

Clinical and Laboratory Features of a Single Family with Mutations in the POLG1 and ANT1 (SLC25A4) Genes [P06.085]

Lauren C. Hyams, Ph.D., Genevieve N. Langley, B.S., John M. Shoffner, M.D.
 Medical Neurogenetics, LLC, Atlanta, GA

Background

Mutations in the adenine nucleotide translocase gene (ANT1 (SLC25A4), OMIM 103220) cause autosomal dominant mitochondrial disease by dysregulation of the permeability transition pore and abnormal ADP/ATP flux between mitochondria and the cytosol. Mutations in the DNA polymerase gamma gene (POLG1, OMIM 174763) cause autosomal dominant or recessive mitochondrial disease by impairing mitochondrial DNA (mtDNA) replication and proofreading exonuclease activity. Mutations in either gene causes disorders presenting with chronic progressive external ophthalmoplegia (CPEO). We present a case of a patient with mutations in both the ANT1 and POLG1 genes. Assessment of mutation pathogenicity is often difficult. However, this unique case allows assignment of pathogenicity to the ANT1 mutation. The POLG1 mutation which has been reported as pathogenic (1) is clearly a neutral polymorphism, thus clarifying the status of this mutation.

Clinical Description

The proband had features typical of patients with ANT1 or POLG1 gene mutations:

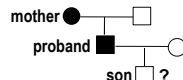
- chronic progressive external ophthalmoplegia (CPEO)
- fatigue with abnormal exercise testing

Mitochondrial Disease diagnosis of patient based on:

- Abnormal histochemistry: ragged-red fiber myopathy
- Abnormal protein chemistry: multiple OXPHOS protein subunits were decreased (both mtDNA and nuclear coded)
- Abnormal OXPHOS enzymology
- Decreased resting metabolic rate (RMR) exercise testing: RMR value was 56% of predicted O₂ utilization which indicates respiratory chain impairment
- Fibroblast fatty acid oxidation: increased medium & long chain acylcarnitines in a pattern consistent with that observed in patients with OXPHOS defects
- Abnormal nuclear gene sequencing: pathogenic POLG1 and ANT1 mutations identified
- NOTE: Quantitation of mtDNA in muscle was normal

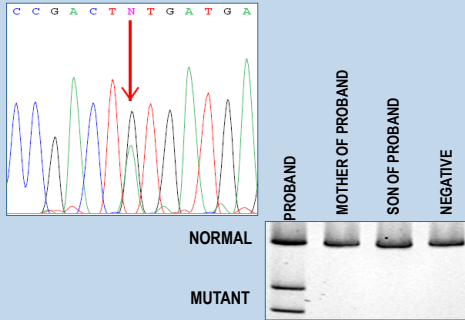
Family History:

- Mother of proband affected - CPEO
- Son of proband unaffected in childhood



POLG1 gene Mutation: (DNA Polymerase Gamma) Tyrosine 831 Cysteine (Y831C)

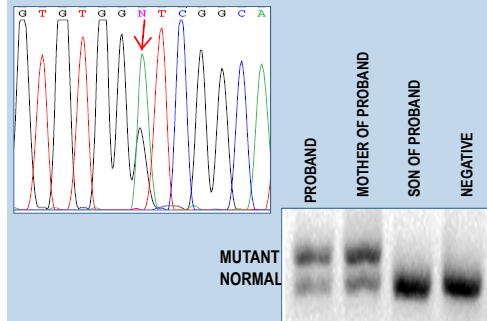
We identified a single heterozygous missense mutation in Exon 16 of the POLG1 gene by direct sequencing: c.2492A>G, Tyrosine (TAT) 831 Cysteine (TGT). This mutation is described as pathogenic in a report of a single family (1). The Tyrosine at codon 831 is NOT conserved across multiple species or within species. Restriction fragment length polymorphism (RFLP) testing of proband muscle DNA and family member leukocyte DNA showed that the mutation is only present in the proband and did not segregate with the disease, suggesting that this variant is a neutral polymorphism and not responsible for the clinical features in this patient. Current literature estimates the frequency of this variant to be approximately 1.1-4% in control populations, supporting our data that this variant should be classified as a neutral polymorphism.



HUMAN	ALPRAVIRHPFD	-----EGLYGAILPQVVTAGT
CHIMP	ALPRAVIRHPFD	-----EGLYGAILPQVVTAGT
DOG	ALPRAVTRHPFD	-----EGRYGAILPQVVTAGT
MOUSE	ALPRVTRHPSED	-----EESHYGAILPQVVTAGT
RAT	ALPRAVTRHPSED	-----EESHYGAILPQVVTAGT
CHICKEN	ELPRAVTRHPYS	-----EEDYGAILPQVVTAGT
ZEBRAFISH	ELPRAVTRHPYS	-----EEDYGAILPQVVTAGT
DROSOPHILA	QLPNEFTGKCP	-----IA--YGAICPQVVVCGT
MOSQUITO	--PGE--	-----DP--YGAICPQVVVCGT
YEAST (Sp)	ELGVPSS	-----SVDGFGIILPCLIPMGT
YEAST (Sc)	PNEFOSLSAKSSLNNE	-----KTNLDLAIIPKIVPMGT
YEAST (Kl)	KSENF--AKTFE--	-----KDLVGLIIPKIVPMGT
FUNGUS (Ec)	PDEAFMDGTF--	-----RDDQVGAIFPKIIPMGT
FUNGUS (Mg)	SAPTASASPKSRNTRKTPQSETKSGE--	-----ILPQIIPMGT
FUNGUS (Nc)	PPSQRFVNKDDASNT	-----PIGGE--VLPQIIPMGT

ANT1 gene Mutation: (Adenine Nucleotide Translocase) Aspartate 104 Glycine (D104G)

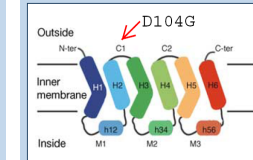
We identified a single heterozygous missense mutation in Exon 2 of the ANT1 gene by direct sequencing: c.311A>G, Aspartate (GAT) 104 Glycine (GGT). This mutation is described as pathogenic in a report of a single Japanese family (2). The Aspartate at codon 104 is STRICTLY conserved across multiple species. The Aspartate-to-Glycine mutation introduces a change in side chain polarity from polar to nonpolar, which may have significant effects on the resulting protein. The surrounding amino acids are conserved as well, indicating the functional importance of the amino acids in this region. RFLP testing of proband muscle DNA and family member leukocyte DNA confirmed the presence of the heterozygous change in the 2 affected family members, but not in the unaffected family member. Segregation of the mutation with disease in this family confirms that this mutation in the ANT1 gene is responsible for the clinical features in both family members.



HUMAN	KYKQFLGGVDRHKQFWRVFAGNLSGGAAGATSLCFVYP
CHIMP	KYKQFLGGVDRHKQFWRVFAGNLSGGAAGATSLCFVYP
DOG	KYKQIFLGGVDRHKQFWRVFAGNLSGGAAGATSLCFVYP
COW	KYKQIFLGGVDRHKQFWRVFAGNLSGGAAGATSLCFVYP
MOUSE	KYKQIFLGGVDRHKQFWRVFAGNLSGGAAGATSLCFVYP
RAT	KYKQIFLGGVDRHKQFWRVFAGNLSGGAAGATSLCFVYP
CHICKEN	KYKQIFLGGVDRHKQFWRVFAGNLSGGAAGATSLCFVYP
ZEBRAFISH	KYKQIFLGGVDRHKQFWRVFAGNLSGGAAGATSLCFVYP
DROSOPHILA	KYKQVFLGGVDRKNTQFTRFYFAGNLSGGAAGATSLCFVYP
MOSQUITO	KYKQVFLGGVDRKNTQFTRFYFAGNLSGGAAGATSLCFVYP

Mechanism of ANT1 defect

ANT1, or the ADP/ATP translocator, is the most abundant protein in the mitochondrial inner membrane. As a homodimer, it forms a gated channel by which ADP is brought into and ATP out of the mitochondrial matrix. ANT1 regulates the adenine nucleotide concentrations in the cytoplasm and within the mitochondria, and mediates signals of nucleo-cytoplasmic energy consumption to the mitochondrial respiratory chain. In addition to the translocase activity, ANT1 is a core structural element of the mitochondrial permeability transition pore and has an important role in mitochondrial-mediated apoptosis (3).



The D104G mutation resides in the highly conserved C1 intermembrane space loop between transmembrane helices 2 and 3 (4). Mutations in this region of the polypeptide may likely effect selection of solutes, namely ADP, entering the channel for transport across the inner membrane and into the matrix.

Summary

1. Two reportedly pathogenic mutations were identified in the ANT1 and POLG1 genes in a patient with progressive external ophthalmoplegia.
2. The ANT1 mutation is pathogenic on the following basis: (1) Aspartate 104 codon and surrounding codons are strictly conserved; (2) mutation of codon 104 to Glycine significantly changes the side chain polarity from polar to nonpolar; (3) the Aspartate 104 to Glycine mutation segregates with disease within this family.
3. ANT1 codon 104 resides in the intermembrane space where it likely plays a significant role in selection of solutes entering the channel.
4. The previously described POLG1 mutation is present only in the proband and appears to be a neutral polymorphism. It is not likely to be pathogenic as previously reported.
5. Assessment of mutation pathogenicity is often difficult. Two mutations in a single family provide a unique opportunity to understand mutation pathogenicity.

Selected References

1. Mancuso, M.; Filosto, M.; Oh, S. J.; DiMauro, S.: A novel polymerase-gamma mutation in a family with ophthalmoplegia, neuropathy, and parkinsonism. *Arch. Neurol.* 61: 1777-1779, 2004.
2. Komaki, H.; Fukazawa, T.; Houzen, H.; Yoshida, K.; Nonaka, I.; Goto, Y.: A novel D104G mutation in the adenine nucleotide translocator 1 gene in autosomal dominant progressive external ophthalmoplegia patients with mitochondrial DNA with multiple deletions. *Ann. Neurol.* 51: 645-648, 2002.
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