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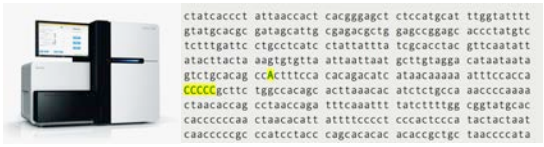
Genetics and Autism



Richard G. Boles, M.D.
Chief Medical & Scientific Officer
NeuroNeeds LLC
Director, CNNH NeuroGenomics Program
National Autism Conference, Great Valley, PA
May 17, 2019

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Disclosure:
Dr. Boles wears many hats

- Clinician treating patients
  - Primary interests in functional disease (autism, cyclic vomiting)
  - Past: Geneticist/pediatrician 20 years at CHLA/USC
  - Present: Director, CNNH NeuroGenomics Program (https://cnnh.org) - press the "Genetics" button
  - Present: In private practice in California (http://molecularmitomd.com)
- Chief Medical & Scientific Officer of NeuroNeeds LLC
  - Present: The company that produces SpectrumNeeds® and QNeeds® (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 multiple companies
- Expert witness in legal cases
  - Present: Medical child abuse, child neglect and custody cases (drboles@molecularmito.com)
  - Vaccine Court, malpractice cases
- Researcher with prior NIH and foundation funding
  - Past: USC faculty for 20 years
  - Present: Study sequence variation that predispose towards neurodevelopmental and functional disorders

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Disclosure & Off-label Indications

In personalized medicine, most treatments are "off label". None of the statements in this talk have been reviewed by the FDA.

A general overview of genetic testing in autism is provided in this talk. Dr. Boles does NOT have a conflict of interest to disclose in terms of this talk.

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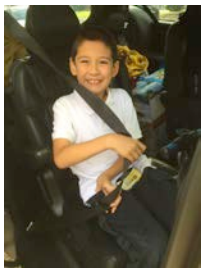
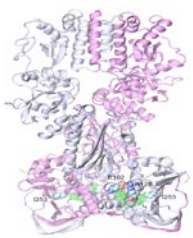
Case Study: (Relatively-Uncomplicated) Autism
Carter, *TRAP1*





- Carter presented at age 1 year following the MMR vaccination, after which he stopped developing speech for the next year.
- He was later diagnosed with autism.
- Biochemical laboratory testing has suggested the possibility of a mitochondrial disorder.
- DNA sequencing revealed the *TRAP1* p.Ile253Val variant.
- *TRAP1* p.Ile253Val is a variant present in about 1% of the general population.
- This gene encodes for a chaperone that protects mitochondrial proteins from oxidative stress.
- Certain variants in this gene are associated with chronic pain, fatigue, and GI symptoms.
- Autism is present in many of the boys with TRAP1-related disease, and this association is statistically significant.

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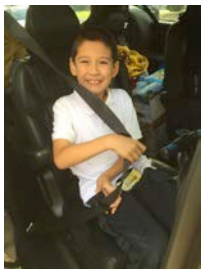
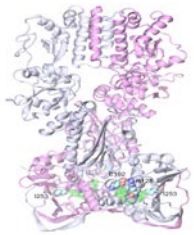
Case Study: (Relatively-Uncomplicated) Autism
Carter, *TRAP1*





- Kytril was predicted by computer modeling to tightly bind in the mutant 253-valine ATP-binding pocket, but not the wild-type 253-isoleucine pocket.
- Following informed consent, this drug was tried in Carter. The anecdotal results were clear and dose-dependent, in that on the drug Carter was:
  - more talkative, expresses himself better
  - more clever
  - more focused*
  - less aggressive*
  - less sensory integration issues*
  - getting along better with his older brother
  - more tolerate of previously-difficult situations

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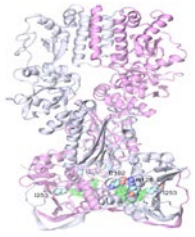
Case Study: Neurotypical
Payton, *TRAP1*

- Cyclic vomiting syndrome from ages 1-10
- Episodes had morphed into daily migraine.
- Chronic pain throughout her body.
- Chronic fatigue syndrome = chief complaint.
- Multiple admissions for bowel clean-outs.
- Excellent student
- Same variant as Carter: p.Ile253Val “common mutation” in the TRAP1 mitochondrial chaperone
- This variant was also found in many other patients with “functional” symptoms such as chronic pain, fatigue and GI dysmotility.
- Essentially all disease manifestations resolved on Kytril therapy.



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"Any sufficiently advanced technology is indistinguishable from magic."

Clarke's Third Law

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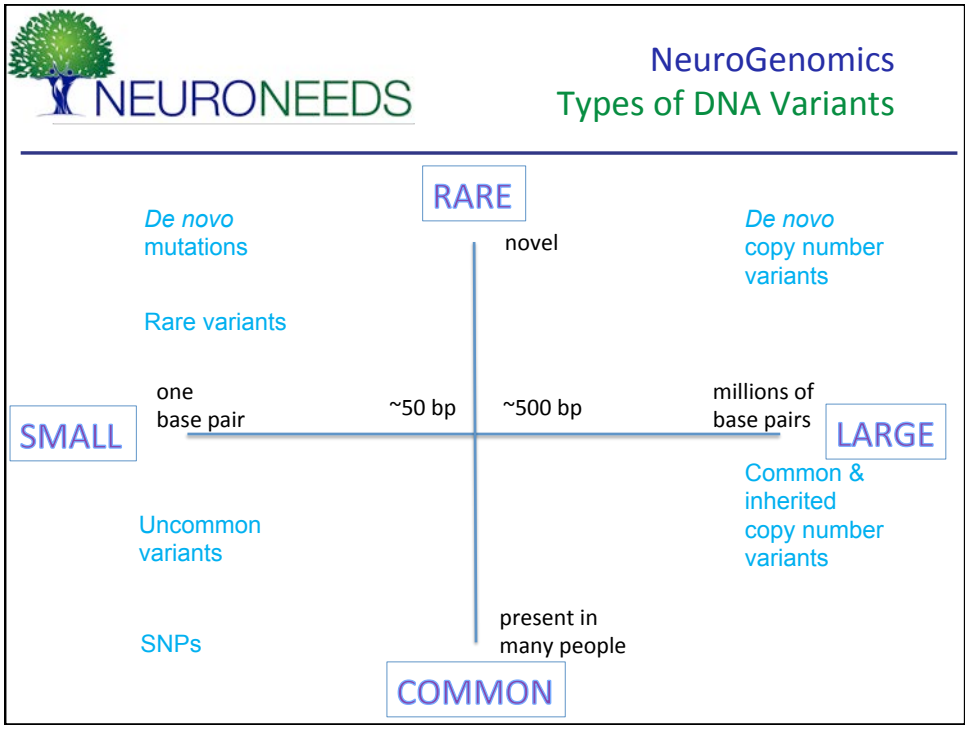
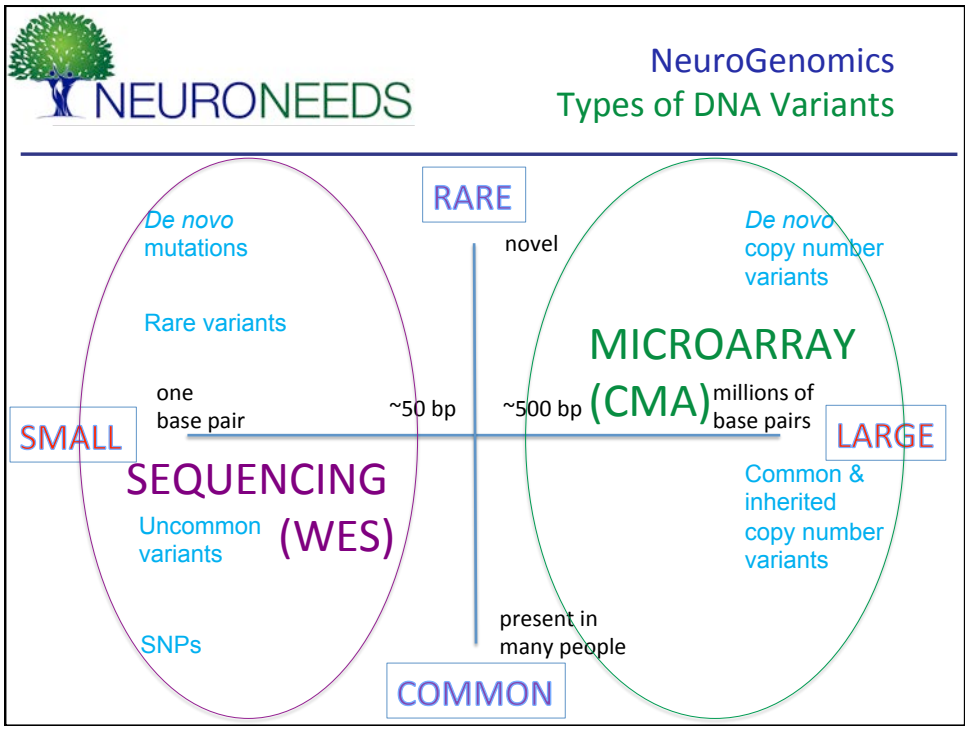
DNA Sequencing
Illumina HiSeq 4000


NEURONEEDS
NeuroGenomics
Types of DNA Variants
RARE
De novo mutations
novel
De novo copy number variants
Rare variants
one base pair
~50 bp
~500 bp
millions of base pairs
SMALL
LARGE
Common & inherited copy number variants
Uncommon variants
present in many people
SNPs
COMMON


NEURONEEDS
NeuroGenomics
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De novo mutations
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De novo copy number variants
Rare variants
MICROARRAY (CMA)
one base pair
~50 bp
~500 bp
millions of base pairs
SMALL
LARGE
SEQUENCING (WES)
Common & inherited copy number variants
Uncommon variants
present in many people
SNPs
COMMON

NEURONEEDS

Autism Genomics
Copy Number Variants (CNVs)

CNV	# studies
16p11.2	79
15q11.2	65
22q11.21	56
15q13.3	51
2p16.3	50
3q29	45
1q21.1	44
17q12	44
Xq28	43
22q13.33	42
7q11.23	41
17p11.2	38
17p13.3	37

- Size: Hundreds to millions of nucleotides
- Can be either *de novo* or inherited
- CNV are generally not detected on exome sequencing, but can be detected by:
  - Chromosomal microarray (CMA) technology.
  - Whole genome sequencing (WGS).

Typical signs of a CNV:
- Intellectual disability
- Other neurological defects
  - seizures
  - hypotonia
- Growth anomaly
- Birth defects
- Dysmorphic features

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NeuroGenomics
Copy Number Variants (CNVs)

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16p11.2	79
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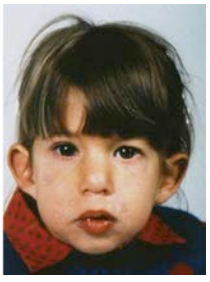
22q13.33	42
7q11.23	41
17p11.2	38
17p13.3	37

DiGeorge, velocardiofacial, Opitz G/BBB syndromes:
COMT for behavioral changes
TBX1 for birth defects, including cardiac



CNVs are commonly found in a normal, or near-normal, parent. They serve as risk factors for disease manifestations, including autism.
Many times, identification of the genetic variant can help guide additional studies and therapy.

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NeuroGenomics
Copy Number Variants (CNVs)

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Xq28	43
22q13.33	42
7q11.23	41
17p11.2	38
17p13.3	37

Angelman syndrome: *UBE3A*
MR/autism, absent speech, epilepsy, ataxia, bizarre laughing, hypopigmentation

Testing:
Requires imprinting testing to detect most cases, and sequencing for the remainder. Some cases can be identified by CMA.

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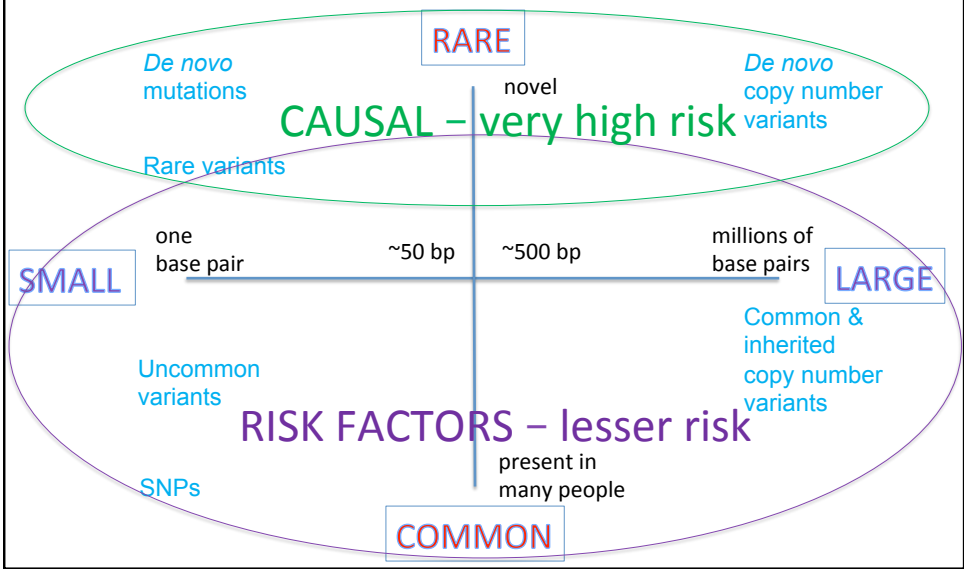
NeuroGenomics
Types of DNA Variants



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Genetic Models of Autism
De Novo Versus Risk Factor

- *De novo* variants:
  - New mutations not present in either parent.
  - Often are the main cause of disease.
  - Present in about one-third of autism patients.
  - Low inheritance risk in additional children, generally < 1%.
- Inherited variants:
  - Inherited from one parent, occasionally both.
  - Generally predispose towards disease in a polygenic manner.
  - Environmental components appear to be common.
  - Family members with these variants can -
    - be completely normal.
    - show *forme fruste* phenotypes.
    - have another neurodevelopmental disorder (e.g. ADHD).
    - have an autistic spectrum disorder.

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Case Study: Complicated Autism
Zach

- Autism, cyclic vomiting syndrome, bowel dysmotility, complex regional pain syndrome, headache, muscle pain, severe exercise intolerance, rhabdomyolysis
- Improvement on mitochondrial cocktail
- Later, genetic testing revealed underlying factors in illness that led to substantial improvements in expressive speech and autistic features.

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Case Study: Complicated Autism
Zach

- A variant likely associated with disease was identified in the CHAT gene.
- This gene encodes for the enzyme, choline O-acetyltransferase, that catalyzes the synthesis of acetylcholine from choline and acetyl-CoA in cholinergic neurons.
- Appears in polygenic disease, particularly with mitochondrial disease.
- Distinct manifestations are:
  - Episodic mental status changes w/o known triggers
  - POTS/dysautonomia
  - Severe reactions to anticholinergic medications
- Anecdotal, yet dramatic, improvement with anticholinesterase inhibitors donepezil (Aricept) and pyridostigmine (Mestinon).
  - Expressive speech improved dramatically on donepezil.

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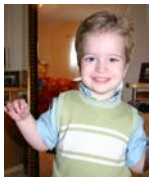
Case Study: Autism and Seizures
Luca



- Profound hypotonia and loss of language skills following a routine vaccination at age 9 months.
- Autism, seizures, severe GI dysmotility and chronic fatigue.
- All further vaccinations were withheld.
- Substantial improved on supplements.
- Later, DNA sequencing revealed a variant that is an excellent candidate for being disease-related:

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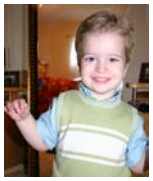
Case Study: Autism and Seizures
Luca



- Profound hypotonia and loss of language skills following a routine vaccination at age 9 months.
- Autism, seizures, severe GI dysmotility and chronic fatigue.
- All further vaccinations were withheld.
- Substantial improved on supplements.
- Later, DNA sequencing revealed a variant that is an excellent candidate for being disease-related:
  - Variants in the *SCN1A* gene are associated with epilepsy, autism, and vaccine-related injury.
- The family has also withheld all vaccinations in his brother, who is unaffected except for hearing loss.
  - The brother was found NOT to carry the SCN1A variant.

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Genetic Sequencing
From Genome to Single Gene

- Whole genome sequencing ("genome", "WGS")
  - Includes the 98% of the DNA that does not encode the exome, yet is poorly understood.
  - Used in research for years, and entering clinical practice as utility increases and costs decrease.
  - Often less expensive that ordering WES + mtDNA + CMA.
- Whole exome sequencing ("exome", "WES")
  - All of the protein coding sequences + splice sites
  - In clinical practice, only a relevant gene list is fully interpreted.
- Large panels
  - Panels of hundreds to thousands of genes that are comprehensive for a specific clinical indication (e.g. autism, epilepsy, mitochondria)
- Small panels
  - Less expensive panels that only include a minority of genes associated with a clinical indication, chosen for being well documented, easy to interpret, higher prevalence and/or driving clinical management.
  - Not well suited for some indications whereas large numbers of genes are indicated (e.g. autism)
  - Good for specific phenotypes caused by a small number of genes, such as Rett syndrome.
- Single gene sequencing
  - Only applicable for typical cases of well-recognized conditions caused by a single/few gene(s), such as Angelman syndrome.
- Repeat length
  - For specific conditions associated with expanded repeat sequences, such as fragile X syndrome.

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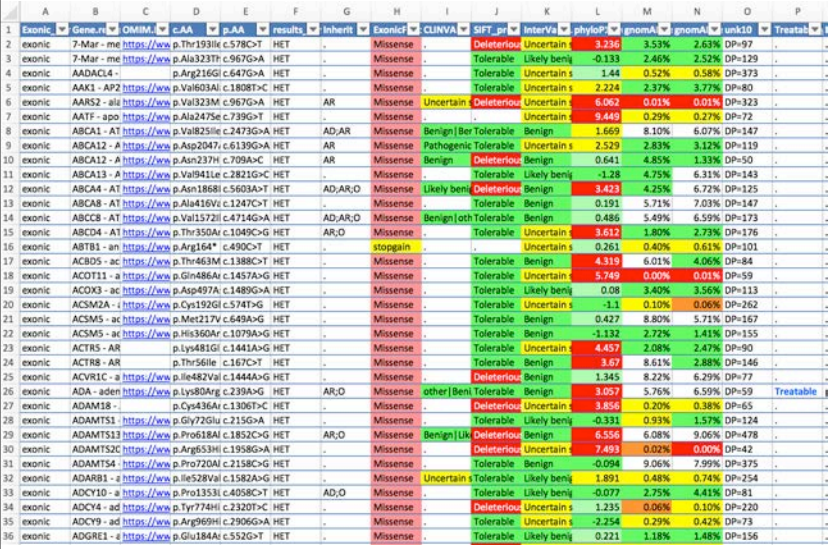
NeuroGenomics
Comprehensive Interpretation



Exonic_	Gene.re	OMIM_I	c.AA	p.AA	results_	Inherit	ExonicF	CLINVA	SIFT_pr	InterVa	phyloP	gnomAI	gnomAI	unk10	Treatab
exonic	7-Mar - me	https://ww	p.Thr193Ile	c.578C>T	HET	.	Missense	.	Deleterious	Uncertain s	3.236	3.53%	2.63%	DP=97	.
exonic	7-Mar - me	https://ww	p.Ala323Th	c.967G>A	HET	.	Missense	.	Tolerable	Likely beni	-0.133	2.46%	2.52%	DP=129	.
exonic	AADACL4 -		p.Arg216Gl	c.647G>A	HET	.	Missense	.	Tolerable	Uncertain s	1.44	0.52%	0.58%	DP=373	.
exonic	AAK1 - AP2	https://ww	p.Val603Al	c.1808T>C	HET	.	Missense	.	Tolerable	Uncertain s	2.224	2.37%	3.77%	DP=80	.
exonic	AARS2 - ala	https://ww	p.Val323M	c.967G>A	HET	AR	Missense	Uncertain s	Deleterious	Uncertain s	6.062	0.01%	0.01%	DP=323	.
exonic	AATF - apo	https://ww	p.Ala247Se	c.739G>T	HET	.	Missense	.	.	Uncertain s	9.449	0.29%	0.27%	DP=72	.
exonic	ABCA1 - AT	https://ww	p.Val825Ile	c.2473G>A	HET	AD;AR	Missense	Benign|Ber	Tolerable	Benign	1.669	8.10%	6.07%	DP=147	.
exonic	ABCA12 - A	https://ww	p.Asp2047	c.6139G>A	HET	AR	Missense	Pathogenic	Tolerable	Uncertain s	2.529	2.83%	3.12%	DP=119	.
exonic	ABCA12 - A	https://ww	p.Asn237H	c.709A>C	HET	AR	Missense	Benign	Deleterious	Benign	0.641	4.85%	1.33%	DP=50	.
exonic	ABCA13 - A	https://ww	p.Val941Le	c.2821G>C	HET	.	Missense	.	Tolerable	Likely beni	-1.28	4.75%	6.31%	DP=143	.
exonic	ABCA4 - AT	https://ww	p.Asn1868I	c.5603A>T	HET	AD;AR;O	Missense	Likely beni	Deleterious	Benign	3.423	4.25%	6.72%	DP=125	.
exonic	ABCA8 - AT	https://ww	p.Ala416Va	c.1247C>T	HET	.	Missense	.	Tolerable	Benign	0.191	5.71%	7.03%	DP=147	.
exonic	ABCC8 - AT	https://ww	p.Val1572I	c.4714G>A	HET	AD;AR;O	Missense	Benign|oth	Tolerable	Benign	0.486	5.49%	6.59%	DP=173	.
exonic	ABCD4 - AT	https://ww	p.Thr350Ar	c.1049C>G	HET	AR;O	Missense	.	Tolerable	Uncertain s	3.612	1.80%	2.73%	DP=176	.
exonic	ABTB1 - an	https://ww	p.Arg164*	c.490C>T	HET	.	stopgain	.	.	Uncertain s	0.261	0.40%	0.61%	DP=101	.
exonic	ACBD5 - ac	https://ww	p.Thr463M	c.1388C>T	HET	.	Missense	.	Tolerable	Benign	4.319	6.01%	4.06%	DP=84	.
exonic	ACOT11 - a	https://ww	p.Gln486Ar	c.1457A>G	HET	.	Missense	.	Tolerable	Uncertain s	5.749	0.00%	0.01%	DP=59	.
exonic	ACOX3 - ac	https://ww	p.Asp497A	c.1489G>A	HET	.	Missense	.	Tolerable	Likely beni	0.08	3.40%	3.56%	DP=113	.
exonic	ACSM2A - i	https://ww	p.Cys192Gl	c.574T>G	HET	.	Missense	.	Tolerable	Uncertain s	-1.1	0.10%	0.06%	DP=262	.
exonic	ACSM5 - ac	https://ww	p.Met217V	c.649A>G	HET	.	Missense	.	Tolerable	Benign	0.427	8.80%	5.71%	DP=167	.
exonic	ACSM5 - ac	https://ww	p.His360Ar	c.1079A>G	HET	.	Missense	.	Tolerable	Benign	-1.132	2.72%	1.41%	DP=155	.
exonic	ACTR5 - AR		p.Lys481Gl	c.1441A>G	HET	.	Missense	.	Tolerable	Uncertain s	4.457	2.08%	2.47%	DP=90	.
exonic	ACTR8 - AR		p.Thr56Ile	c.167C>T	HET	.	Missense	.	Tolerable	Benign	3.67	8.61%	2.88%	DP=146	.
exonic	ACVR1C - a	https://ww	p.Ile482Val	c.1444A>G	HET	.	Missense	.	Deleterious	Benign	1.345	8.22%	6.29%	DP=77	.
exonic	ADA - aden	https://ww	p.Lys80Arg	c.239A>G	HET	AR;O	Missense	other|Beni	Tolerable	Benign	3.057	5.76%	6.59%	DP=59	Treatable
exonic	ADAM18 -		p.Cys436Ar	c.1306T>C	HET	.	Missense	.	Deleterious	Uncertain s	3.856	0.20%	0.38%	DP=65	.
exonic	ADAMTS1	https://ww	p.Gly72Glu	c.215G>A	HET	.	Missense	.	Tolerable	Likely beni	-0.331	0.93%	1.57%	DP=124	.
exonic	ADAMTS13	https://ww	p.Pro618Al	c.1852C>G	HET	AR;O	Missense	Benign|Like	Deleterious	Benign	6.556	6.08%	9.06%	DP=478	.
exonic	ADAMTS2(	https://ww	p.Arg653Hi	c.1958G>A	HET	.	Missense	.	Deleterious	Uncertain s	7.493	0.02%	0.00%	DP=42	.
exonic	ADAMTS4 -	https://ww	p.Pro720Al	c.2158C>G	HET	.	Missense	.	Tolerable	Benign	-0.094	9.06%	7.99%	DP=375	.
exonic	ADARB1 - a	https://ww	p.Ile528Val	c.1582A>G	HET	.	Missense	Uncertain s	Tolerable	Likely beni	1.891	0.48%	0.74%	DP=254	.
exonic	ADCY10 - a	https://ww	p.Pro1353(	c.4058C>T	HET	AR;O	Missense	.	Tolerable	Likely beni	-0.077	2.75%	4.41%	DP=81	.
exonic	ADCY4 - ad	https://ww	p.Tyr774Hi	c.2320T>C	HET	.	Missense	.	Deleterious	Uncertain s	1.235	0.06%	0.10%	DP=220	.
exonic	ADCY9 - ad	https://ww	p.Arg969Hi	c.2906G>A	HET	.	Missense	.	Tolerable	Uncertain s	-2.254	0.29%	0.42%	DP=73	.
exonic	ADGRE1 - a	https://ww	p.Glu184A(	c.552G>T	HET	.	Missense	.	Tolerable	Likely beni	0.221	1.18%	1.48%	DP=156	.

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NeuroGenomics
Comprehensive Interpretation

Call	Gene	OMIM	p.	c.	Zygo	Inherit	Type	ClinVar	SIFT	InterVar	PhyloP-vert	gnomAD-ALL	gnomAD-NFE	DP	Pathway	Disease
PossModif	AARS2 - alan	https://	p.Val323Met	c.967G>A	HET	AR	Missense	Uncertain sign	Deleterious	Uncertain sign	6.062	0.01%	0.01%	DP=323	mitochondria	Combined Oxidative P
PossModif	AFAP1 - actir	https://	p.Ser664Leu	c.1991C>	HET	.	Missense	.	Deleterious	Uncertain sign	8.849	0.09%	0.14%	DP=287	cytoskeleton	not in OMIM
PossModif	ALX1 - ALX h	https://	p.Arg64Leu	c.191G>T	HET	AR	Missense	Likely benign	Deleterious	Uncertain sign	3.818	0.43%	0.69%	DP=76	homeoprotein	Frontonasal dysplasia
PossModif	ARFGEF3 - Al	https://	p.Ser1631Gly	c.4891A>	HET	.	Missense	.	Tolerable	Uncertain sign	6.848	0.00%	0.00%	DP=63	estrogen	not in OMIM
PossModif	ARHGEF10 -	https://	p.Gly1094Ser	c.3280G>	HET	AD	Missense	.	Deleterious	Uncertain sign	8.766	0.00%	0.00%	DP=217	myelination	slowed nerve conducti
Incidental	BMPR1A - bc	https://	p.Val450Met	c.1348G>	HET	AD	Missense	Uncertain sign	Tolerable	Uncertain sign	6.748	0.01%	0.00%	DP=282	morphogenesis	Polyposis syndrome
PossModif	CAMSAP1 - c	https://	p.Ser115Phe	c.344C>T	HET	.	Missense	.	Deleterious	Uncertain sign	7.744	0.07%	0.14%	DP=248	cytoskeleton	not in OMIM
PossModif	CD300E - CD	https://	p.Arg153*	c.457C>T	HET	.	stopgain	.	.	Uncertain sign	0.454	0.02%	0.03%	DP=100	immunity?	not in OMIM
PossModif	CEP152 - cen	https://	p.Ile394Val	c.1180A>	HET	AR	Missense	Uncertain sign	Tolerable	Uncertain sign	-0.257	0.24%	0.34%	DP=61	centromere	Microcephaly 9, prima
PossModif	CERCAM - ce	https://	p.Gln187Glu	c.559C>G	HET	.	Missense	.	Tolerable	Uncertain sign	6.123	0.06%	0.11%	DP=169	Immunity?	not in OMIM
PossModif	CIT - citron rl	https://	p.Arg1172His	c.3515G>	HET	AR	Missense	.	Deleterious	Uncertain sign	7.905	0.01%	0.02%	DP=126	kinase	Microcephaly 17, prim
PossTxModif	CNR1 - canni	https://	p.Thr467Met	c.1400C>	HET	.	Missense	.	Deleterious	Uncertain sign	9.597	0.00%	0.00%	DP=73	cannabinoid receptor	pain, sleep, memory a
Incidental	CYP2C9 - cyt	https://	p.Ile359Leu	c.1075A>	HET	AD	Missense	drug response	Deleterious	Benign	0.278	6.36%	6.86%	DP=167	drug metabolism	poor metabolizer warf
Poten AR dz	CTBP2 - C-ter	https://	p.Arg169Cys	c.505C>T	HET	.	Missense	.	Deleterious	Uncertain sign	4.3	0.00%	0.00%	DP=281	transcription	not in OMIM
	CTBP2 - C-ter	https://	p.Ala175Val	c.524C>T	HET	.	Missense	.	Tolerable	Uncertain sign	9.581	0.00%	0.00%	DP=287		
PossModif	EIF2B3 - euk	https://	p.Pro24Leu	c.71C>T	HET	AR	Missense	.	Deleterious	Uncertain sign	9.509	0.02%	0.03%	DP=105	translation	Leukoencephalopathy
PossModif	FBXO28 - F-b	https://	p.Leu58Val	c.172C>G	HET	.	Missense	.	Tolerable	Uncertain sign	3.659	0.01%	0.01%	DP=443	ubiquitination	not in OMIM
PossTxModif	FECH - ferroc	https://	p.Gly55Cys	c.163G>T	HOMO	AD;AR	Missense	other	Tolerable	Likely benign	0.874	2.22%	1.84%	DP=219	heme metabolism	erythropoietic protopo
Incidental	GGCX - gamn	https://	p.Gly576Ala	c.1727G>	HET	AR;O	Missense	.	Deleterious	Uncertain sign	5.468	0.00%	0.00%	DP=135	blood clotting	Pseudoxanthoma elast
	GGCX - gamn	https://	p.Arg359Cys	c.1075C>	HET	AR;O	Missense	.	Deleterious	Uncertain sign	5.467	0.00%	0.00%	DP=143		
PossModif	GLI53 - GLI5	https://	p.Gly158Ala	c.473G>C	HET	AR	Missense	other	Deleterious	Likely benign	5.02	0.74%	0.91%	DP=179	transcription	Diabetes mellitus, neo
Incidental	HS6ST1 - hep	https://	p.Arg382Trp	c.1144C>	HET	AD	Missense	Likely pathoge	Deleterious	Uncertain sign	5.539	0.19%	0.05%	DP=160	heparan sulfate	(Hypogonadotropic hy
PossModif	IGHMBP2 - ir	https://	p.Gln51Glu	c.151C>G	HET	AR;O	Missense	other|Uncerta	Tolerable	Uncertain sign	3.853	0.61%	0.83%	DP=179	transcription	Charcot-Marie-Tooth D
PossModif	KLC3 - kinesi	https://	p.Leu402Pro	c.1205T>	HET	.	Missense	.	Deleterious	Uncertain sign	6.146	0.01%	0.03%	DP=83	cell transport	not in OMIM
PossTxModif	MEFV - MEFV	https://	p.Arg408Gln	c.1223G>	HET	AD;AR	Missense	other|other|(	Tolerable	Benign	-0.571	1.32%	0.82%	DP=72	proinflammatory	Familial Mediterranea
	MEFV - MEFV	https://	p.Pro369Ser	c.1105C>	HET	AD;AR	Missense	other|other	Deleterious	Benign	1.688	1.45%	0.82%	DP=252	.	
Incidental	MPL - MPL p	https://	p.Arg426Gln	c.1277G>	HET	AD;AR;O	Missense	.	Deleterious	Uncertain sign	3.748	0.00%	0.00%	DP=189	hematopoietic growth factor	AD: Thrombocythemia
Incidental	MXI1 - MAX	https://	p.Arg157*	c.469C>T	HET	AD	stopgain	.	.	Likely pathoge	4.975	0.00%	0.00%	DP=269	transcription	Neurofibrosarcoma; (P
Incidental	MYO6 - myo	https://	p.Thr845Ile	c.2534C>	HET	AD;AR	Missense	other	Deleterious	Likely benign	3.819	0.26%	0.46%	DP=148	Vesicle & organelle transport	AD: Deafness, autoson
PossModif	NIN - ninein	https://	p.Arg429Gln	c.1286G>	HET	AR	Missense	.	Deleterious	Uncertain sign	2.82	0.01%	0.01%	DP=104	centromere	?Seckel syndrome 7
PossTxModif	OGDH - oxog	https://	p.Pro98Leu	c.293C>T	HET	AR	Missense	.	Deleterious	Uncertain sign	7.524	0.00%	0.01%	DP=206	mitochondria	Alpha-ketoglutarate de
PossModif	OTUD7B - OT	https://	p.Ala23Val	c.68C>T	HET	.	Missense	.	Deleterious	Uncertain sign	9.397	0.01%	0.01%	DP=88	ubiquitination	not in OMIM
PossTxModif	PAH - phenyl	https://	p.His100Arg	c.299A>C	HET	AR	Missense	Uncertain sign	Tolerable	Uncertain sign	1.73	0.03%	0.05%	DP=161	neurotransmission	Phenylketonuria
PossModif	PCDHGB1 - p	https://	p.Asn552Ser	c.1655A>	HET	.	Missense	.	Deleterious	Uncertain sign	8.984	0.00%	0.00%	DP=199	cell-cell adhesion	not in OMIM
PossModif	PHF21A - PH	https://	p.Thr604Ile	c.1811C>	HET	.	Missense	.	Deleterious	Uncertain sign	5.503	0.02%	0.03%	DP=167	transcription	not in OMIM

NEURONEEDS

How do I get the advantages of genetic testing for my child? What tests to order?

Mutant baby chinchillas 8-days old (x2) or 4-hours old

NEURONEEDS

Genetic Testing Costs

- Prices have dropped drastically for sequencing:
  - Self-pay exome prices are as low as $1,000.
  - mtDNA and CMA prices are as low as $500 each.
  - Self-pay genome prices are as low as $1,800.
- Many laboratories will take insurance:
  - Look for labs that will run insurance, but have a low-cost self-pay option if insurance fails.
  - Insurance coverage is more likely with:
    - a Letter of Necessity.
    - appropriate ICD10 codes
- Interpretation is the most difficult step:
  - Consider the assistance of a clinical genomicist.

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NEURONEEDS

Neurodevelopmental Disorders Testing Recommendations

- Recommendations for Testing:
  - Whole exome sequencing ("exome", "WES")
    - Test the parents (triome) versus only the patient (singleton)
  - CNV testing by chromosomal microarray (CMA) or sequencing equivalent
  - Mitochondrial DNA (mtDNA) sequencing and CNV testing
  - Fragile X syndrome repeat testing
  - Pharmacogenetic (PGx) testing
- Whole genome sequencing:
  - Covers all of the above
  - Trio versus singleton
- Angelman syndrome testing by methylation in select cases
- EXPERT INTERPRETATION

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NEURONEEDS

Neurodevelopmental Disorders Testing Recommendations

Should you test only the patient (singleton), or the parents as well (trio)?

- Advantages of trio testing:
  - *De novo* variants
  - Phasing for recessive variants
  - Following the condition within the family
- Disadvantages of trio testing:
  - Double the price
  - Logistics of obtaining samples from both parents
- Recommendation:
  - Trio for severe cases of neurodevelopmental disorders (ASD, ID, epilepsy) and/or birth defects

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NEURONEEDS

Neurodevelopmental Disorders Testing Recommendations

Only your physician can determine what test is right for your child and order testing.

However, in most cases with autism, trio whole genome sequencing (WGS) is the correct test, in my opinion.

Who will interpret that test is the main question.

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CNNH
The Center for Neurological and Neurodevelopmental Health®

Logistics – Who Can Read My DNA?
CNNH NeuroGenomics Program

- Peer-to-Peer Counselling Service:
  - Genetic testing is extremely complicated, and very few physicians can fully understand the information provided or how to apply that information to improve treatment outcomes.
    - The purpose of this service is to provide this information to treating physicians.
  - This Service includes a telemedicine conversation between the physician and Dr. Boles.
    - The family can listen in and ask questions if physically present in a state whereas Dr. Boles holds a medical license: Arizona, California, Florida, New Jersey, Pennsylvania, and South Carolina.
  - Different levels of the Service
    - Standard: Review up to 4 reports, including exome (WES) or genome (WGS).
    - Comprehensive: Complete reevaluation and reinterpretation from raw sequence data.
- Telemedicine Genetics Consultations:
  - Provides full evaluations as done in his private practice in California, except by telemedicine.
  - The patient physically must be present in a state whereas Dr. Boles holds a medical license: Arizona, California, Florida, New Jersey, Pennsylvania, and South Carolina.
- For more information:
  - Laurie Ebenau, NeuroGenomics Program Coordinator
  - Phone: (855) 852-8150 x2005; fax: (855) 266-6180
  - neurogenomics@cnnh.org
  - https://cnnh.org (press the “Genetics” button)

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CNNH
The Center for Neurological and Neurodevelopmental Health®

Logistics – Who Can Read My DNA?
CNNH NeuroGenomics Program

- Mintz et al.: *"Peer-to-Peer": A program to Connect a Clinical Genomicist and the Treating Neurologist to Correlate Genotype with Phenotype"*; AAN 2019.
  - The Program's coverage to date:
    - 17 patients
    - 8 physicians in 7 states and 3 nations
    - 6 different genetic labs.
  - Results-to-date on re-analysis of raw sequence files:
    - 94% (16/17): altered physicians' management
    - 52% (9/17): Received a pathogenic genetic diagnosis
    - 88% (15/17): Recommended changes in therapy
    - 41% (7/17): Indications for additional testing
    - 23% (4/17): Indications for additional outside consultations

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NEURONEEDS

Genetic Testing in NDD/Autism
My Most Recent 7 Patients 3/19

Age/ Sex	Phenotype	Genotype	Intervention
13M	Autism, absent speech, hypotonia, GI symptoms, immune dysfunction	*De novo* CHD1 GRIN1, TRAP1	NMDA antagonists Mito-cocktail
16M	High fxn autism, chronic pain and fatigue, cyclic vomiting	Lg heteroplasmic mtDNA deletion; CACNA1S missense	Mito-cocktail, acetazolamide
15M	Autism, OCD, autonomic dysfxn, episodes of rage	Frameshift in X-linked GRIA3	Venlafaxine – acts on AMPA receptor
8M	ADHD, stereotypical episodes of severe leg pain	Stop gain in SCN4A VUS: mtDNA, SCN10A	Mito-cocktail, acetazolamide
16M	Autism, autonomic over-activity, anxiety, constipation, migraine	*De novo* 84-kb deletion 8p23.1 USP17L2, USP17L17 mtDNA for migraine	Mito-cocktail
23M	Autism, epilepsy, dyspraxia, OCD, anxiety, hypotonia	Deletion of 67% of ARHGEF9 (collybistin, X-linked)	GABA and glycine agonists
23M	Changed personality to progressive psychotic behaviors, with prominent and multiple delusions	7.4 Mb duplication 5p15.2-14.3 *(31 genes, 3 genes in ubiquitination)*	Mito-cocktail Mg, Zn

NEURONEEDS

Genetic Testing in Autism
Summary

How Do Results Improve Patient Care?

TREATMENT

1. Right Therapy: Detect uncommon etiologies/factors that are best treated without drugs.
2. Right Drug: Help choose among potential drug therapies based on mechanism.
3. Right Dose: Help choose the correct dose based on drug metabolite report.

COUNSELING

A name, anticipatory guidance, prognosis, recurrence risks

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NEURONEEDS

Like the Fates of Ancient Greece,
Is Our Destiny Written in Our DNA?

- Genetics determines risk, not outcome!
- Genes are regulated by the environment.
- *Metaphor – one hand in a card game:*
  - Genetic testing is the hand one is dealt.
  - Environmental factors are the hands of the other players.
  - Genetic testing is the careful reading of the cards in your hand.
  - The outcome of the hand depends on the cards we are dealt, the cards of our opponents, and HOW WE PLAY OUR HAND.

Play your hand to the fullest potential, know the cards in your hand!

Get genetic testing.

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Resources: For More Information

NeuroNeeds®
https://www.neuroneeds.com
For more information on nutritional therapy for neurodevelopmental and functional disorders.

CNNH NeuroGenomics Program
https://cnnh.org (click "Genetics" button)
neurogenomics@cnnh.org
For more information on the Peer-To-Peer Program to help you to understand and utilize genetic testing results.

Molecular and Mitochondrial Medicine
http://molecularmitomd.com ("DNA Testing" menu)
For more information on Dr. Boles' practice and on DNA testing.

Thank You,
Richard Boles, M.D.