport & energy metabolism comprise the genetic predisposition of CVS.

MECHANISM OF DISEASE: CVS

Diagram of a mitochondrion



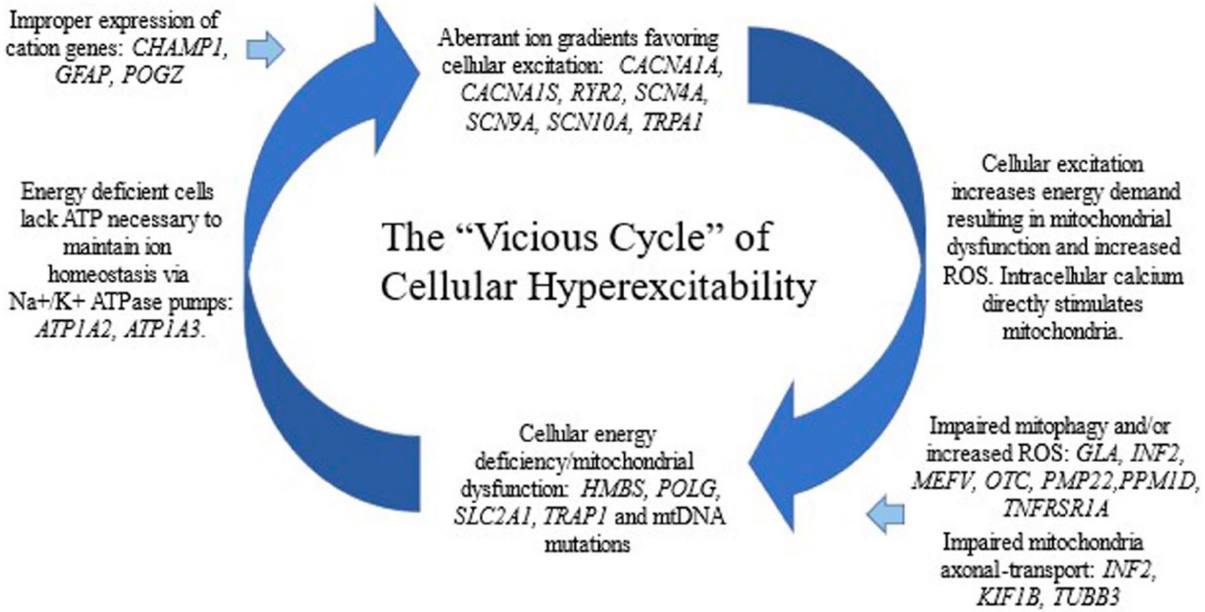
Abnormal energy metabolism (mitochondrial dysfunction) is near universal in people with CVS per Dr. Boles' published research.

Dr. Boles' published articles on CVS and energy metabolism:

- PMID: 37234784
- PMID: 25934187
- PMID: 25332060
- PMID: 21846334
- PMID: 20109231
- PMID: 19368653
- PMID: 19220304
- PMID: 17275670
- PMID: 15643622
- PMID: 15368478
- PMID: 12884425
- PMID: 10490048
- PMID: 9357417

Mitochondrial dysfunction in CVS is well established.

MECHANISM OF DISEASE: CVS

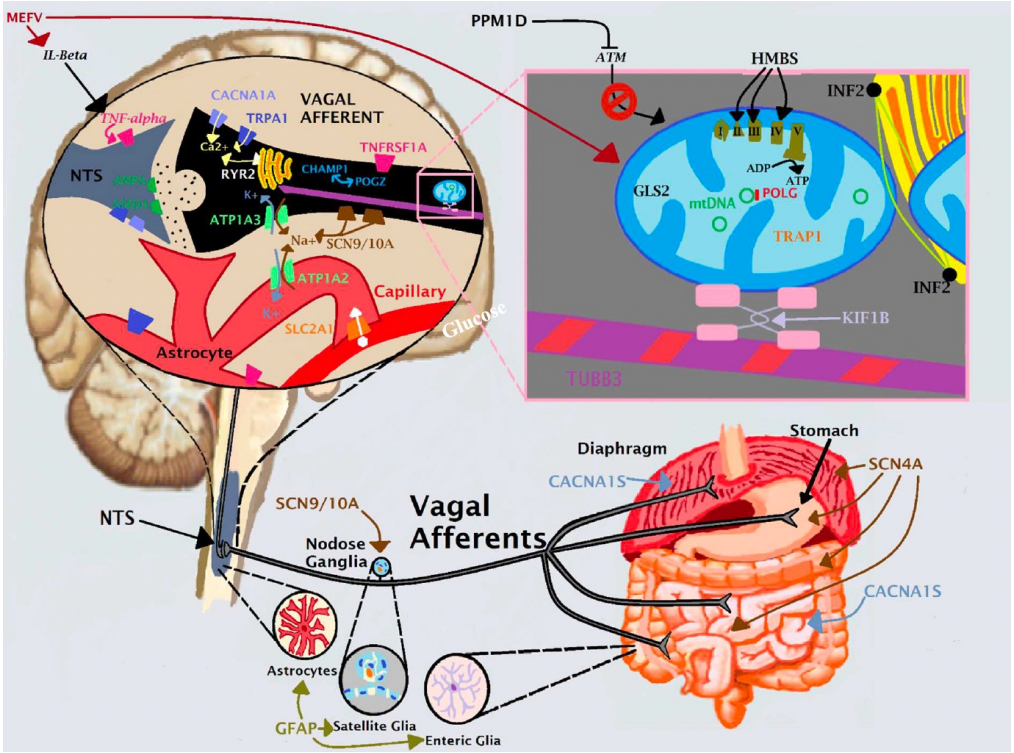


Bar et al, 2023

FIGURE 1
Our cellular model for CVS.

Cation transport & energy metabolism: shared genetic risks for CVS and autism.

MECHANISM OF DISEASE: CVS



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- The site of CVS is likely in and near the vagal afferents, including in the brain stem.

CASE REPORT 2

- Ryan presented as a teenager with episodes of vomiting, headache, and dizziness for 5 days (3 days in the hospital each episode).
  - Episodes occurred every 90 days, range 88-92 days).
  - Ketosis during episodes (ketones of urine dipstick).
- Issues between episodes: cognitive delays, chronic fatigue, frequent urination, GERD, constipation, choking episodes, and obstructive sleep apnea
  - Random aggressive/explosive behaviors were more of a problem than CVS.
- Failed treatment with coenzyme Q10, carnitine, amitriptyline, and propranolol.

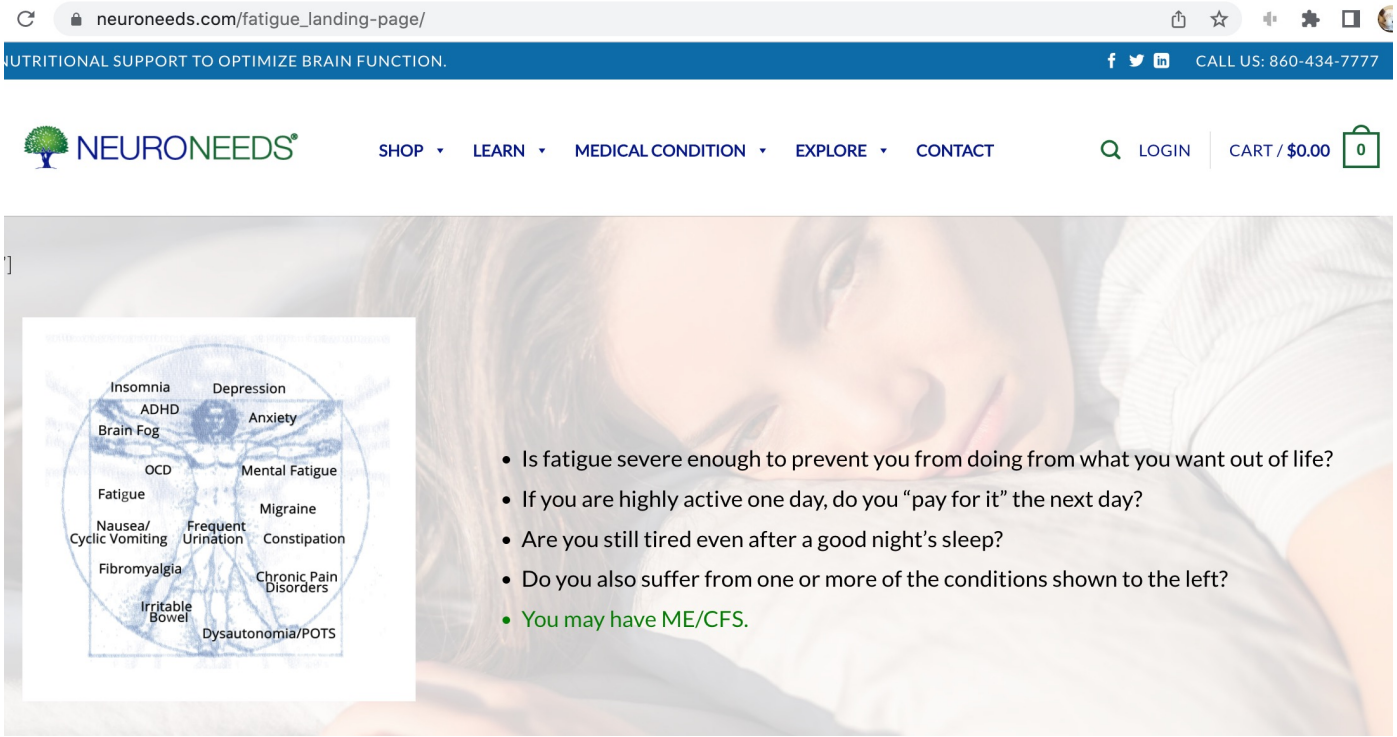
A “clockwork”-like case of CVS, refractory to standard treatment for years.

CASE REPORT 2

- Genetic testing revealed a variant of interest in the *SCN4A* gene (present in 1.2% of the population) encoding the alpha subunit of the voltage-gated type IV sodium channel.
  - This variant has been shown in a published study to have aberrant channel function.
- Cyclic vomiting episodes resolved since starting acetazolamide, which acts on the *SCN4A* channel.
- Aggressive behaviors are now only triggered, not spontaneous.
  - Ryan has been able to thrive in a supervised group home for years.

A common variant identified on sequencing led to successful therapy.

MYALGIC ENCEPHALOMYLITIS/ CHRONIC FATIGUE SYNDROME (ME/CFS)



- Common
- Disabling
- Co-morbidities
- Genetic components
- Environmental triggers

PILOT ME/CFS GENOMICS STUDY

- 18 unrelated individuals:
  - Age range 5-38 yrs (children: 2, teens: 5, 20s: 9, 30s: 2)
  - Female: 11, male: 7
  - Fatigue: 16/18 ME/CFS (2 likely, yet insufficient data)
  - GI: 18/18 (nausea/GP/CVS: 14, bowel dysmotility/IBS: 12)
  - Dysautonomia: 17/18 (POTS/dizziness: 14)
  - Pain: 16/18 (headache: 14 - mostly migraine, limb pain: 11)
  - Cognitive: 15/18 (AD/HD: 9, autism: 6 mostly high-fxn)
  - Sleep disorders: 14/18 (parasomnia: 9)
  - Neurological: 12/18 (muscle weakness: 8)
  - Psychiatric: 12/18 (anxiety: 10, depression: 7)
  - Immunological: 11/18 (frequent infections: 7, MCAS: 4)
  - Urological: 7/18 (frequent urination: 6)

Patients ≥ 50%, 33-50%

ME/CFS has the same main co-morbidities as CVS, and other functional disorders.

PILOT ME/CFS GENOMICS STUDY

Pathogenic/Likely Pathogenic variants: 12/18 (67%)
Clinical additional diagnostic variants in 9/18 (50%)
Total number of diagnostic variants: 21 in 18 patients
Diagnostic variant (one of the above): 16/18 (89%)
Ion channel gene variants predominate in 11/18 cases, including 8/18 with a diagnostic variant.
Mitochondrial metabolism gene variant in 7/18 cases.

Cation channel or energy metabolism: 15/18

Clinical diagnostic variants: 9/18 (all presumed inherited)
Patient has a clinical diagnosis associated with that gene.
Variant is plausible disease causal/associated.

- CACNA1A p.Asn838Thr: Migraine w aura, hemiplegia
- CACNA1A del 9(CAG): Migraine w aura
- CACNA1S p.Asn649His: Periodic paralysis
- SCN9A p.Phe142Ser: Amplified pain syndrome
- SCN9A p.Ala749Val: Widespread chronic pain
- CACNA1A p.His2225dup; CACNA1S p.Pro1839Ser; ATP1A2 p.Arg564Gln: Hemiplegic migraine
- KCNJ18 p.Thr140Met: Periodic paralysis
- *POGZ p.Cys652Arg: White-Sutton syndrome*
- *PHKA2 IVS27+1G>A: Glycogen storage disorder*

Ion channels, ion pumps, *de novo variant*

Cation transport/mitochondria comprise the shared genetic predisposition of ME/CFS, CVS, & ASD.

AUTISM STUDY: PUBLISHED IN 1/2024

International Journal of *Molecular Sciences*

MDPI

Article

Reanalysis of Trio Whole-Genome Sequencing Data Doubles the Yield in Autism Spectrum Disorder: De Novo Variants Present in Half

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The latest, published in January 2024 in a prestigious, open-access journal (IF 5.6).

AUTISM STUDY: RESULTS, GENETIC

1	essentially negative	*de novo*	***EHMT1***	RYR2	yes
2	essentially negative	autosomal recessive, CompHet *in trans*		*IVD* (*AR-CompHet*)	yes
3	essentially negative	*de novo*	*PGAM5*	CDH15	no
4	essentially negative	*de novo*	*COL4A1*	TRAP1	no
5	essentially negative	*de novo*	*TRPM2*		no
6	sister autism; siblings & mother ADHD	X-linked, mother carrier		*THOC2* (*XL-Mat*), dup with CHL1, RIC1 (AR-CompHet)	no
7	brother LD	*de novo*	*del 5 SFARI genes*		yes
8	essentially negative	*de novo*	*MTMR4*	2p16.1p16.1 dup with FANCL, 9p13.3p13.3 dup	no
9	essentially negative	*de novo*	***del with UBE3A***	**FRMPD4** (XL)	yes
10	slow speech in twin sister, now normal				N/A
11	affected sister; parents with small fiber neuropathy	*de novo*	*USP20*	CLPX, POLRMT, **RELN**, SCN10A	no
12	essentially negative	*de novo*	*SLC41A2*	FLNA (XL)	no
13	brother LD, ADHD, possible autism	autosomal recessive, homozygous		*SLC1A4* (*AR-Hom*)	yes
14	essentially negative			**SCN9A**, **CPT2**	N/A
15	essentially negative	*de novo*	***KCNB1***	MT-TW	yes
16	essentially negative	X-linked, mother carrier		*USP9X* (*XL-Mat*), CIC	no
17	essentially negative	*de novo*	*GRIK1*		no
18	brother LD; father possible autism			**GABRA1**, tetrasomy at 14q32q33 with 26 genes	N/A
19	essentially negative			**CACNA1A**, **RIMS1**	N/A
20	essentially negative				N/A
21	sister ADHD	*de novo*	*OCM*	SCN10A	no
22	sister ADHD; mother ADD	X-linked, mother carrier		*NLGN4X* (*XL-Mat*), 4p16.1p16.1 dup with SORCS3, 9p24.3p24.3 dup with DOCK8, MT-ND3	yes
23	essentially negative			POLA1 (XL), **PAH** (AR-CompHet), 22q11.21 11.5-kb del, 14q32.33 27-kb dup, MT-CYB	N/A
24	essentially negative			7p22.3p22.3x1, **DYNC1H1**	N/A
25	essentially negative	*de novo*	***ANKFN1***	**SCN2A**, **ASH1L**	no

26	essentially negative	*de novo*	*CEP170*, *NUP210*		no
27	essentially negative			**SCN9A**, RYR2	N/A
28	essentially negative	*de novo*	***ANKRD11***	TRAP1	yes
29	essentially negative				N/A
30	brother OCD, autism	*de novo*	***RIF1***, *AGO3*	PRKCA	no
31	essentially negative				N/A
32	affected brother; father probable LD	*de novo*	*Large del with GGNBP2*	**SETD1B**, **DEAF1**	yes
33	only extended relatives affected	autosomal recessive, homozygous		*EIF3F* (*AR-Hom*), SCN10A	yes
34	father ADD	autosomal dominant, paternal		***TCF20*** (*AD-Pat*), MT-TC	no
35	essentially negative	*de novo*	*KRAS*	MTHFR, HSPG2	yes
36	mother & dizygotic twin with autism	autosomal dominant, maternal		***RERE*** (*AD-Mat*)	yes
37	essentially negative			GBE1 (AR-CompHet)	N/A
38	essentially negative			MTHFR, **TSC2**, MT-CYB	N/A
39	father possible autism	*de novo*	*YTHDF1*	CUX2, 148-kb del with IMMP2L	no
40	essentially negative	*de novo*	*GRB10*, *STAT1*	RYR2	no
41	essentially negative	*de novo*	*GOLGB1*		no
42	essentially negative	*de novo*	***GABRA1***	16q23.1q23.1, 736.50-kb with ADAMTS18	yes
43	essentially negative	*de novo*	*SP8*	MT-CYB	no
44	mother & sister ADHD				N/A
45	essentially negative			SHROOM4 (XL)	N/A
46	brother ADHD			**KMT2E**, RYR2, MT-CO3	N/A
47	essentially negative			SCN4A, KCND2, 7q31.31q31.31x1 del with IGHG2	N/A
48	sister ADHD, ID, seizures, MELAS	*de novo* + mtDNA	***SPEN***	*MT-CO1*	yes
49	brother autism	autosomal recessive, CompHet *in trans*	Large del with 53% PRODH	***ZNF292*** (*AR-ComHet*), Xq22.3q22.3x1, 54 base pairs; includes TBC1D8B	no
50	essentially negative	*de novo*	*HSPA1A*		no

Column 3/white: No Primary Diagnostic Variant
Column 3/yellow: *De novo* PDV
Column 3/other colors: Inherited PDV

Column 2/indigo: Significant NDD in a first-degree relative

- Precise diagnoses were made in 68% of cases; primarily in *de novo* variants.

AUTISM STUDY: GENES WITH PDVs

Participant Number	Gene	Report[1]	Disorder	NDD per HGMD[2]	Protein Function	Cation Transport	Redox State	Amino Acids	Ubiquitin	Neuro-transmitter	Gene Expression	Cell Division
On Lab Report												
1	EHMT1	Uncertain	Known		Histone methyltransferase						Yes	
2	IVD	Candidate	Known		Amino acid metabolism			Yes				
7	del SFARI x5[3]	POSITIVE	Known		Ubiquitination/cation channel/cholinergic receptor[3]	Yes	Yes		Yes	Yes		
9	UBE3A[3]	POSITIVE	Known		Ubiquitination		Yes		Yes			
13	SLC1A4	POSITIVE	Known		Amino acid transport			Yes				
15	KCNB1	POSITIVE	Known		Potassium transporter	Yes						
22	NLGN4X	Likely Negative[4]	Known		Neuronal cell-cell interactions, glutamate receptors					Yes		
28	ANKRD11	Likely Positive	Known		Transcription						Yes	
32	GGNBP2[3]	POSITIVE	Known		Growth suppressor?							
33	EIF3F	Uncertain	Known		Translation initiation factor						Yes	
35	KRAS	Likely Negative[4]	Known		Ras protein, GTPase activity, regulation of cell proliferation							Yes
36	RERE	Likely Negative[4]	Known		Transcription						Yes	
42	GABRA1	Likely Negative[4]	Known		GABA receptor, chloride channel					Yes		
48	SPEN	Likely Positive	Known		Transcription						Yes	
48 (PDV 2)	MT-CO1	Likely Negative[4]	Known		Energy metabolism		Yes					
Not on Report												
3	PGAM5		Novel	1	Programmed cell death, mitophagy		Yes					
4	COL4A1		Known		Collagen, structural							
5	TRPM2		Very rare[5]		Calcium channel, oxidative stress	Yes	Yes					
6	THOC2		Very rare[6]		Transcription						Yes	
8	MTMR4		Novel	1	Ubiquitination, vescular fusion, phagocytosis				Yes			
11	USP20		Novel	2	Ubiquitination, inflammatory signaling				Yes			
12	SLC41A2		Novel	3	Cation 2+ transporter, including magnesium	Yes						
16	USP9X		Known		Ubiquitination, separating sister chromatids, axonal growth				Yes			Yes
17	GRIK1		Novel	3	Glutamate ionotropic receptor kainate type	Yes				Yes		
21	OCM		Novel	0	Calcium buffering	Yes						
25	ANKFN1		Novel[7]	1	Orientation of mitotic spindle and cell polarity							Yes
26	GEP170		Very rare[8]		Centrosome component							Yes
30	RIF1		Novel	3	Cell check point							Yes
30 (PDV 2)	AGO3		Novel	3	Transcription						Yes	
34	TCF20		Known		Transcription						Yes	
39	YTHDF1		Novel	1	Binds m6A-containg mRNAs						Yes	
40	GRB10		Novel	5	Involved in multiple cell signaling cascades							
41	GOLGB1		Novel	3	Golgi crosslinking							
43	SP8		Novel	0[19]	Transcription						Yes	
49	PRODH		Known	0[19]	Amino acid metabolism			Yes				
50	HSPA1A		Novel		Chaperone		Yes					

- Every PDV reported was in a different gene.
  - There are hundreds, likely thousands of genes associated with autism.
- However, there are only a small number of common pathways in the pathophysiology of autism:
  - *Cation transport*
  - *Redox state/mitochondrial energy metabolism*
  - *Amino acids (metabolism & transport)*
  - *Ubiquitination (protein degradation pathway)*
  - *Neurotransmission*
  - *Gene expression (neuron\brain specific* and general)
  - Cell division

Italics = of particular importance in neuron/brain

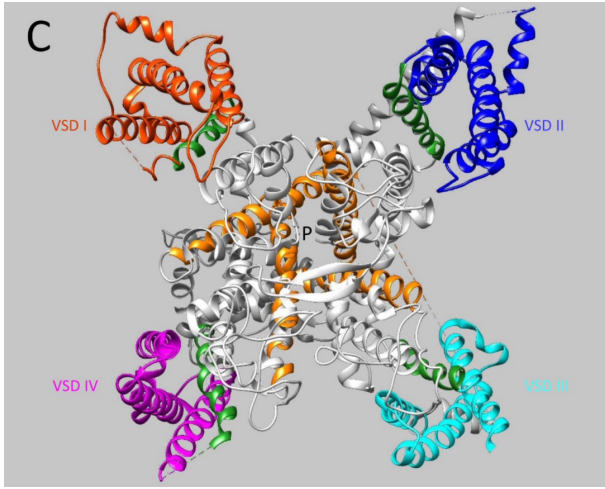
Green – generally treatable, potentially treatable

There are 7 common pathways leading to autism; 4-6 of which are treatable.

TREATMENT CAVEATS - CATIONS

- Multiple studies (+ ours) have identified aberrant cation transport in autism.
- Despite being a treatable pathway, this is **not** generally recognized among autism treatment providers.
- Variants generally cause leaky cation channels, resulting in cellular hyperexcitability.
- Treatment often consist of dietary supplementation and//or prescription medications that are specific antagonists to the channel with the identified genetic variant.
- However, there are some therapies which often are efficacious for variants in multiple different genes:
  - *Diet & Supplements:*
    - Energy metabolism-acting: mito-cocktail
    - Cation channel-acting: potassium, magnesium, (calcium removal)
    - Neurotransmitter-acting:
      - GABA-acting: GABA, L-theanine, magnesium, zinc, B6
      - Glutamate-acting: magnesium, zinc
      - Serotonin-acting: 5-HTP
  - *Medications:*
    - Channel-acting: acetazolamide, bumetanide
    - GABA-acting: gabapentin, *benzodiazepines*
    - Glutamate-acting: memantine, amantadine
    - Serotonin acting: SSRIs, SNRIs, amitriptyline

CACNA1A



https://doi.org/10.3389/fnsyn.2021.634760

Cation transport is a frequent target of therapy; diet/supplements are key.

TREATMENT CAVEATS - CATIONS

- High potassium foods and/or supplements are a common treatment for many cation channelopathies.
- Effective dosing is usually 20-40 meq TID
- One meq = 40 mg KCl
- Nausea is the most-common adverse effect.
- Pomegranate juice is high in K.
- Check blood levels; aim for 4.5-5.3.

Many cation transport disorders can be treated with potassium.

TREATMENT CAVEATS - MITOCHONDRIA

- Energy metabolism is one of the key autism pathways
- Cation transport, another key pathway, leads to energy depletion
- Energy depletion is seen in most cases of autism.
- See multiple studies by Drs. Frye and Rossignol

SEE THE DATA

Click on a nutrient to learn more:

- Alpha lipoic acid
- Choline
- Chromium
- Coenzyme Q10
- Creatine
- Inositol
- L-carnitine/Acetyl-L-carnitine
- Magnesium
- Molybdenum
- Omega-3 fatty acids (DHA & EPA)
- Selenium
- Vitamin B1 (thiamine)
- Vitamin B2 (riboflavin)
- Vitamin B5 (pantothenic acid)
- Vitamin B6 (pyridoxine)
- Vitamin B7 (biotin)
- Vitamin B9 (folate)
- Vitamin B12 (cobalamin)
- Vitamin C
- Vitamin D
- Zinc

As mitochondrial dysfunction is common in autism as discussed above, the following nutrients might be helpful in autism since they are mitochondrial targeted:

- Alpha Ketoglutarate
- Arginine
- Citrate
- Malate
- Quercetin & Rutin*
- Vitamin A/Beta-carotene*
- Vitamin B3 (nicotinamide)
- Vitamin E
- Vitamin K

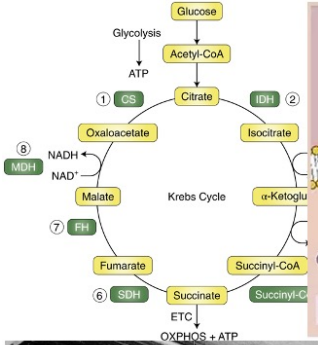
There are far too many references demonstrating the use of mitochondrial-targeted supplements in autism. See: https://www.neuroneeds.com/autism-landing-page

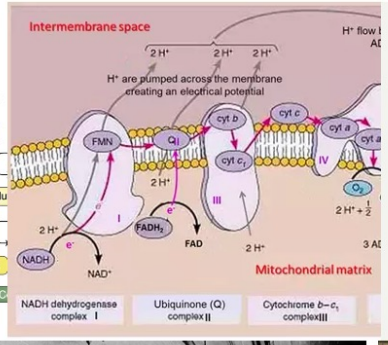
Mitochondrial/energy metabolism is a frequent target of therapy; tx is dietary.

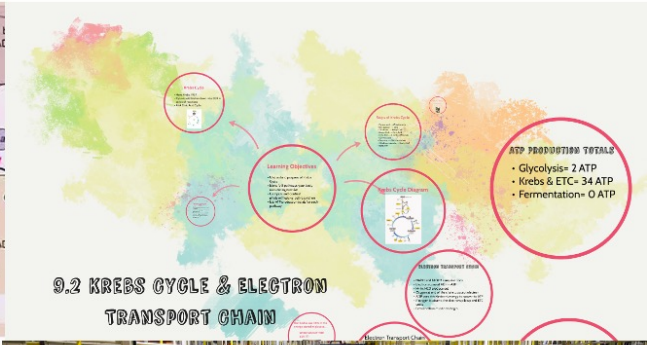
Bahamas
November,
2022

Apex predators

ENERGY IS PRODUCED ON AN ASSEMBLY LINE







Combination products have higher efficacy AND tolerance than individual supplements.

EXPERT OPINION: SPEAKER'S APPROACH

- A high-powered mitochondrial cocktail multivitamin and mineral supplement,
  - Recommended dose based on patient's weight.
  - Check blood levels of carnitine (aim for free carnitine > 30 or 40).
  - 25-hydroxy-vitamin D level per your physician (> 30 or 40).
- Ubiquinol form of coenzyme Q10:
  - Recommended dose is the one that leads to a target blood level.
  - Check blood levels 2 weeks later (optimal range 4.0-7.0, which is well above "normal").
- Essential fatty acid supplement:
  - Krill oil is best for brain health.
  - As high dosing of omega-3s from fish oil is good for general/cardiac health, consider:
    - Both fish oil and krill oil supplements.
    - A supplement that combined fish & krill oil together.
- Consider additional zinc (total dose to 25-50 mg/day in adolescents/adults, less in children).
- Consider GABA-enhancing supplementation (GABA, L-theanine, 5-HTP, Mg, B6) if anxiety, hyperactivity, or insomnia are issues.
- Diet and supplements are BOTH necessary, and synergistic.
- *These recommendations are general, and not meant to circumvent or contradict your physician's advice. It is recommended that ALL treatments be discussed with your physician.*

The above addresses the main known treatable pathways often affected in ASD, CVS, & ME/CFS.

THE MITOCHONDRIAL COCKTAIL MADE EASY

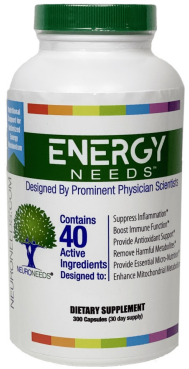
- High-powered mitochondrial supplements designed by a collaborative of prominent physicians and scientists.
  - Cofactors: alpha-lipoic acid, L-carnitine, and coenzyme Q10
  - Vitamins: (multiple) B vitamins, vitamin C, vitamin D
  - Minerals: chromium, magnesium, molybdenum, selenium, zinc
  - Other: creatine
- They are often used to optimize nutrition for a wide range of disorders associated with mitochondrial dysfunction.
- These products also double as a high-powered multi-vitamin and mineral supplement.
- Powder and capsule forms are available in some brands.

- These products are designed to offer a high-powered mitochondrial cocktail, which is best combined with optimized general nutrition.
- Additionally, these products are designed to be a safe nutritional supplement for anyone regardless of the underlying genetics, signs or symptoms, severity of mitochondrial dysfunction, age, and/or general health.
- However, as with any supplement, it is recommended to discuss with your physician.

Combination mitochondrial cocktail products: Choose one to address energy & cations.

THE MITOCHONDRIAL COCKTAIL MADE EASY

Berry & Lemon flavors



Capsules

SpectrumNeeds® (powder), 33-active ingredients
EnergyNeeds® (capsules), 40-active ingredients

- High-powered mitochondrial supplements designed by a collaborative of prominent physicians and scientists.
  - Cofactors: alpha-lipoic acid, L-carnitine, and coenzyme Q10
  - Vitamins: (multiple) B vitamins, vitamin C, vitamin D
  - Minerals: chromium, magnesium, molybdenum, selenium, zinc
  - Other: creatine

- They are often used to optimize nutrition for a wide range of disorders associated with mitochondrial dysfunction.
- Both products also double as a high-powered multi-vitamin and mineral supplement.

- These products are designed to offer a high-powered mitochondrial cocktail, which is best combined with optimized general nutrition.
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- However, as with any supplement, it is recommended to discuss with your physician.

Combination mitochondrial cocktail products: Choose one

THE CASE FOR CoQ10/UBIQUINOL

- Many chronic conditions, especially those involving mitochondrial function, can increase the body's demand for coenzyme Q10 (coQ10), leading to deficiency.
- CoQ10 supplementation is often recommended for a variety of different medical conditions, including autism and ADHD.
- Multiple studies showing deficiency or benefit from coQ10 – see neuroneeds.com.
  - CoQ10 is sold in two different forms, ubiquinone and ubiquinol. Ubiquinol is about five times more bioavailable.
  - All ubiquinol products are in gel capsules, with one exception.
  - Most are in soy oil; limonene oil products are available.

Most products have very poor absorption; only truly bioavailable as ubiquinol in oil.

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QNeeds® soft gel capsules provide additional coQ10 in the ubiquinol form to help boost antioxidant support. It comes in limonene oil, the natural oil of lemon peel, which boosts bioavailability without soy, which is present in most commercially-available preparations.

Most products have very poor absorption; only truly bioavailable in oil.

THE CASE FOR OMEGA-3 FATTY ACIDS

- Omega-3 fatty acids are low in people on most modern diets who do not consume a lot of seafood.
- Omega-3s are critical for proper brain function.
- Omega-3 supplementation was found to be beneficial in autism and ADHD in multiple studies – see neuroneeds.com.
- **Most omega-3 brands do NOT directly cross into brain!**

- Combination products are available that blend krill and fish oils sources of omega-3 fatty acids including DHA & EPA.
- Krill oil omega-3s are phospholipid bound, allowing for direct access into brain.
- Fish oil provides high dosing of omega-3s, which is important for general health, including that of heart, skin & hair.
- Krill oil also includes astaxanthin, a powerful antioxidant.
- Phosphatidylserine is available from sunflower seed oil; one product combines this with fish+krill.

Choose a krill-containing product for direct brain uptake.

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LEARNING OBJECTIVES

Learn that:

1. ***Diagnosis:*** Whole genome sequencing (WGS) can provide precise diagnostic information for most people with chronic functional symptomatology such as pain, fatigue, and nausea/vomiting; also, autism.
   a. The majority of precise diagnoses are NOT on the laboratory report and require additional review by an expert.
   b. Trio sequencing (including parents) is needed for neurodevelopmental disorders such as autism to find *de novo* mutations.
2. ***Treatment:*** Many of the known pathways in which genetic variants predispose towards autism, cyclic vomiting, & ME/CFS are treatable.
   a. WGS can provide individualized treatment options for most people.
   b. Cation transport & energy metabolism are the main common pathways.
   c. There are many good treatments for both cation transport & energy metabolism disorders, most of which are dietary supplements.
   d. Combination products are available that cover all of the main treatable pathways.

Modern genomics is a powerful tool to treat disease!

“TAKE-HOME” MESSAGE

- There are known common pathways underlying functional and neurodevelopmental disorders.
  - Cation transport and energy metabolism are primary pathways.
    - These two pathways are highly interrelated.
  - Both of these, and other important pathways (neurotransmitters, amino acids & neuroinflammation), are treatable!
  - Treatment is predominately dietary.
- The above knowledge on dietary supplementation can be used to help tailor therapies for people of all ages with these common disorders.
  - The above applies whether or not modern genetic testing was done or can/will be done.
  - Discuss genetic testing in severe, problematic, or refractory cases.

Come talk to us at our booth.

FOR MORE INFORMATION

drboles@molecularmito.com

To learn the options and/or to make an appointment:
lvaheydrbolesc@gmail.com

For NeuroNeeds products:
https://www.neuroneeds.com

Thank You, *Richard G. Boles, M.D.*