Time to *CHAT* with Your Patients? Can a Mutation in Choline-O-Acetyltransferase Exacerbate Mitochondrial Disease?

Wong, S [1], Sheldon, K [2], Boles, R [1,2] [1]*Children's Hospital Los Angeles, Los Angeles, CA,* [2]*Courtagen Life Sciences, Inc., Woburn, MA*

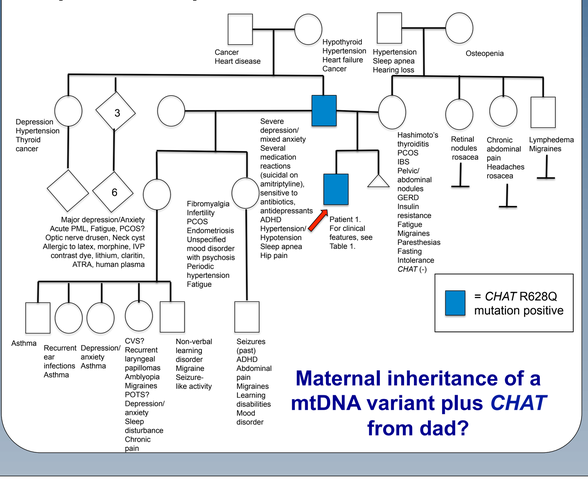
Introduction

- Mitochondrial disease/dysfunction manifests as heterogeneous symptoms that are variable in severity and presentation among members of the same family.
- In particular, autonomic dysfunction and adverse response to medications are common presentations in mitochondrial disease.
- While some family members may have a relatively mild presentation, it is common that one individual, usually the proband, manifests with more severe disease.
- Although heteroplasmic mutations of mitochondrial DNA may explain this phenomenon, in some individuals there is no identified heteroplasmy that can account for this variability.
- It has been hypothesized that modifying mutations are present in the nuclear DNA that may exacerbate the severity and clinical presentation of certain individuals. Thus, we sequenced the 1,092 mito-exome in 9 mito-probands.

Methods

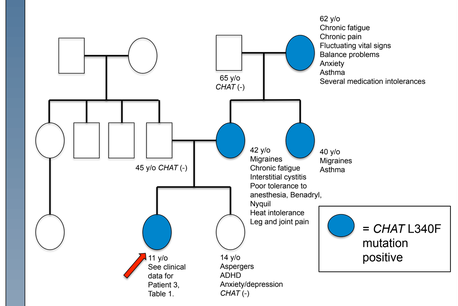
- Sequencing was recommended in patients with clinical diagnoses of mitochondrial disease/ dysfunction who were evaluated at the Children's Hospital Los Angeles Medical Genetics Clinic by a single clinician while seen in clinic over a 6-month period.
- The family histories of these patients were evaluated for matrilineal inheritance as determined by PMID 18192313.
- The mitochondrial exome (1,092 nuclear-encoded proteins) was sequenced in the most-severely affected family member, which was always the proband, using Next Generation sequence methodology (nucSEEK™, available from Courtagen Life Sciences) in 9 patients who had maternally inherited pedigrees. Mitochondrial DNA sequencing had previously been performed in these 9 patients by commercially-available assays. For further details on the Courtagen assay, see poster #0856.
- Patient 1 was studied on 4 different dates before and after donepezil HCL (Aricept treatment). A Vivometrics LifeShirt was worn during 5 minutes of supine rest on four different occasions. Pre-ejection period of the heart (sympathetic marker) and respiratory sinus arrhythmia (parasympathetic marker) were calculated from signals obtained from the thorocardiography (using ventricular volume curves) and inductance plethysmography transducers/bands (50Hz sampling rate), and ECG (200Hz sampling rate) of the Lifeshirt (PMID 14531161). R-waves were sampled at 1KHz; calculations of RSA used the peak to trough/valley method (44). All data were analyzed in Vivologic softwere, version 3.1.

Figure 1. Family pedigree of Patient 1. Analysis of the *CHAT* gene has only been completed on the patient and parents.



Maternal inheritance of a mtDNA variant plus *CHAT* from dad?

Figure 2. *CHAT* mutation segregation in the pedigree of Patient 3.



An example of pseudo-maternal inheritance?

Table 1. Clinical and laboratory data in 9 probands evaluated with matrilineal inheritance pedigrees and mitochondrial dysfunction. Note that the *CHAT* cases (orange background) are indistinguishable from the others except by the reactions to medications, and possibly more autonomic dysfunction.

Patient Data	Gastrointestinal symptoms	Chronic pain	Constitutional/ Immunological	Autonomic dysfunction	Neurological/Muscular//Sleep	Cognitive/Behavioral/Psychiatric	Unusual Medication Reactions	Biochemical and Tissue Laboratory Data	Mitochondrial DNA sequencing	Mitochondrial nuclear DNA sequencing
Patient 1: 13-year-old male ***CHAT***	Cyclic vomiting, abdominal migraine, GERD, pseudo-obstruction. TPN	Migraine headaches muscle pain with exertion, cramps, allodynia	Chronic fatigue, decompensation episodes	Night sweats, profuse nasal drainage, urinary incontinence, pallor, heat/cold intolerance, postural orthostatic tachycardia syndrome	Mixed seizures, Absence seizures with car rides, stroke-like episodes, Hyper-reflexive movements with sleep, gross and fine motor delay, mild hypotonia, ptosis, exotropia, tremors	Episodic encephalopathy (decreased cognitive abilities with increased emotionality), history of cognitive regression, ADHD-like/ autistic behaviors, depression, phobias, anxiety	Keppra: unusual behaviors, chewing on clothes; amitriptyline (on dose >75 mg): inappropriate urination, suicidal gestures; Prilosec: nausea and facial swelling; Trileptal: irritability, psychotic breaks/odd behaviors, possible drop seizures; Tegretol: irritability, losing cognition, gait abnormalities, overall lassitude; Wellbutrin: symptoms like CNS depression, irritability; Benadryl: slowing respiration, worsened nausea, lethargy; atenolol with Florinef: POTS symptoms worsened.	Anion-gap metabolic acidosis Low plasma free carnitine	9070T>G (Ser182Ala) ATP6 gene	*CHAT* R628Q, *TRAP1*
Patient 2: 19-year-old male ***CHAT***	Cyclic vomiting, pseudo-obstruction, GERD, G-tube, cecostomy tube	Complex regional pain syndrome, migraines	Chronic fatigue	Heat/cold intolerance, photophobia, difficulty regulating temperature, changes in limb color and temperature, severe night sweats, hyperventilation, frequent urination	Rhabdomyolysis, sleep disorder (only sleeps in short blocks), clumsiness, seizures, freezing episodes	Autism, developmental disability, tics, aggressive behavior	Amoxicillin: tongue swelling; Moban: eyes rolled upwards, head and neck back and upper body tense; Clonipine: freak out, aggressiveness, then crash. All meds have caused slowed respiration, unable to stand upright, blue lips, and almost loss of consciousness. Abilify, Celexa: shaking episodes.	Frequent ketosis, Anion-gap metabolic acidosis, Generalized increases in multiple urine organic acids, Generalized increases in multiple plasma acylcarnitines, Low plasma free carnitine	2257C>T in 16S rRNA gene, homoplasmy 2280 C>T in 16S rRNA gene, homoplasmy	*CHAT* L340F, *CBR4*
Patient 3: 11-year-old female ***CHAT***	GERD, chronic abdominal pain, gastroparesis, IBS, pseudo-obstruction	Migraine headaches, back, palms, arches, joints, leg pain, complex regional pain syndrome, allodynia	Chronic fatigue, recurrent paronychia, prolonged healing with wounds, frequent infections	Swallowing difficulties, abnormal sweating with blotchiness, temperature instability, UTI-like symptoms, Raynaud's, postural orthostatic tachycardia syndrome, dry eyes/blurriness	Absence seizures, Insomnia, limb movement sleep disorder,	Forgetfulness, confusion, sometimes difficulty concentrating, phobias, anxiety, change in personality when ill (aloofness, irritability).	Prednisone: violent vomiting; Ativan: strange facial expression, paranoia and hallucination, distraught and agitated behavior; Erythromycin: mood instability, GI pain, headache, and joint pain.	Frequent ketosis Minimal testing done	6253T>C in the COI gene (M>T), D-loop, HV2 T>C at position 55, 80% heteroplasmy, D-loop, HV2 57T>G, 80% heteroplasmy	*CHAT* L340F
Patient 4: 16-year-old female	Cyclic vomiting syndrome, GERD, abdominal pain	Migraine headaches, parathesias, chest pain, muscle cramps	Chronic fatigue	Difficulty urinating, stroke-like episode	Staring spells, strabismus	Depression	None reported	Frequent ketosis, Anion-gap metabolic acidosis, Generalized increases in multiple plasma acylcarnitines	11253T>C in ND4 gene, homoplasmy	*PLGRKT*
Patient 5: 13-year-old female	Cyclic vomiting syndrome, GERD, gastroparesis, pseudo-obstruction	Migraine headaches	Chronic fatigue syndrome, juvenile rheumatoid arthritis	Palpitations, syncope, photophobia	Insomnia	None reported	None reported	Frequent ketosis Anion-gap metabolic acidosis Low plasma free carnitine	mtDNA sequencing negative	*TRAP1, NOA1*
Patient 6: 9-year-old male	GERD, severe dysmotility, GJ tube, severe fasting intolerance, hypoglycemia s/p TPN	Migraine headaches, parathesias	Chronic fatigue	Skin mottling and increased heart rate	Episodes of abnormal movements, insomnia	Anxiety, speech delay, episodic decreased speech, autistic-like behavior, facial recognition difficulties	None reported	Frequent ketosis, Anion-gap metabolic acidosis, Generalized increases in multiple urine organic acids, Muscle biopsy with abnormal ultrastructure, including mitochondrial pleomorphia, and accumulation/proliferation; zero activity for NADH cytochrome C reductase with normal citrate synthase.	5686A>T tRNA-N gene, homoplasmy	*ATAD3A, WFS1*
Patient 7: 11-year-old female	None reported	Back pain	Chronic fatigue	None reported	Ataxic hemiparesis, cerebral palsy, decreased motor strength, strabismus	Asperger-like, ADHD	Strattera: caused manic-like episode; Supplement containing B2, Mg, Taurine, and N-acetyl cysteine: caused tics.	Elevated serum lactate Borderline low Coenzyme Q10	1888 G>A in 16S rRNA 26% heteroplasmy, 1751 A>G tRNA-ASP homoplasmy, 3820C>A L172I ND1 16% heteroplasmy	*TRAP1, ACLY*
Patient 8: 12-year-old male	Pseudo-obstruction, abdominal pain, G-tube, C-tube, intolerant to mammalian and poultry products	Migraine headaches, parathesias	Chronic fatigue, Chronic variable immune deficiency, PANDAS	High pain tolerance, decreased temperature sensation, knuckle swelling, temperature instability, stroke-like episodes	Staring spells, muscle weakness, ataxia, eye muscle weakness, difficulty writing	Obsessive-compulsive disorder, autistic spectrum	None reported	Generalized increases in multiple urine organic acids, Generalized increases in multiple plasma acylcarnitines, Low plasma free carnitine	Homoplasmy 9621G>A (p.A139T in the COIII gene).	*SLC25A42, ABCC9*
Patient 9: 18-year-old female	Dysmotility, gastroparesis	Migraine headaches, whole body, leg, and chest chronic pain	Chronic fatigue	Irregular heart rate, skipped beats, tachycardia, daily periodic fever	Insomnia, Muscle spasms, twitching, giving out	Episodes of confusion, now unable to drive	Amitriptyline: suicide ideation.	Low plasma free carnitine Minimal testing done	Polymorphism in control region at 73, 195, 499; 4793 A>G 2.5% heteroplasmy	*TRAP 1, MARC1, MTO1*

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Want to *CHAT*?

Have a patient that fits the phenotype that you would like tested?

Contact Richard.boles@Courtagen.com

Physiological Data

- The **respiratory sinus arrhythmia (RSA)**, an indirect measurement of parasympathetic activity derived from R-R heart-rate variability, is substantially below age-matched norms, but increased a full standard deviation on 20 mg donepezil HCl (Aricept), an anticholinesterase inhibitor (P = 0.015).
- The **pre-ejection period (PEP)** is an indirect measure of sympathetic activity and a lower PEP is indicative of higher sympathetic tone. The PEP was normal, but increased above the normal range on donepezil HCl (Aricept) as expected.

Table 2. Respiratory sinus arrhythmia (RSA) and pre-ejection period (PEP) measurements of Patient 1 with *CHAT* mutation R628Q before and after treatment with donepezil

	2009 (before treatment initiated)	2011 (before treatment initiated)	2013 on 10 mg donepezil HCl (Aricept)	2013 on 20 mg donepezil HCl (Aricept)	Age-matched comparison group
RSA (ln[ms2])	2.41 (-4.5 SD)	2.29 (-4.6 SD)	3.13 (-3.8 SD)	3.31 (-3.6 SD)	7.1 ± 1.04
PEP (ms)	95 (-1.0 SD)	100 (-0.5 SD)	154 (+5.5 SD)	131 (+3.0 SD)	105.1 ± 10.3

References: PMID 15718639, 15133982, 15133978

The *CHAT* Box

The data behind the case of clinical relevance

CHAT encodes for choline-O-acetyltransferase, the enzyme that synthesizes acetylcholine. It is of special importance in the parasympathetic nervous system, acting as both the pre-synaptic and post-synaptic neurotransmitter.

Why variants 340L>F and 510R>Q in the CHAT gene are highly likely to be associated with clinical symptoms:

1. **Prevalence:** ***Uncommon*** - 0.51% and 0.11% of the general population, respectively.
2. **Evolutionary conservation:** ***Highly conserved*** - 38/38 and 41/42 vertebrae species to lamprey on the UCSC Genome Browser.
3. **Computer Algorithms:** ***Predicted deleterious to protein function*** – by MutationTaster, PolyPhen2, and SIFT.
4. **Phenotype:** ***Distinct in the 3 patients*** - substantial reactions to drugs with known anti-cholinergic effects, in addition to "typical" mitochondrial disease manifestations.
5. **Treatment Response:** ***Dramatic*** - in 2 patients treated with anticholinergic esterase inhibitor donepezil (Aricept), including complete reversal of previous cognitive regression, better verbal communication, and improvement in other autistic features.
6. **Physiological Data:** ***Very reduced parasympathetic activity*** - statistically significant decrease in RSA in one patient to date, with significant improvement on donepezil HCl (Aricept) (P = 0.015).

Conclusions

- Extreme variable expressivity within a family is a well-documented phenomenon in mitochondrial disease, even when there is not an identified heteroplasmic mutation. Oftentimes, as is the case in our three families, the proband is moderately to severely affected while several relatives are substantially less affected. This observation has prompted the frequent assumption that this variability must be due to "modifying genes".
- We now have the technical capability via NextGen sequencing to identify these modifying genes. This is of potential relevance to treatment, as the primary mutation may not highly amenable to therapy, but a modifying factor might be.
- *CHAT* is a good example of a modifying gene of mitochondrial disease in which its identification leads to specific therapy that demonstrates substantial efficacy. We were fortunate that there is an objective parameter to monitor and substantiate an observed clinical effect.
- Analysis of *CHAT* should be considered in cases of mitochondrial disease or dysfunction where these is an unusual response to medications with anticholinergic effects or substantial dysautonomia. Identification of a mutation would be helpful before anticholinergic medications are given.