Author’s response to reviews

Title: Compound heterozygous mutations in glycyl-tRNA synthetase are a proposed cause of systemic mitochondrial disease

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Author's response to reviews: see over
Dear Prof Scaglia,

Please accept the revised version of our manuscript entitled: “Compound heterozygous mutations in glycyl-tRNA synthetase are a proposed cause of systemic mitochondrial disease”. We have made all of the revisions and/or provided clarification to each point raised by the Reviewers.

REVIEWER #1 (Minor Essential Revisions):

1. To clarify, both ubiquinone and ubiquinol were started at age 10 years?

RESPONSE: Yes, both medications were started at 10 years old. The patient was initially started on ubiquinone (along with vitamin B50 and carnitine). However 1-month later, ubiquinol 100mg; 2x/day was added. Three months later, creatine monohydrate 1.25 g daily was also added.

2. The case report could be condensed without losing substantial meaning.

RESPONSE: This has been done.

3. It appears that the brothers did not carry either mutant allele, but this is not stated explicitly.

RESPONSE: The boys were non-identical twins and were 2-1/2-years younger than their affected sister. Each brother was found to possess 1 mutant allele. One brother possessed the maternally-inherited allele. The other brother possessed the paternally-inherited allele. The boys were very active (cycling, cross country skiing) with reported no symptoms of a peripheral neuropathy. Complete clinical examination of each boy was performed by a Pediatric Neurologist. This examination as well as baseline nerve conduction studies were entirely normal.

4. If the brothers do not harbor a mutant allele, why was nerve conduction testing done? How old are they?

RESPONSE: Nerve conduction testing was done as each boy possessed 1 mutant allele. NCS of each boy was well within normal limits. The boys 2-1/2-years younger than their affected sister.

5. Was electron microscopy performed on the muscle biopsy sample?

RESPONSE: Yes, electron microscopy was performed on the muscle biopsy sample. No abnormality was reported in muscle fiber morphology including mitochondria number and/or appearance.
6. How common is it to have normal mitochondrial ETC functional studies in muscle biopsies from patients with either dominant GARS mutations or mutations in other ARS? How do you explain the normal ETC function as GARS mutations might be expected to affect multiple complexes?

**RESPONSE:** Regrettably, this information is not known. Published manuscripts documenting the clinical, electrodiagnostic and genetic details of dominant GARS mutations have not provided information about ETC or OxPHOS enzyme activity in muscle. Muscle biopsy in early-onset cases have reported muscle to demonstrate neurogenic changes (James et al, 2006) or combined neurogenic and myopathic changes (Eskui et al, 2012) in light of their patient`s muscle biopsy showing scattered atrophic and hypertrophic fibers with type I fiber predominance. Given the involvement of both nerve and muscle in the latter report it would have been particularly helpful to have known the ETC function.

7. Did the parents undergo echocardiogram?

**RESPONSE:** Yes, both mother and father had a transthoracic echocardiogram. Their echocardiograms were completely normal with no evidence of cardiomyopathy. The father`s LV ejection fraction was 66%. The mother`s LV ejection fraction was 70%. Neither parent had a clinical history of cardiac disease: no chest pain, no palpitations, no shortness of breath and no exercise intolerance. Clinical examination of both mother and father was also normal.

**REVIEWER #1 (Major Compulsory Revisions):**

1. Dominant mutations in the anticodon binding domain seem to be associated with a worse phenotype associated with early onset as reported by Eskuri et al in J Periph Nerv Syst. 2012 Mar;17(1):132-4. Since both parents carry variants that occur at a highly conserved site within the anticodon binding domain, could the authors speculate why both parents (and in particular the mother) present with such a mild phenotype? The normal clinical and neurological phenotype of the mother is puzzling (although no EMG was performed). It would have been useful to do an EMG to provide her with better counselling. In some cases the phenotype can present in the last decades of life both typically mutations in this domain tend to be associated with worse outcome.

**RESPONSE:** GARS mutations appear to demonstrate considerable variability in phenotype severity, even when same mutation is possessed by multiple family members. In the report by James et al, 2006 where Family #2 had a mutation in the anticodon binding domain (c.2260C>T, S581L) there was striking variability in the phenotype severity among two family members possessing this mutation. The mother had a phenotype that was more typical of CMT2D. She presented to orthopedic service in her late 20’s with foot deformity that was first noted in late-adolescence. Her milder phenotype stands in contrast to that of her son whose symptoms became apparent at 4 years old due to his abnormal gait and uneven wearing pattern of his shoes.

Our proband`s father shares an identical mutation to that reported in James et al, 2006. At 55 years old, this gentleman had been unaware of any symptoms of polyneuropathy and remained active with cycling
and skiing. Although nerve conduction studies and electromyography confirmed him to have a mild sensorimotor polyneuropathy no deficits could be seen on his exam. This finding adds further evidence to the fact that no definite genotype-phenotype correlation exists in CMT2D patients possessing heterozygous GARS mutations since tremendous variability exists in the age of onset, even among those with mutations affecting the anticodon binding domain.

Our proband’s mother (like her husband) also reports no clinical symptoms of polyneuropathy and has a normal neurological examination. Given that she is only 47 years old it is possible that electrophysiological and/or clinical signs of a polyneuropathy with axonal features may develop in the decades ahead.

2. The authors do not expand on the potential association between the left ventricular left compaction and the presence of these two variants in the GARS gene. Could there be another gene in the exome sequencing data that could be responsible for the presence of left ventricular non-compaction independently of the GARS variants? Exome sequencing data are currently showing that several patients may have complex phenotypes due to the presence of mutations in several genes.

RESPONSE: We reviewed the data from the exome carefully and with the knowledge that hypertrophic cardiomyopathies are often dominant mutations and frequently associated with dysfunction of respiratory chain enzyme function.

Our patient had mitochondria DNA sequencing as well as duplication/deletion analysis performed on muscle tissue. We are therefore confident that there was no mitochondrial DNA mutation that could account for her cardiomyopathy or other symptoms.

We reviewed the exome data with special attention to all genes that have been previously reported to cause either a dominant or recessively inherited hypertrophic or dilated cardiomyopathy. The OMIM list was expanded since we originally submitted our manuscript on 20 Oct 2013. We identified our patient to have a sequence variant in the MIB1 (mindbomb E3 ubiquitin protein ligase 1) gene which has recently been linked to non-compaction cardiomyopathy (see: Luxan G et al. Nature Medicine. 2013;19(2):193-201). The MIB1 sequence variant has not been previously reported but did occur in a highly conserved region of this gene. We sequenced the proband’s mother and father’s DNA and confirmed that the father carries the same sequence variant, although he shows no evidence of cardiomyopathy on clinical examination or echocardiogram. Thus, we cannot be certain if our patient’s non-compaction cardiomyopathy is due to incomplete penetrance of our proband’s sequence variant in the MIB1 gene or associated with the GARS gene findings. Even if the cardiomyopathy is due to a second gene, there is still substantial evidence for mitochondrial dysfunction in this girl given her persistent lactic acidosis, elevated serum alanine, exercise-induced myalgia and white matter changes on MRI brain.

3. Although the authors state that there was as subjective improvement with the addition of creatine monohydrate, there was no comment whether clinical improvement was observed with the addition of B50 complex, carnitine and coenzyme Q10.
RESPONSE: The patient was first started on coenzyme Q10 (i.e. ubiquinone), vitamin B50 and carnitine in July 2010 (at 10y 2mos old). In mid-Aug 2010 ubiquinol was added. In Nov 2010 creatine monohydrate 1.25 g daily was added. The patient and family did not report any subjective improvement with ubiquinone, vitamin B50, carnitine or ubiquinol. Symptom improvement was only noted with creatine monohydrate.

MINOR essential revisions:

1. Please in background section change the name of disease: CMT2A2 should be changed to CMT2A

RESPONSE: This has been done.

2. Why was ubiquinol used at the same of ubiquinone? Is this a mistake?

RESPONSE: The patient was on both medications at the same time (please see comment #3, above). The patient was on ubiquinone (coenzyme Q10) for 1 month before ubiquinol was added.

Thank you once again for your time and consideration,

Hugh McMillan, MD, MSc, FRCPC, FAAN