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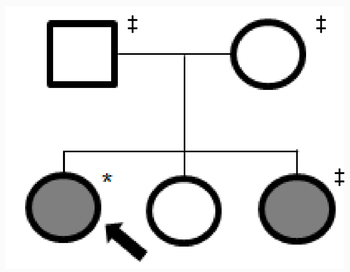
Clinical Exome Sequencing Leads to the Diagnosis of Mitochondrial Complex I Deficiency in a family with global developmental delays, ataxia, and cerebellar and pons hypoplasia

ABSTRACT

Exome sequencing was performed on a 14 year-old female with familial ataxia, global developmental delays, and cerebellar and pons hypoplasia. The family history was remarkable for a 3 year-old sister with a similar phenotype. Nearly a decade of molecular, cytogenetic, and biochemical testing was uninformative. Exome sequencing revealed compound heterozygous alterations of the *NUBPL* gene (c.311T>C; p.L104P & c.815-27T>C). The c.311T>C missense alteration is located at a highly conserved amino acid. The c.815-27T>C alteration is located at a highly conserved nucleotide and previous in vitro analyses demonstrated splicing defects. The affected sister manifested both alterations; each parent carried one alteration. Alterations within the *NUBPL* gene occur in an autosomal recessive fashion in association with mitochondrial complex I deficiency syndrome (CI deficiency) (MIM_252010). The *NUBPL* gene was first discovered in association with disease in 2010 and has only been reported in two other families, both of which displayed remarkable clinical overlap with the family herein. Exome sequencing is an especially powerful tool to aid in the diagnosis of CI deficiency given the extreme clinical and genetic heterogeneity making establishing a clinical diagnosis exceedingly difficult. Further, the underlying mutation has not been discovered in about half of patients with CI deficiency, thought to be due to yet undiscovered associated genes. Diagnostic exome sequencing led to the successful identification of the *NUBPL* alterations and, after years of unsuccessful analyses, led to a molecular diagnosis for the family.

Figure 1: Family History



Legend:
Shading indicates affected
** Whole exome sequencing*
‡ Co-segregation analysis

Table 1: Variant Filtering Based on Inheritance Model & Interpretation

	Post-Inheritance Model Filtering	Post-Medical Review[1]		Notable Candidate Genes[2]
		Post-alteration review	Post-clinical association review	
Autosomal Dominant Genes (Alterations)	33 (33)	33 (33)	2 (2)	**0 (0)**
Autosomal Recessive Genes (Alterations)	7 (14)	6 (12)	1 (2)	**1 (2)**
X-linked Recessive Genes (Alterations)	0 (0)	0 (0)	0 (0)	**0 (0)**
X-linked Dominant Genes (Alterations)	3 (3)	3 (3)	0 (0)	**0 (0)**
Y-linked Genes (Alterations)	N/A	N/A	N/A	**N/A**
TOTAL GENES (Alterations)	**43 (50)**	**42 (48)**	**3 (4)**	**1 (2)**

[1]Post-medical review filtering involves the manual removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign
[2]Notable Candidate Genes: Gene alterations selected for co-segregation.

METHODS

- Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from the patients and sister. Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).
- The Human Gene Mutation Database (HGMD; Stenson, 2009), OMIM, and several other databases were used to search for previously described gene mutations and polymorphisms. A molecular geneticist performed interpretive filtering based on the deleterious nature of the candidate alterations literature search and analysis of the relevance of the candidate genes' function in relation to the patient's phenotype.
- Each candidate variant was analyzed by Sanger sequencing for mutation confirmation and co-segregation studies were performed for the family.

Table 2. *NUBPL* Alterations and Familial Co-segregation Results

Gene (RefSeq ID)	Protein	Alteration	Exon #	Genotype	Alteration Type	Patient	Mother	Father	Affected Sister
NUBPL (NM_025152)	Nucleotide binding protein-like	**c.3518C>T (p.P1173L)**	Exon 4	Heterozygous	Missense	**+/-**	**+/-**	**-/-**	**+/-**
		c.815-27T>C	Intron 9	Heterozygous	Splice	**+/-**	**-/-**	**+/-**	**+/-**

[1]GRCh37

Table 3. Clinical overlap with previous cases

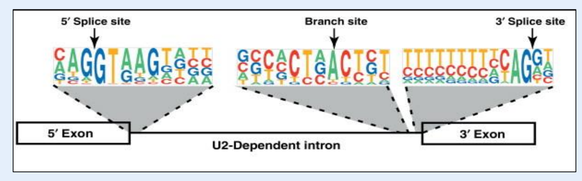
NUBPL: Mitochondrial complex I deficiency (MIM_252010)

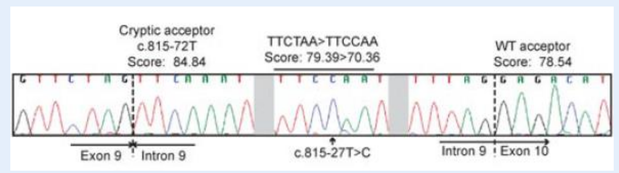
	Calvo et al. 2010	Tenisch et al. 2012	*Current Patient*
Gender	Male	Male	*Female*
Current Age	8 yrs	23 yrs	*14 yrs*
Age of onset	2 yrs	2-3 yrs	*6 mo*
Presenting Symptom	Developmental Delay		*Developmental Delay*
Ataxia	Yes	Yes	*Yes*
Speech	Impaired	Slurred	*Impaired*
Ocular Sx	Nystagmus Squint	Nystagmus	*Nystagmus*
Seizures	Staring spells		*Yes*
MRI	Leukodystrophy	T2 hyperinstensity of cerebellum and brainstem	*Cerebellar and pons atrophy*
Plasma Lactate	Normal		*Normal*
CNS Lactate	Elevated	Elevated	*Elevated*
Muscle Bx	~20% Ragged Red Fibers		*Pending*
Complex I Deficiency	Skeletal Muscle and Fibroblasts	Skeletal Muscle	*Pending*
NUPBL alterations:	p.G56R	p.G56R	*DOES NOT CARRY*
	c.815-27T>C	c.815-27T>C	*c.815-27T>C*
	Ins/Del Rearrangement	c.205_205delGT	*p.L104P*

Mutations | Variant, Likely Pathogenic | Variant, Likely Benign

Figure 2. c. 815-27C>T is a common branch point mutation

Population	Alleles C	Alleles T	Alleles Total	Genotypes C/C	Genotypes C/T	Genotypes T/T	Genotypes Total
European American	31	6,511	6,542	0	31	3,240	3,271
	0.47%	99.53%	100%	0%	0.95%	99.05%	100%
			N/A				N/A
African American	5	2,945	2,950	0	5	1,470	1,475
	0.17%	99.83%	100%	0%	0.34%	99.66%	100%
			N/A				N/A
Total	36	9,456	9,492	0	36	4,710	4,746
	0.38%	99.62%	100%	0%	0.76%	99.24%	100%
			N/A				N/A





Patient	Country of origin	c.DNA[b]	Protein	Exon	State	Inheritance
1	Argentina	c.166G>A	p.Gly56Arg	2	Homozygous[c]	Unknown
		c.815–27T>C[d]		Intron 9	Homozygous[c]	Unknown
2	Germany	c.166G>A	p.Gly56Arg	2	Heterozygous	Paternal
		c.667_668insCCTTGTGCTG	p.Glu223Alafs*4	8	Heterozygous	Maternal
		c.815–27T>C[d]		Intron 9	Heterozygous	Paternal
3 and 4	Canada	c.166G>A	p.Gly56Arg	2	Heterozygous	Paternal
(sibs)		c.313G>T	p.Asp105Tyr	4	Heterozygous	Maternal
		c.815–27T>C[d]		Intron 9	Heterozygous	Paternal
5	United States	c.166G>A	p.Gly56Arg	2	Heterozygous	Paternal
		693+1G>A[e]	p.?	Intron 8	Heterozygous	Unknown[f]
		815–27T>C[d]		Intron 9	Heterozygous	Paternal
6	Netherlands	c.166G>A	p.Gly56Arg	2	Heterozygous	Maternal
		c.579A>C	p.Leu193Phe	7	Heterozygous	Paternal
		c.815–27T>C[d]		Intron 9	Heterozygous	Maternal
7[g]	Australia	c.166G>A	p.Gly56Arg	2	Heterozygous	Paternal
		240-kb deletion (exons 1–4); 137-kb duplication (exon 7)		1–4 and 7	Heterozygous	Maternal
		c.815–27T>C[d]		Intron 9	Heterozygous	Paternal

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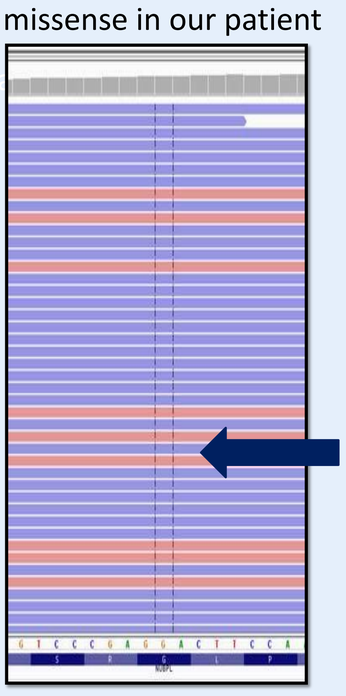
815-27C>T not *in cis* with missense in our patient



RESULTS

CLINICAL HX: Proband initially presented at 6 months of age with hypotonia an developmental delay born to non-consanguineous parents. Now at 14 years of age with seizures, severe ataxia, and cognitive impairment. No diagnosis despite years or previous testing.

FAMILY HX: Similarly affected sister, now 5 years of age, and one unaffected sister (**Figure 1**). No other family history.

INFORMATICS & ANALYSIS: Inheritance model filtering based on autosomal and X-linked dominant and recessive models revealed 43 genes (50 unique alterations). Manual review one notable gene (two alterations) with potential clinical relevance (**Table 1**).

MOLECULAR DIAGNOSIS: Automated fluorescence dideoxy sequencing confirmation confirmed the two alterations: *NUBPL:* c.3518C>T (p.P1173L) and c.815-27T>*C* (**Table 2**).

- The c.815-27T>*C* alteration is thought to represent one of the most common autosomal recessive OXPHOS mutations observed to date (Tucker, 2012) (**Figure 2**). *In vitro* analyses via mRNA, protein expression, and RT-PCR analyses demonstrate that the alteration reduces mRNA expression and protein levels, and results in the production of three distinct transcripts (Tucker, 2012).

CLINICAL RELEVANCE: The patient's clinical presentation is consistent with that of previously-reported patients with *NUBPL* alterations who present with Mitochondrial Complex I deficiency (MIM_252010) (Calvo, 2010) (**Table 3**).

- Recent publication of 5 additional families with pathognomonic MRI findings (**Figure 2**).
- Branch point mutation is seen in all families
  - In *cis* with missense alteration in **all** reported cases except this one
  - Recurrent mutation vs. founder haplotype

CLINICAL INTERVENTION: Treatment initiated with Mitochondrial coenzymeQ cocktail therapy. Future potential for EPI-743 clinical trials (complex I enhancer)

CONCLUSIONS

- Clinical diagnostic exome identified compound heterozygous mutations in *NUBPL* consistent with the diagnosis of mitochondrial complex I deficiency
- The identified mutations are highly consistent with the patient's clinical symptoms which provides an opportunity for clinical intervention and surveillance as well as recurrence risks estimation and family planning.

REFERENCES:

- Calvo et al. (2010) *Nature Genetics* 2010 Oct;42(10):851-8
- Tucker et al (2012) *Human Mutation* 2012 Feb;33(2):411-8.
- Kevelam et al (2013) *Neuroloy* 2013 Apr 23;80(17):1577-1583
- HGMD® [Internet]: Stenson PD, *et al*. (2009) *Genome Med* **1**(1):13.
- PolyPhen [Internet]: Adzhubei IA, *et al*. (2010) *Nat Methods* **7**(4):248-249.
- SIFT [Internet]: Ng PC & Henikoff S (2006) *Hum Genet.* **7**:61-80.

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