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Case Report

A new pathogenic POLG variant

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ABSTRACT

POLG gene mutations are the most common causes of inherited mitochondrial disorders. The enzyme produced by this gene is responsible for the replication and repair of mitochondrial DNA. To date, around 300 pathogenic variants have been described in this gene. The resulting clinical outcomes of *POLG* mutations are widely variable in both phenotype and severity. There is considerable overlap in the phenotype of the so-called POLG syndromes with no clear genotype-phenotype correlation. Here we describe a newly discovered pathogenic variant in the *POLG* gene in a 7-year-old male that died of uncontrollable refractory status epilepticus. Genetic epilepsy panel sequencing identified two variants in the *POLG* gene, the common p.A467T pathological mutation and a novel p.S809R POLG variant found *in trans* with the p.A467T POLG that accompanied a severely reduced mitochondrial DNA level in the patient's tissues.

1. Introduction

Mitochondria are membrane-bound organelles present in all nucleated eukaryotic cells. Mitochondria create ATP for cellular energy via oxidative phosphorylation. The biology and genetics of mitochondrial oxidative phosphorylation are complex with the components that make up the structure and control the function of the mitochondria being encoded for by both mitochondrial and nuclear genomes. Any abnormality in these components can reduce the functional output of the oxidative phosphorylation system producing a wide range of diseases with marked genetic and phenotypic heterogeneity. Pathogenic variants have been described in both the mitochondrial and nuclear genomes. Current literature details at least 300 disease-causing genes linked to mitochondrial dysfunction and this number continues to rise [1].

The gene *POLG* (**Ensembl:ENSG00000140521; MIM:174763**), located on nuclear chromosome 15q, encodes for DNA polymerase gamma (POLG), an enzyme involved in the replication and repair of mitochondrial DNA [2]. *POLG* encodes the catalytic subunit, p140, of the mitochondrial DNA polymerase (Pol g). The p140 catalytic subunit is complexed to the dimeric p55 accessory subunit encoded by the *POLG2* gene [3]. The p55 subunit functions as a processivity factor enhancing the complex affinity to DNA and allowing for very long stretch of DNA synthesis [4]. Mutations in the *POLG* gene are the most common causes of inherited mitochondrial disorders [5], with around 300 known pathogenic variants currently described [6,7]. Mutations in this gene cause a spectrum of disorders with overlapping phenotypes, collectively described as *POLG* syndromes [8]. These syndromes can include developmental delay, hypotonia [9,10] seizures (generalized, focal motor, myoclonic, and status epilepticus) [11–16], stroke-like episodes [11], movement disorders (chorea, parkinsonism) [14,17,18], myopathy (ptosis, external ophthalmoplegia, weakness, fatigue) [11,14,17,19,20], ataxia [11,13,14,16], peripheral neuropathy [11,21,22], psychomotor regression [6], cardiomyopathy [11,13], and endocrinopathies (diabetes mellitus, primary ovarian or testicular failure) [11,14,17,23,24]. Patients can present at any time from early childhood to late adulthood, depending on phenotype, typically with pediatric-onset disorders demonstrating mitochondrial DNA depletion and adult disorders showing accumulation of multiple mitochondrial DNA deletions on muscle analysis [10,20,24,25,27,30,31]. The phenotypes of POLG are commonly categorized into six syndromes.

- Alpers-Huttenlocher syndrome (AHS) is one of the most common and severe phenotypes. Children can have normal development for the first few months or even years of life but may have some degree of developmental delay that becomes apparent by age three years. Seizures are often the first sign of AHS and these commonly are partial or secondarily generalized tonic-clonic seizures but children may present with status epilepticus. The epilepsy is resistant to

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antiepileptic drugs and often becomes intractable. Encephalopathy develops in a severe, progressive fashion and hepatic failure occurs with a wide range of variability. Valproic acid is contraindicated as rapid progression to end-stage liver failure is a well-described outcome in those treated with valproic acid or its derivatives [6,11,15,25–27].

- Childhood myocerebrohepatopathy spectrum (MCHS) presents between the first few months to three years of life with developmental delay, lactic acidosis, myopathy, failure to thrive, pancreatitis, hearing loss, and dementia. MCHS can also include hepatic failure and renal tubular dysfunction [10].
- Myoclonic epilepsy, myopathy, and sensory ataxia (MEMSA) includes disorders previously described as spinocerebellar ataxia with epilepsy (SCAE) but now is used to refer to disorders with epilepsy, myopathy, and ataxia without ophthalmoplegia. Patients typically first present in young adulthood with cerebellar ataxia secondary to sensory neuropathy. Myopathic weakness usually develops in proximal muscle groups but can be distal. Many patients will go on to develop focal epilepsy with progression to generalized refractory epilepsy [6].
- Ataxia neuropathy spectrum (ANS) includes phenotypes previously referred to as mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia, neuropathy, dysarthria, and ophthalmoplegia (SANDO). Patients with ANS commonly have ataxia and varying degrees of sensorimotor neuropathy. Many also develop headaches, seizures, myoclonus, blindness, ophthalmoplegia, psychiatric disorders, and liver failure [10,15,28,29].
- Autosomal recessive progressive external ophthalmoplegia (arPEO) is characterized by progressive extraocular muscle weakness. Strabismus and ptosis are often the only signs early on in the disease course [6].
- Autosomal dominant progressive external ophthalmoplegia (adPEO) involves more systemic features such as generalized myopathy and in some cases sensorineural hearing loss, depression, ataxia, axonal neuropathy, parkinsonism, and hypogonadism [17,21,22,29,31,32].

Regardless of whether or not patients fall neatly into one of the described syndromic categories, patients with POLG syndromes generally present with epilepsy. Myoclonus is the most common presented seizure type and patients often develop status epilepticus or epilepsy partialis continua. The seizures become refractory to treatment and worsen prognosis [33]. Valproic acid is contraindicated as it can precipitate or accelerate liver failure by inhibition of fatty acid beta-oxidation which impairs mitochondrial function [11,12,15,26]. Cognition declines at a variable rate with more rapid periods of decline during acute infectious illnesses. Somnolence, loss of receptive and expressive language, and memory deficits are commonly seen with neurodegeneration [6]. Magnetic resonance imaging (MRI) of the brain often demonstrates T2 and FLAIR hyperintensities in the cortical and subcortical regions, particularly in the occipital lobes. Similar lesions are also seen in the thalamus and cerebellum [33]. Electroencephalography (EEG) findings are variable but occipital discharges and slowing are frequently seen, as are rhythmic high-amplitude delta with superimposed spikes (RHADS) [33]. The diagnostic gold standard for POLG related disorders is gene sequencing. Life expectancy is usually 3 months to 12 years from symptom onset. Here we describe a patient with a newly described pathogenic *POLG* variant in *trans* with a known pathogenic variant. Clinically, his phenotype had overlapping features of both AHS and MEMSA.

2. Case

Our patient was a 7-year-old male with an unremarkable birth and early childhood. His parents reported an explosive-onset of seizures at the age of 5 years. Seizures were difficult to control from the onset and at the time of his initial evaluation his parents reported he had failed numerous anti-epileptic medications and had therefore stopped all anti-epileptics. His parents reported daily myoclonic seizures, generalized tonic-clonic seizures, and episodes of behavioral arrest suspicious for absence seizures. Functionally, his parents reported that he was able to walk, eat, swim, and play even though he was less verbal than prior to seizure onset and was falling behind in school. Physical examination was notable for generalized rigidity and a dystonic gait. Prolonged EEG recording was performed, demonstrating over 50 generalized-onset seizures within a 24-h period. Seizure semiology was myoclonic, absence, and tonic-clonic. Conservative management was attempted with anti-epileptic therapy with a decrease in daily seizures and an increase in cognitive function.

Three months later, the patient developed status epilepticus prompting admission to our pediatric intensive care unit. He was febrile with a presumed viral infection. The status epilepticus was refractory to multiple anti-seizure drugs (ASDs) and EEG showed continuous epileptiform discharges in the right fronto-centro-temporal and right temporo-parietal areas. He was placed in chemically-induced burst suppression with pentobarbital. MRI brain showed confluent T2 hyperintensities in the right caudate head, right thalamus, and left parietotemporal cortex with restricted diffusion in the right thalamus. Follow-up MRI one week later showed new abnormal signal changes in the left ventral medulla. There were no significant renal, gastrointestinal, hepatic, or endocrine abnormalities. Despite aggressive ASD management, our patient continued to seize and mental status did not improve. After his third pentobarbital coma (total of >14 days in pentobarbital induced burst suppression with no improvement), the decision was made to terminally extubate under DNR code status. The patient died the day after extubation.

Genetic epilepsy panel obtained prior to his last admission identified a pathogenic variant, c.1399G > A (p.A467T), and a variant of unknown significance (VUS), c.2425A > G (p.S809R), in the *POLG* gene. VUS were also identified in *SCN2A*, *SCN9A*, and *CNTNAP2* genes but none had features suggestive of pathogenicity. The child's mother was found to have the c.2425A > G variant but not the c.1399G > A variant. Father was not available for testing. Muscle biopsy from the vastus lateralis revealed no structurally pathologic findings but mitochondrial DNA content was 23% of the mean value of age and tissue-matched controls. Analysis for mitochondrial DNA deletions was not performed.

3. Discussion

Our patient presented with a clinical phenotype including overlapping features of both AHS and MEMSA. The c.1399G > A (p.A467T) variant is one of the most common *POLG* disease mutations occurring in ~0.6% of certain populations [21] and is considered to be a "founder" mutation. The wild type residue, Ala467, resides in the DNA-interacting thumb domain and is important for the p55 subunit interaction that allows for highly processive DNA synthesis [34]. Alteration to Ala causes disruption of the p140-p55 interaction, reduced processivity and decreased DNA polymerase activity [34]. The p.A467T mutation has been identified alone in adPEO and in combination with other pathogenic variants in every other phenotypic form of POLG syndrome. The undescribed c.2425A > G variant, causing an amino acid change from Ser to Arg is suspected to be pathogenic as it is located in a highly conserved region of *POLG* (Fig. 1). The Ser809 residue located in the thumb domain which is important for DNA binding on one side and interacting with the accessory subunit on the other side (Fig. 2A) [35,36]. The Ser side chain is positioned to interact with the peripheral thumb domain alpha-helix which makes contact with the accessory subunit (Fig. 2B). A change of this residue to Arg (Fig. 2C) could alter the binding capacity of the enzyme to the p55 accessory subunit or DNA or could affect the enzyme's stability thus producing the phenotype seen in this child. Heterozygotes would likely be able to partially compensate for this reduced processivity with a functional wild-type enzyme; however, homozygotes or compound heterozygotes would not be able to

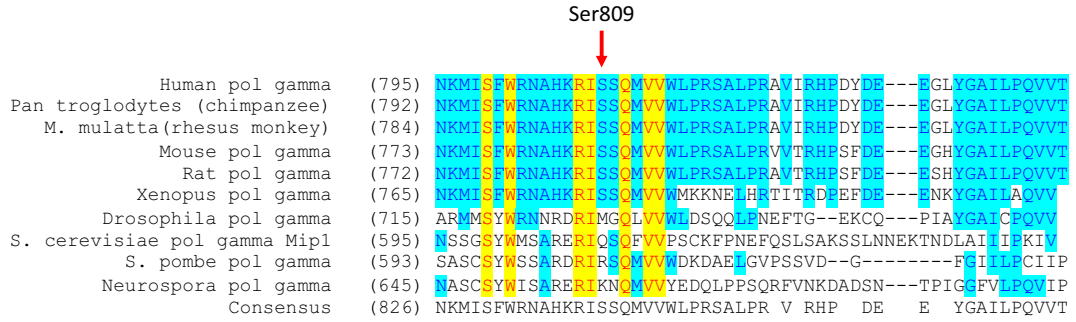

Fig. 1. Conservation of POLG amino acid sequence from across eukaryotes. Yellow highlighted amino acids are invariant across all POLG sequences, while blue highlighted amino acids are conserved in animal POLG sequences. The Ser809 residue is depicted by the arrow.

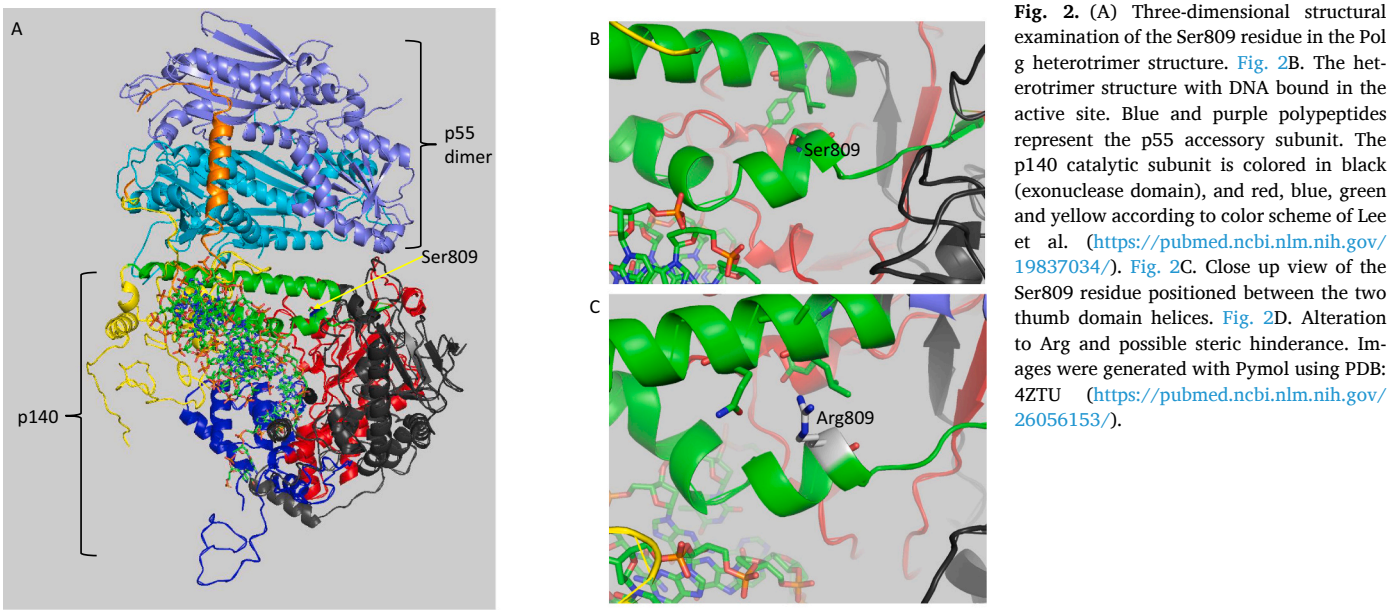

Fig. 2. (A) Three-dimensional structural examination of the Ser809 residue in the Pol g heterotrimer structure. Fig. 2B. The heterotrimer structure with DNA bound in the active site. Blue and purple polypeptides represent the p55 accessory subunit. The p140 catalytic subunit is colored in black (exonuclease domain), and red, blue, green and yellow according to color scheme of Lee et al. (https://pubmed.ncbi.nlm.nih.gov/19837034/). Fig. 2C. Close up view of the Ser809 residue positioned between the two thumb domain helices. Fig. 2D. Alteration to Arg and possible steric hinderance. Images were generated with Pymol using PDB: 4ZTU (https://pubmed.ncbi.nlm.nih.gov/26056153/).

compensate. Our patient's muscle biopsy demonstrated a severe reduction in mitochondrial DNA content to 23% of control, suggesting an inability to properly process mitochondrial DNA in this patient. Although tragic, this case serves to describe a new pathogenic variant in the *POLG* gene associated with disease.

Details of the contributions of individual authors

S. Nicholas Russo, MD: Designed, conceptualized, and drafted the manuscript.

Ekta G. Shah, DO: Edited, conceptualized, and critically reviewed the manuscript for intellectual content.

William C. Copeland, PhD: Drafted, conceptualized, edited, and critically reviewed the manuscript for intellectual content.

Mary Kay Koenig, MD: Conceptualized, edited, and critically reviewed the manuscript for intellectual content.

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Details of ethics approval

Ethics approval was not required.

A patient consent statement

Patient consent was obtained for this manuscript.

Declaration of Competing Interest

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Data availability

No data was used for the research described in the article.

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