[Gene 499 (2012) 209–212](http://dx.doi.org/10.1016/j.gene.2012.02.034)

Contents lists available at [SciVerse ScienceDirect](http://www.sciencedirect.com/science/journal/03781119)

Gene

journal homepage: [www.elsevier.com/locate/gene](http://www.elsevier.com/locate/gene)

Short communication

*POLG* mutation in a patient with cataracts, early-onset distal muscle weakness and atrophy, ovarian dysgenesis and 3-methylglutaconic aciduria

Mir Reza Bekheirnia [a](#_bookmark0), Wei Zhang [a](#_bookmark0), Tanya Eble [a](#_bookmark0), Alecia Willis [a](#_bookmark0), Aziz Shaibani [b](#_bookmark1),[c](#_bookmark2), Lee-Jun C. Wong [a](#_bookmark0),

Fernando Scaglia [a](#_bookmark0), Shweta U. Dhar [a](#_bookmark0),[b](#_bookmark1),[⁎](#_bookmark3)

a *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*

b *Nerve and Muscle Center of Texas, Houston,TX, USA*

c *Department of Medicine, Baylor College of Medicine, Houston, TX, USA*

# a r t i c l e i n f o

*Article history:*

Accepted 19 February 2012

Available online 3 March 2012

*Keywords:*

*POLG*

3-Methylglutaconic aciduria Muscle weakness

Cataracts

Ovarian dysgenesis

# a b s t r a c t

Mutations in *POLG* account for one of the most frequent nuclear encoded causes of mitochondrial disorders to date. Individuals harboring *POLG* mutations exhibit fairly heterogeneous clinical presentations leading to in- creasing difﬁculties in classifying these patients into deﬁned clinical phenotypes. This study aims to investi- gate the molecular basis of a mitochondrial cytopathy in a patient with 3-methylglutaconic aciduria and to expand the clinical phenotype associated with *POLG* mutations.

Clinical, molecular and genetic analyses as well as neurophysiological examinations were carried out for a 23-year-old woman of mixed Caucasian and Latin American ancestry with a history of cataracts diag- nosed at age 1year, she had onset of distal muscle weakness at age 2years progressing to atrophy and ovarian dysgenesis at puberty. The patient was found to have 3-methylglutaconic acid with normal 3 hydroxyisovaleric acid on urine organic acid analysis. *POLG* sequencing was done and a heterozygous var- iant, c.2851T>A (p.Y951N) was found which is predicted to be deleterious. There are limited reports of *POLG* mutations in individuals with 3-methylglutaconic aciduria. This case report of a young woman with a heterozygous mutation in *POLG*, presenting with muscle weakness and atrophy at a young age aims to aid clinicians in similar challenging diagnostic situations as well as enhances our understanding of *POLG*-related disease phenotypes.

Published by Elsevier B.V.

1. Introduction

Human mitochondrial DNA (mtDNA) is continuously replicated in dividing cells and postmitotic tissues by the nuclear-encoded DNA polymerase gamma (POLG). POLG is the sole mtDNA polymerase and plays a major role in maintaining mtDNA integrity ([Milone and](#_bookmark6) [Massie, 2010](#_bookmark6)). POLG is a heterotrimer and consists of a catalytic sub- unit (p140) and 2 smaller identical accessory subunits (p55). The cat- alytic subunit is encoded by *POLG*, whereas the accessory subunits are encoded by *POLG2*. *POLG* maps to 15q25 and *POLG2* maps to 17q21.

*Abbreviations:* POLG, Polymerase gamma; MRI, Magnetic resonance imaging; EKG, Electrocardiogram; EEG, Electroencephalogram; GALT, Galactose-1-phosphate uridy- lyltransferase; *GJB1*, Gap junction beta-1; *MPZ*, Myelin protein zero; DNA, Deoxyribose nucleic acid; STR, Short tandem repeat; SIFT, Sort Intolerant from Tolerant.

⁎ Corresponding author at: Department of Molecular and Human Genetics, Baylor

College of Medicine, 1709 Dryden Rd, Rm 17.20, Houston, TX 77030, USA. Tel.: +1 713 798 3568; fax: +1 713 798 6450.

*E-mail address:* [dhar@bcm.edu](mailto:dhar@bcm.edu) (S.U. Dhar).

The catalytic subunit plays a critical role since it contains both poly- merase and proofreading exonuclease activities ([Longley et al.,](#_bookmark5) [2001](#_bookmark5)). Mutations in *POLG* represent the most common causes of auto- somally inherited mitochondrial diseases in children and adults ([Wong et al., 2008](#_bookmark11)). The ﬁrst mutation in *POLG* was discovered in 2001 in a Belgian family with Progressive External Ophthalmoplegia (PEO) ([Van Goethem et al., 2001](#_bookmark12)). The phenotype of patients with *POLG* mutations is often heterogeneous and, as observed in other mi- tochondrial disorders, may lead to involvement of several organ sys- tems. The reported *POLG* mutations are inherited either in an autosomal recessive or autosomal dominant manner ([DiMauro et](#_bookmark5) [al., 2006; Horvath et al., 2006](#_bookmark5)). Diagnosis in a clinical setting may pose a challenge to clinicians and molecular testing including se- quencing of *POLG* in suspected cases can help to reach a diagnosis and institute proper counseling. In this report we present an adult pa- tient with a complicated medical history where multiple evaluations had failed to arrive at a diagnosis until a novel *POLG* mutation, brieﬂy described in an earlier paper by [Tang et al. (2011)](#_bookmark9) was found. The clinical presentation of this patient and its association with a *POLG* mutation will add to our current knowledge and understanding of *POLG*-related disease phenotypes.

0378-1119/$ – see front matter. Published by Elsevier B.V. doi:[10.1016/j.gene.2012.02.034](http://dx.doi.org/10.1016/j.gene.2012.02.034)

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1. Subject and methods
   1. *Study subject*

A 23-year-old woman of mixed Caucasian and Mexican descent was evaluated in the Adult Genetics clinic for a history of bilateral cat- aracts, ovarian dysgenesis and distal muscle weakness and atrophy. Several studies including genetic testing were undertaken over a pe- riod of several years to determine a diagnosis, until a ﬁnal diagnosis was reached that explained her presentation.

* 1. *Non-genetic investigations*

At age 18years, she had a syncopal episode at school and under- went a diagnostic work-up that included an echocardiogram, EKG, cardiac MRI, brain MRI without contrast and EEG. At age 19years, due to progressive muscle weakness, electromyogram (EMG) and nerve conduction velocities (NCV) were carried out. Based on those results, a skeletal muscle biopsy was undertaken to understand the muscular atrophy. In the Genetics clinic, biochemical testing was sent for acylcarnitine analysis, plasma amino acids, urine organic acids, lactic acid levels, liver function tests and basic metabolic panel. An echocardiogram to determine structural anomalies of the heart was also ordered. A galactose 1-phosphate level was also obtained to rule out GALT deﬁciency due to the history of bilateral cataracts, ovarian dysgenesis and neurological compromise.

* 1. *Genetic testing*

DNA tests for spinal muscular atrophy and myotonic dystrophy were sent. Sequencing of *GJB1* and *MPZ* was also performed as part of the evaluation for Charcot–Marie Tooth disease. An oligonucleotide- based chromosomal microarray analysis to rule out genomic copy number variants was sent. Based on her overall clinical picture at

age 23years, it was decided to pursue a work-up for mitochondrial disease. Since mutations in *POLG* are associated with variable presen- tations that include but are not limited to encephalopathy, parkin- sonism, stroke-like episodes, exercise intolerance, ataxia–neuropathy and PEO (progressive external ophthalmoplegia) and limb myopathy, *POLG* sequencing was pursued. Subsequently, MitoMet array (copy

number analysis of metabolic and mitochondrial related genes) was also performed to evaluate for deletions in the *POLG* gene.

1. Results
   1. *Clinical history of the subject*

The subject was healthy at birth with no signiﬁcant problems dur- ing the ﬁrst year of life. At the age of 1year, she developed vision problems and was found to have bilateral cataracts which were re- moved. At the age of 2years, she manifested increasing difﬁculties with motor movements of both hands that led to delayed ﬁne motor skills which continued to progress, leading to the inability to use both of her hands, with consequent atrophy of arm and hand muscles. At the age of 16years she had a gynecological evaluation due to absent menarche which resulted in the diagnosis of ovarian dysgenesis. Hormone replacement therapy was initiated with appro- priate response. At the age of 18years, she had a syncopal episode at school. At the age of 23years, she presented with a seizure like epi- sode of unknown etiology. The episode consisted of generalized

tonic–clonic convulsions without loss of bowel or bladder continence

or tongue bite; however, she developed post-ictal confusion. Two months later, she was evaluated at the Adult Genetics clinic. At that time, she was attending college and exhibited an intact cognitive function. Family history was non-contributory for any history of

similar conditions. The patient has one sibling, a 24year old brother, who was diagnosed with depression.

* 1. *Clinical ﬁndings in the subject on physical examination (see Supplemental ﬁgure online)*

The patient was a thin young woman with a height of 170.2cm (75th–90th percentile) and a weight of 53.9kg (25th percentile). She had remarkable atrophy of arm and hand muscles but no facial dysmorphic features. Pupils were irregular and sluggish bilaterally. On neurological examination she was alert and oriented. There were no cranial nerve deﬁcits and her coordination was normal. There was severe atrophy of arm and hand muscles including biceps, tri-

ceps, forearm and hands with claw hand deformity and digitalization of the thumbs. However, deltoid muscles had normal bulk and tone. Lower extremities were diffusely thin and feet arches were normal. Motor examination in upper extremities revealed her power to be 0/5 at wrist ﬂexors and extensors, hand and forearm and 0/5 at bi- ceps; however, it was 5/5 at deltoid muscles and 3/5 at triceps. At the lower extremities, her power was 5/5 in proximal muscles includ- ing hip ﬂexors, quadriceps and hamstrings and 4/5 at tibialis anterior. She displayed absent reﬂexes in upper extremities, whereas her lower extremities had normal symmetric reﬂexes at knees and an- kles. The rest of the examination was unremarkable.

* 1. *Results of non-genetic investigations*

Echocardiogram, EKG, cardiac MRI, brain MRI without contrast and EEG done at the age of 18years did not detect any abnormalities. NCV revealed diffusely decreased amplitude of compound motor ac- tion potentials with otherwise normal motor and sensory responses including sural responses. EMG revealed mixed short and long dura- tion motor unit potentials with normal ﬁring frequency suggestive of a chronic myopathic process affecting the distal muscles although the pattern could also be seen in patients with chronic neurogenic conditions. Skeletal muscle biopsy revealed severe myoﬁber degener- ation and on electron microscopy there was evidence of end stage muscle disease that precluded interpretation.

Urine organic acid analysis showed presence of 3-methylglutaconic acid with normal 3 hydroxyisovaleric acid excluding the possibility of 3-methylglutaconic aciduria type I. All other biochemical tests were normal.

* 1. *Results of genetic testing*

*POLG* sequencing revealed a heterozygous variant, c.2851T>A (p.Y951N). Mitomet array was normal excluding a deletion on the other *POLG* allele. Neither parent carried the c.2851T>A (p.Y951N) *POLG* variant. As there are reports of multiple mtDNA deletions in both dominant and recessive forms of *POLG1*1, a repeat muscle biopsy was considered to look for deletions and to characterize the muscle involvement better. However, the patient declined a repeat biopsy.

All other genetic tests were normal.

1. Discussion

The clinical presentation associated with *POLG* mutations is very heterogeneous. The different clinical phenotypes include: 1) Alpers syndrome 2) PEO with or without Limb Myopathy 3) PEO, Parkinson- ism, and Early Menopause 4) Ataxia Neuropathy Spectrum (ANS) dis- orders 5) Myocerebrohepatopathy Spectrum disorders (MCHS) and

6) Myoclonus Epilepsy Myopathy Sensory Ataxia (MEMSA) ([Wong](#_bookmark11) [et al., 2008](#_bookmark11)). In this report we describe a 23-year-old woman with cataracts, ovarian dysgenesis and early-onset distal muscle weakness. This patient does not ﬁt into any of the prior classiﬁcations although there are some similarities with the phenotypes reported previously.

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Individuals with early menopause, as well as patients with peripheral neuropathy and/or cataracts have been described in the literature ([Milone and Massie, 2010](#_bookmark6)). The presence of ovarian dysgenesis seems to be a variable phenotype similar to early menopause and pre- mature ovarian failure ([Ledig et al., 2010](#_bookmark5)). Premature ovarian failure is a heterogeneous group of disorders characterized by amenorrhea and high serum gonadotropins in women less than 40years old. Ovar- ian dysgenesis which is characterized by the loss of follicles before puberty, describes the most severe premature ovarian failure out- come ([Ledig et al., 2010](#_bookmark5)). Multiple different genetic and non-genetic factors are known to play a role in the development of premature ovarian failure and ovarian dysgenesis ([Ledig et al., 2010](#_bookmark5)). Although premature ovarian failure has been described in patients with *POLG* mutations ([Tong et al., 2010](#_bookmark10)), ovarian dysgenesis has not been reported before and to the best of our knowledge, this is the ﬁrst re- port that links ovarian dysgenesis with a *POLG* mutation.

Although cataracts are not a common clinical ﬁnding associated with mitochondrial disorders, it has been reported in a mitochondrial disorder called Sengers syndrome ([Atiq et al., 2004](#_bookmark5)) which is charac- terized by congenital cataracts, lactic acidosis, skeletal myopathy and hypertrophic cardiomyopathy. In addition, cataracts have also been described in Autosomal Dominant PEO (adPEO) caused by *POLG* mu- tations ([Luoma et al., 2004](#_bookmark7)). The mechanism is not known although it has been speculated that deﬁciency of glutathione may cause a signif- icant increase in membrane permeability which in addition to the susceptibility of the lenses to oxidative damage may lead to inactiva- tion of the Na+/K+ pump. This effect in turn may result in ionic changes and cataract development ([Reddy et al., 1988](#_bookmark8)).

The onset of muscle weakness in this patient at such a young age is unusual for an autosomal dominant *POLG* mutation. Although distal myopathy has been previously described in *POLG*-related diseases ([Giordano et al., 2010](#_bookmark5)), it had an adult-onset. Skeletal muscle weak- ness and exercise intolerance can accompany PEO ([Luoma et al.,](#_bookmark7) [2004](#_bookmark7)); however, most of the previous reports with either neuropathy or myopathy caused by a heterozygous *POLG* mutation have been reported in older subjects ([Milone and Massie, 2010; Wong et al.,](#_bookmark6) [2008](#_bookmark6)). Inter-familial variation in the severity of muscle weakness has been reported ([Luoma et al., 2004](#_bookmark7)); however, this level of distal muscle atrophy at a very young age, observed in the current report is an intriguing ﬁnding associated with *POLG* mutations specially among heterozygous variants. This ﬁnding expands the clinical spec- trum associated with *POLG-*associated disorders.

Epilepsy is commonly observed in children and adults with reces- sive *POLG* mutations. Seizures may occur either in the setting of Alpers syndrome, in association with PEO, ataxia, and neuropathy, or as an isolated manifestation of *POLG* mutations ([Atiq et al.,](#_bookmark5) [2004](#_bookmark5)). Partial and secondary generalized seizures can occur. An oc- cipital EEG focus has been reported in the majority of the patients. Epilepsy is a very important factor inﬂuencing mortality in patients with *POLG* mutations. Abnormal brain MRI ﬁndings may develop and cerebral infarct-like lesions have been reported ([Deschauer et](#_bookmark5) [al., 2007; Engelsen et al., 2008; Tzoulis et al., 2006](#_bookmark5)). Onset of seizures in adulthood as observed in our patient is more likely to be associat- ed with a heterozygous mutation. However, it should be noted that the proband had only one seizure episode and her course could be unpredictable. It is not well known which antiepileptic drugs should be used as ﬁrst or second line agents in *POLG*-disease and many pa- tients need combined antiepileptic therapy. However, it is well established that sodium valproate should be avoided in *POLG*- disease or suspected *POLG*-disease due to the risk of hepatotoxicity and possible liver failure ([Kollberg et al., 2006; Tzoulis et al., 2006](#_bookmark5)). The need to avoid valproic acid as an antiepileptic therapy has been emphasized to our proband.

A heterozygous *POLG* variant c.2851T>A (p.Y951N) was identiﬁed

in this patient. Computational predictive programs, SIFT and Poly- Phen, suggest that this change may be pathogenic. The critical role

of tyrosine at position 951 of POLG in the same patient is recently summarized by [Tang et al. (2011)](#_bookmark9). As it has been previously dis- cussed, tyrosine at this position is highly conserved across different species. It should be noted that Y951 is one of the crucial residues in- volved in binding incoming nucleotides based on the structure analy- sis provided in [Fig. 1](#_bookmark4). Enzyme kinetic analysis using a mutant p.Y951A had nearly no polymerase activity of POLG, but maintained the same DNA binding afﬁnity to a DNA template ([Lim et al., 2003](#_bookmark5)). Further- more, p.Y951F, which lacks a phenolic hydroxyl atom, interferes with binding and incorporating incoming deoxynucleotides ([Lim](#_bookmark5) [et al., 2003](#_bookmark5)). Thus, the presence of the hydroxyl atoms of tyrosine at position 951 appears to be fundamental to incorporate the de- oxyribonucleotide triphosphate (dNTPs) and to facilitate the nucleotidyl transferase reaction. According to the classiﬁcation of pathogenic mutations proposed in the original *POLG* structure de- termination publication ([Lee et al., 2009](#_bookmark5)), p.Y951N change would belong to class I mutations which interfere with the catalytic active-site conﬁguration of the POLG enzyme leading to impaired enzymatic activity. Moreover, another class I mutation (p.Y955C change), located at the polymerase catalytic domain of POLG, has been associated with adPEO. This mutation has been shown to be associated with decreased binding afﬁnity to incoming nucleotides, and increased error-rate of polymerase reaction ([Graziewicz et al.,](#_bookmark5) [2004; Ponamarev et al., 2002](#_bookmark5)). Structurally, the amino acid Y955 is located adjacent to Y951 and their side chain atoms are tightly packed by hydrophobic interactions. Thus, the p.Y955C change is likely to have a direct inﬂuence on the conformation of Y951. It has been proposed that both Y951 and Y955 are parts of the nucle- oside binding site of POLG ([Lee et al., 2009](#_bookmark5)) and given their inti- mate chemical interactions, the classiﬁcation of the observed de novo variant in the case herein presented is in line with the dom- inant phenotype observed and is consistent with other mutations observed in this critical region.

In addition to the evidence for a phenotypic effect caused by an

autosomal dominant mutation in our proband, there are also reports of autosomal dominant *POLG* mutations in the literature ([Wong et](#_bookmark11) [al., 2008](#_bookmark11)). To determine whether the detected mutation was patho- genic, the parents were tested. Since they had a normal phenotype, presence of this mutation in either of them would point in favor of a familial variant. Parental testing revealed that neither parents were carriers, thus establishing that this was a de novo event in

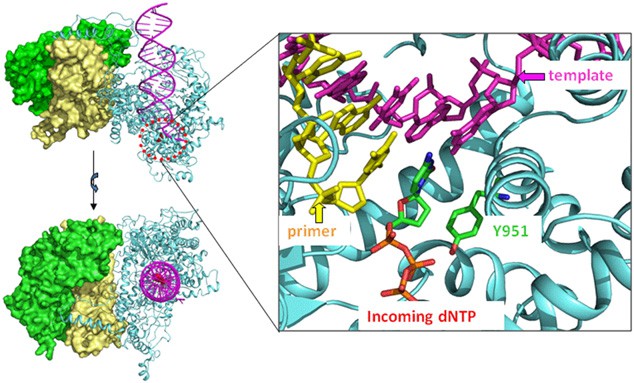


Fig. 1. The complex structure model of human mitochondrial DNA polymerase was constructed based on the holoenzyme and an extended DNA template from crystal structure of T7 polymerase complex (PDB accession code: 3IKM and 1T8E). On the left panel (a), the schematic representation of the complete structure: one POLG A sub- unit, which is represented by ribbon diagram colored in cyan, two monomers of POLG B shown as surface representation colored in green and pale yellow. The modeled DNA strand is shown as ribbon colored in purple. On the right panel (b), the zoomed in snapshot shows the partial active site with Y951 at the center and the close interaction with the incoming dNTP.

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the patient. One might have considered the possibility of non- paternity hence we performed identity testing with 15 unlinked STR markers which excluded non-paternity with a calculated proba- bility of paternity greater than 99%.

Another possibility was that the patient had one mutated allele and a second deleted allele which would establish an autosomal re- cessive pattern of inheritance. To clarify this further, a MitoMet array was performed which did not demonstrate any copy number variation on the other copy of the *POLG* gene, thus establishing that the de novo variant, p.Y951N is likely a dominant mutation. However, a second mutant allele deeply embedded in the introns or in the pro- moter region cannot be ruled out.

This patient presented with 3-methylglutaconic acid with normal

3 hydroxyisovaleric acid on urine organic acid analysis. 3- Methylglutaconic aciduria (3-MGCA) has 5 distinctive clinical types ([Gunay-Aygun, 2005](#_bookmark5)). An elevated amount of 3-hydroxyisovaleric acid (3-HIV) is seen in type I in contrast to normal levels in the other types. In 3-MGCA Type IV, which is mostly connected with mi- tochondrial dysfunction, most patients present early in life with non- speciﬁc neurological features like psychomotor retardation and muscle tone abnormalities; however, there have been reports of sen- sorineural hearing loss and ocular abnormalities including cataracts as observed in our patient ([Gunay-Aygun, 2005](#_bookmark5)). A potential associa- tion between 3-MGCA and *POLG* mutations has been published previ- ously by [Wortmann et al., 2009](#_bookmark13). The authors studied 18 children with 3-MGCA type IV with 4 clinical subgroups (encephalomyopathic, hepatocerebral, cardiomyopathic, myopathic). In the children with a hepatocerebral phenotype most with complex I deﬁciency, 3 out of

6 carried either homozygous or compound heterozygous *POLG*- mutations, indicating autosomal recessive inheritance ([Wortmann](#_bookmark13) [et al., 2009](#_bookmark13)). This current report is yet an evidence of an association between 3-MGCA and *POLG*-disease.

In summary, the clinical presentation of *POLG* mutations is fairly heterogeneous and it is difﬁcult to classify patients into speciﬁc syn- dromes. Here we present a new clinical presentation with cataracts, ovarian dysgenesis and an unusual early onset of distal muscle atro- phy and weakness as a result of a heterozygous *POLG* mutation. How- ever, we cannot rule out the possibility of a deep intronic mutation or a change in the promoter. The possibility of a digenic cause for the ob- served phenotype as well as distal myopathy of other etiology should also be considered. This report is also an evidence of association be- tween 3-MGCA and an apparently heterozygous *POLG* mutation.

* 1. *Structural modeling of POLG-nucleotide template and* *molecular analysis*

The structural model of human mitochondrial DNA polymerase was constructed based upon the 3.24Å protein structure (PDB acces- sion code: 3IKM) and an extended DNA template from the solved crystal structure of T7 DNAP-DNA complex (PDB accession code: 1T8E) ([Brieba et al., 2004; Lee et al., 2009](#_bookmark5)). The DNA template, primer and incoming nucleotide were positioned based on the alignment of active site residues of polymerase to ensure the accuracy of the modeling. The ﬁnal structure is a heterotrimeric protein made of one POLG molecule and two monomers of POLG2, with a modeled

DNA template, primer and incoming nucleotides. This model pro- vides stereochemical information for the key residues around the ac- tive site of POLG. The missense change was evaluated by examining the chemical interactions in the context of the complex structure.

Supplementary materials related to this article can be found on- line at doi:10.1016/j.gene.2012.02.034.

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