

Novel *TOP3A* Variant Associated With Mitochondrial Disease

Expanding the Clinical Spectrum of Topoisomerase III Alpha–Related Diseases

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Abstract

Objectives

Topoisomerase III alpha plays a key role in the dissolution of double Holliday junctions and is required for mitochondrial DNA (mtDNA) replication and maintenance. Sequence variants in the *TOP3A* gene have been associated with the Bloom syndrome–like disorder and described in an adult patient with progressive external ophthalmoplegia. The purpose of this report is to expand the clinical phenotype of the *TOP3A*-related diseases and clarify the role of this gene in primary mitochondrial disorders.

Methods

A 44-year-old woman was referred to our hospital because of exercise intolerance and creatine kinase increase. Muscle biopsy and a targeted next-generation sequencing (NGS) analysis were performed.

Results

A histopathologic assessment documented a mitochondrial myopathy, and a molecular analysis revealed a novel homozygous variant in the *TOP3A* gene associated with multiple mtDNA deletions.

Discussion

This case suggests that *TOP3A* is one of the several nuclear genes associated with mtDNA maintenance disorder and expands the spectrum of its associated phenotypes, ranging from a clinical condition defined Bloom syndrome–like disorder to canonical mitochondrial syndromes.

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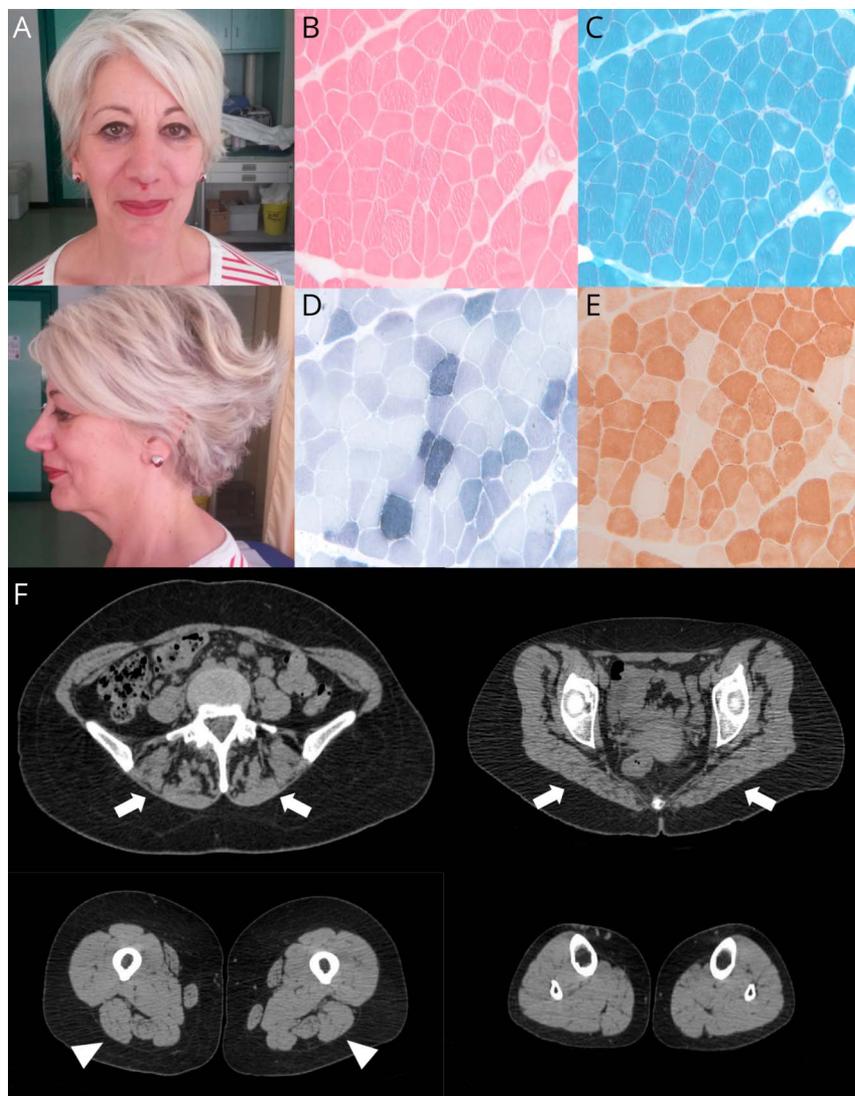
The *TOP3A* gene encodes topoisomerase III alpha (TOP3A), which exists in a nuclear and mitochondrial isoform,¹ with a key role in promoting the dissolution of double Holliday junctions and in mitochondrial DNA (mtDNA) replication and maintenance.² Recently, pathogenic variants in the *TOP3A* gene have been associated with the adult-onset progressive external ophthalmoplegia (PEO) syndrome complicated by cerebellar features² and with Bloom syndrome-like disorder characterized by clinical features and laboratory findings consistent with mitochondrial dysfunction.^{3,4} We report on a novel variant in the *TOP3A* gene in a patient with mtDNA maintenance disorder.

Case Presentation

The proband is a 59-year-old woman born at term to non-consanguineous Italian healthy parents. The neonatal period

was unremarkable. The patient's younger sister died of dilated cardiomyopathy when she was aged 17 years; the remaining family members (2 brothers and a sister) are clinically unaffected. The patient was referred to the Neurology Unit of our Hospital at the age of 44 years because of exercise intolerance and creatine kinase increase (1,999 IU/L; n.v. 30–170). Her medical history comprises a premature menopause at age 36 years and a pacemaker implantation for second-degree atrioventricular block at the young age of 37 years. The medical examination revealed the absence of deep tendon reflexes and short stature. In addition, the patient had a narrow face with a prominent nose due to the scarcity of subcutaneous fat (Figure 1A). Furthermore, the high level of glycated hemoglobin (42 mmol/mol, n.v. 23–41) was suggestive of diabetes. Lactate at rest was normal (1.6 mmol/L). EMG and nerve conduction study revealed a myogenic pattern associated with sensory axonal neuropathy. The histopathologic assessment of the patient's deltoid muscle biopsy,

Figure 1 Clinical, Muscle Biopsy, and Muscle CT Findings



(A) Physical examination showing a narrow face with a prominent nose due to the scarcity of subcutaneous fat. Serial skeletal muscle sections (deltoid) documenting (B) mild variation in fiber size with linear "cracks" (hematoxylin and eosin stain) and classic (C) ragged-red or (D) ragged-blue fibers, respectively, on modified Gomori trichrome and succinate dehydrogenase staining, which appeared (E) pale with COX stain (magnification 10x); Oil Red-O stain did not document lipid accumulation (figure not shown); (F) CT showing minor symmetrical degenerative changes and hypotrophy of the muscles of the posterior thigh compartment (arrowheads) and mild involvement of the paraspinal and gluteal muscles (arrows).

performed at the age of 45 years, showed mitochondrial proliferation with classic ragged-blue fibers, which appeared pale with cytochrome c oxidase (Figure 1B–E), indicating an underlying mitochondrial etiology. Audiological investigations detected a moderate high-frequency sensorineural hearing loss. CT muscle performed at the age of 53 years showed minor symmetrical degenerative changes and hypotrophy of the muscles of the posterior thigh compartment and mild involvement of the paraspinal and gluteal muscles (Figure 1F). Over the past few years, the patient has experienced a significant worsening of muscle fatigue and myalgia. The last neurologic evaluation, 15 years after the first, showed mild axial and proximal lower limb weakness.

Molecular Analysis

A first analysis of mtDNA excluded the common pathogenic m.3243A > G and m.8344A > G variants; on the contrary, long-range PCR revealed multiple mtDNA deletions in muscle samples (Figure 2A). A targeted next-generation sequencing analysis, using a Trusight One Expanded panel (a clinical exome from Illumina, which covers coding regions of 6,794 genes), revealed a novel homozygous variant in the *TOP3A* c.(247A > C) (Figure 2B), which is predicted to produce a p.(Thr83Pro) amino acid substitution in a highly conserved region (Figure 2C). Moreover, segregation analysis revealed that the healthy parents were heterozygous for the *TOP3A*

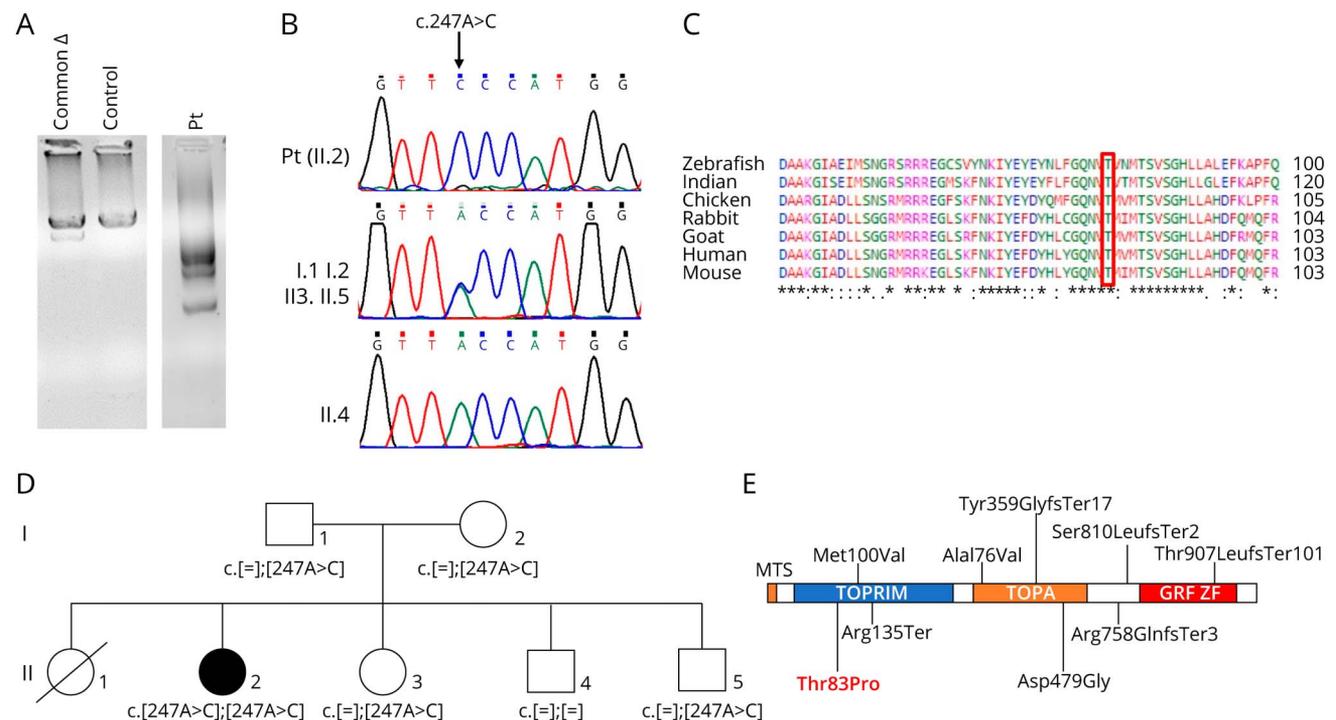
variant, and the healthy siblings were either heterozygous or wild type for the mutation (Figure 2D).

Discussion

The pathogenic variants described in the *TOP3A* gene were recently associated with PEO plus phenotype and multiple mtDNA deletions or with Bloom syndrome–like syndrome.^{2,4}

In this study, we report a novel homozygous *TOP3A* variant in a patient with multiple mtDNA deletions and late-onset mitochondrial disease characterized by multisystem involvement with predominant heart and skeletal muscle manifestations, such as exercise intolerance, myalgia, and myopathic weakness. This variant falls in a domain of the protein that is predicted to be functionally important (PM1; Figure 2E), and it is absent in the public available database (PM2). Furthermore, the mutation identified is predicted to produce an amino acid substitution in a highly conserved region (PP3). Additional typical “mitochondrial symptoms” included sensorineural hearing loss, peripheral neuropathy, diabetes, and premature menopause. Different from what is reported by Nicholls et al.,² the patient here described had no PEO or CNS involvement. Regarding the other phenotype associated with sequence variants in *TOP3A* defined as Bloom syndrome–like disorders, we observed similarities and

Figure 2 Molecular Studies of the Family Included in the Study and the Clinical Spectrum of *TOP3A*-Related Disease



(A) Long-range PCR showing multiple mitochondrial DNA (mtDNA) deletions in the index patient (Pt) in comparison with a control (Ctrl) and a patient positive for “common mtDNA deletion” (common Δ); (B) Electropherograms encompassing the mutated genomic region in *TOP3A* for patient (Pt, II.2), father (I.1) and mother (I.2), the heterozygous member of the family (II.3 and II.5), and the wild-type brother (II.4); (C) Protein alignments showing aminoacid residue conservation across the vertebrates; (D) pedigree of the family, in black the proband; (E) *TOP3A* domain organization: MTS: mitochondrial targeting sequence (AA 1-20); TOPRIM: topoisomerase/primase domain (AA 35-169); TOPA (AA 172-569); GRF ZF (AA 895-939); in black, a representation of the previously reported mutations; in red, the mutation reported in this article.

differences.^{3,4} In particular, the short stature, the dilated cardiomyopathy, the narrow face with prominent nose for paucity of subcutaneous fat (Figure 1A), and the absence of malignancies are in common with the patients described, but unlike them, our case presented a late onset of disease without cafe-au-lait macules or growth retardation.

This study has some limitations. Although we have documented histochemical and molecular evidences of mitochondrial dysfunction, further models of functional studies would have strengthened the pathogenic role of the detected *TOP3A* variant.

In conclusion, our report suggests that *TOP3A* is one of the several nuclear genes associated with mtDNA maintenance disorders.⁵ Regarding the clinical manifestations, pathologic variants in this gene are associated with a continuum of heterogeneous phenotypes, ranging from a clinical condition defined Bloom syndrome-like disorder to canonical mitochondrial syndromes.

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Appendix Authors

Name	Location	Contribution
Guido Primiano, MD, PhD	Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy	Designed and conceptualized study; major role in the acquisition of data; analysis of the data; and drafted the manuscript.

Appendix (continued)

Name	Location	Contribution
Alessandra Torracco, PhD	Bambino Gesù Research Hospital, Rome, Italy	Major role in the acquisition of data; analysis of the data; and revised the manuscript for intellectual content.
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Serenella Servidei, MD	Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy	Designed and conceptualized study; analysis of the data; and revised the manuscript for intellectual content.

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