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Short Communication

Early onset and severe clinical course associated with the m.5540G>A mutation in *MT-TW*☆

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ABSTRACT

We report a patient harboring a *de novo* m.5540G>A mutation affecting the *MT-TW* gene coding for the mitochondrial tryptophan-transfer RNA. This patient presented with atonic–myoclonic epilepsy, bilateral sensorineural hearing loss, ataxia, motor regression, ptosis, and pigmentary retinopathy. Our proband had an earlier onset and more severe phenotype than the first reported patient harboring the same mutation. We discuss her clinical presentation and compare it with the only previously published case.

1. Introduction

Since the first mutations in mtDNA were described, more than 200 pathogenic point mutations and rearrangements have been found in patients with mitochondrial cytopathies [1]. Mitochondrial tRNA genes are hotspots for mutations associated with multi-organ involvement, making them a major

Abbreviations: CoQ10, coenzyme Q10; COX, cytochrome *c* oxidase; CS, citrate synthase; EEG, electroencephalogram; MRI, magnetic resonance imaging; mtDNA, mitochondrial DNA; NADH, nicotinamide adenine dinucleotide dehydrogenase; NGS, next generation sequencing; SCA, spinocerebellar ataxia; tRNA, transfer RNA; $tRNA^{Trp}$, tryptophan-transfer RNA.

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contribution to mitochondrial disorders caused by mtDNA mutations, due to their essential role in mitochondrial protein synthesis [2].

We report the second case of a patient carrying a *de novo* m.5540G>A mutation affecting the mitochondrial $tRNA^{Trp}$ gene. This patient presented with an early onset and severe progressive encephalopathy associated with sensorineural hearing loss.

2. Case report

We present the case of a 10-year-old female, born after an uneventful pregnancy as the second child of a non-consanguineous healthy couple. Her family history did not reveal other family members afflicted with neuromuscular or hearing disorders. She had normal neurodevelopment until age 6 years. At that time, she presented with atonic–myoclonic epilepsy, associated with ptosis of the right eye. Brain MRI revealed bilateral basal ganglia calcifications. The EEG reported abnormal multifocal spike discharges with background slowing. However, the EEG pattern later evolved, revealing both multifocal and generalized spikes. Initially, the epilepsy was treated successfully with zonisamide. At age 7 years, the patient exhibited progressive gradual decline of truncal and appendicular ataxia, tremor, spasms, muscular weakness, regression of gross and fine motor skills, and bilateral sensorineural hearing loss. Verbal skills and cognition were normal. Carnitine, coenzyme Q10 (CoQ10) and a B vitamin complex supplementation were trialed. However, no improvement in symptomatology was observed prompting their discontinuation. At age 10 years, she was evaluated at the Texas Children's Hospital Pediatric Genetic Clinic. She was receiving multiple anticonvulsants to control her epilepsy. Clinical examination was remarkable for low weight (17.1 Kg, <3rd percentile), short stature (125.7 cm, 2nd percentile), axial hypotonia, appendicular hypertonia, contractures of the fingers, muscle weakness, hyperreflexia of the lower extremities, decreased muscle mass, ataxic gait, dysmetria, horizontal nystagmus, and bilateral ptosis greater on the right upper lid. Ophthalmologic examination found a symmetric diffuse granular retinal pigment epitheliopathy from the macular areas to the far periphery, without pigment migration into the retina. The retinal vessels and the optic discs appeared normal. The patient was lost to follow up.

3. Results

Initial laboratory studies revealed normal urine organic acid analysis, plasma amino acids, and acylcarnitine profile. Plasma lactic acid was elevated at 3.3 mmol/L (0.2–2 mmol/L). The clinical presentation suggested a mitochondrial cytopathy. A skeletal muscle biopsy was performed. Ragged blue fibers were found with NADH staining. Trichrome staining was normal. There was weak COX staining in type 2 fibers. Electron microscopy reported increased number and pleomorphism of mitochondria. Spectrophotometric analysis of mitochondrial respiratory chain enzymatic activities on skeletal muscle [3–6] showed partial reductions in complex II+III and rotenone sensitive complex I+III activities (Table 1). Analysis of a panel of common mtDNA point mutations and single deletions performed in blood did not detect deleterious mutations. Comprehensive analysis of the circular mitochondrial genome by NGS revealed a 25.6% heteroplasmic mutation, m.5540G>A, in blood. Heteroplasmy of this mutation in skeletal muscle was 51.4%. The m.5540G>A is located in the anti-codon stem of mitochondrial $tRNA^{Trp}$ and changes the original G:C pairing to A:C

Table 1
Mitochondrial respiratory chain complex enzyme analysis on skeletal muscle.

ETC complexes	Activity (%)[a]
Complex I	522 (186, 144)
Complex I + III	3.91 (43, 33)
Complex II	12.11 (149, 115)
Complex II + III	3.37 (69, 53)
Complex IV	48.7 (167, 129)
Citrate synthase (CS)	363 (129, 100)

[a] Activity: nmol/min/mg protein. First number in the parentheses is % of normal mean activity, the second number in the parentheses is % normalized against citrate synthase (CS) activity.

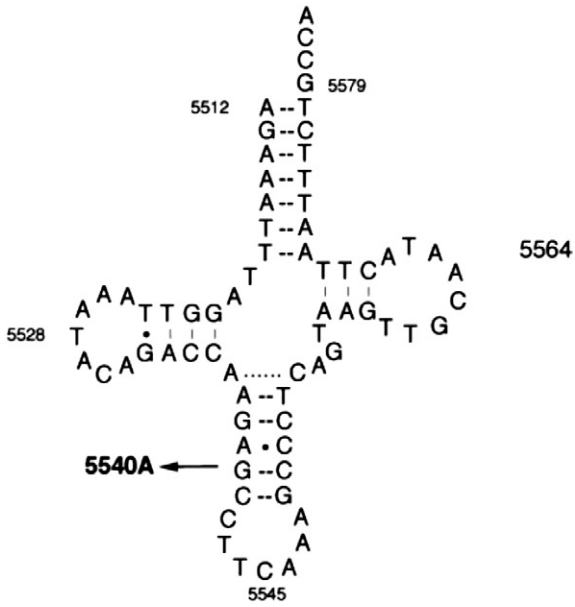

Fig. 1. Schematic representation of the mitochondrial $tRNA^{Trp}$ secondary structure, showing the m.5540G>A mutation.

mispairing (Fig. 1). Analysis of the mitochondrial genome from the blood of the patient's mother did not detect the mutation.

4. Discussion

Mutations in the *MT-TW* gene coding for the mitochondrial $tRNA^{Trp}$ are associated with diverse phenotypes, such as neonatal onset mitochondrial encephalomyopathy [7], mitochondrial myopathy [8], Leigh syndrome [9], dementia and chorea [10], and a neurogastrointestinal syndrome [11]. Here we present the case of a female patient harboring the m.5540G>A transition involving the *MT-TW* gene. This particular mutation has been reported once previously [12]. As in our proband, that patient manifested a neurological phenotype. However, the onset of neurological disease occurred in the second decade of life. That subject exhibited SCA and sensorineural hearing loss, peripheral neuropathy, dysmetria, dysdiadochokinesia, elicited nystagmus, areflexia of lower extremities, pes cavus, muscle hypotrophy, apallesthesia, and lactic acidosis. The first features of dementia began in the third decade of life. EEG demonstrated diffuse background slowing and multifocal spike waves. Brain MRI showed marked atrophy of brain and cerebellum, diffuse white matter abnormalities and basal ganglia hypointensities. That patient was reported to have a 98% heteroplasmy level on skeletal muscle, and the authors demonstrated by single fiber PCR that the COX-negative fibers had a significantly higher percentage of heteroplasmy than their surrounding normal fibers. The patient died at age 41 years of bronchopneumonia.

A comparison of clinical features between these two patients reveals some remarkable differences (Table 2). Despite having a lower heteroplasmy in skeletal muscle (51.4%), our patient developed signs of neurodegeneration and cerebellar dysfunction at an earlier age, and exhibited a worse clinical course and a faster clinical progression. This finding suggests that the level of heteroplasmy in skeletal muscle may not always be a good predictor of the severity of the neurological disease. In addition, our patient may have a higher level of heteroplasmy in the central nervous system. Likewise, the patient from the previous report may have had a different tissue distribution of the mutation heteroplasmy, leading to later onset and milder clinical phenotype. Furthermore, other factors such as nuclear and environmental modifiers and specific mitochondrial haplotypes may be responsible for the observed clinical heterogeneity. It is noticeable that in our case muscle morphology was more sensitive than the analysis of mitochondrial respiratory chain

Table 2
Comparison between our proband and the previously reported case.[a]

	Proband	Silvestri et al.
Age of onset of major neurological features	6 yrs (atonic–myoclonic epilepsy)	Adolescence (spinocerebellar ataxia)
Hearing loss	7 yrs sensorineural, bilateral, progressive	6 yrs sensorineural
Epilepsy	6 yrs	No
Ataxia	7 yrs, progressive	Adolescence, progressive
Dysmetria	Yes	Yes
Dysdiadochokinesia	Yes	Yes
Tremor	Resting and intentional tremor on trunk, head and extremities	Not reported
Nystagmus	Jerky saccades and very irregular pursuit movements	Elicited. Normal eye movements
Ptosis	Yes	Not reported
Paresis	In lower extremities	Not reported
DTR	Normal in upper extremity, brisk in lower extremities	Areflexia of lower extremities
Cognition	Normal	Dementia during third decade of life
Pigmentary retinopathy	Yes	Not reported
Brain MRI	Basal ganglia calcifications	Marked cortical and cerebellar atrophy, diffuse white matter abnormalities and basal ganglia hypointensities
Muscle biopsy	Ragged blue fibers with NADH staining. Signs of denervation and reinnervation. EM: Mitochondrial proliferation and pleomorphism of mitochondria without matrix inclusions.	Severe mitochondrial myopathy with numerous COX-negative ragged red fibers
Heteroplasmy level (skeletal muscle)	51.4%	98%
Mitochondrial enzyme assays	Partial reduction in RC complexes I + III and II + III	COX deficiency (11%)

COX: citochrome *c* oxidase, NADH: nicotinamide adenine dinucleotide dehydrogenase, RC: respiratory chain.
[a] [12].

enzymatic activities. Although we have been able to demonstrate a deficiency in complex I + III activity by running a complete panel of mitochondrial enzymes, the reduction did not meet a major criterion (residual activity of <20%) based on Walker modified criteria. This finding may be due to the fact that, with a relatively low mutational load in skeletal muscle, a severe biochemical defect may be present only in a few muscle fibers and may not be detectable by a spectrophotometric assay. Moreover, our patient has certain findings not described in Silvestri's paper, such as epilepsy, hypertonia, muscle spasms, ptosis, and pigmentary retinopathy. The earlier report emphasized dementia late in the third decade of her life and severe cortical and cerebellar atrophy [12]. The brain MRI in our proband did not reveal these findings, perhaps reflecting that she is younger, or that these features may require time to evolve. Both patients shared several neurologic features including childhood-onset sensorineural hearing loss and multiple signs of cerebellar dysfunction (ataxia, nystagmus, tremor, dysmetria, and dysdiadochokinesia).

COX deficiency and COX negative fibers are the most frequent biochemical and histochemical features of *MT-TW* mutations, including Silvestri's patient [2,7,8,11–17]. Although our proband did not have COX deficiency on mitochondrial enzyme assays, she had reduced staining for COX in type 2 fibers. These findings may reflect the fact that two COX subunits, COI and COIII, have a relatively higher content of tryptophan (3.1% and 4.6%, respectively) than the average content of the other mtDNA-encoded subunits (2.6%) [8]. The absence of ragged red fibers in our patient could be explained by a muscle biopsy done at a young age [18].

Multiple sequence alignment with CLUSTALW reveals that this mutation affects a highly conserved nucleotide in the anticodon arm stem (Fig. 1). According to RNAalifold analysis [19] (http://rna.tbi.univie.ac.at/cgi-bin/RNAalifold.cgi), this nucleotide participates in a highly conserved base pair. The RNAfold software [20] (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi), predicted that this transition may alter the secondary structure of the tRNA. Currently, this mutation is not registered in mtDB [21] (http://www.mtdb.igp.uu.se/) and is reported as pathogenic in MITOMAP (http://www.mitomap.org/MITOMAP). It is classified as definitively pathogenic with a score >30 according to the scoring criteria devised by Scaglia and Wong [2].

In conclusion, this case further supports the pathogenic effect of the m.5540G>A mutation, and its association with a neurological phenotype. Moreover, the shared clinical presentation of cerebellar ataxia and sensorineural hearing loss could suggest a discernible phenotype that should be considered during the diagnostic assessment of patients with genetic ataxias. Tissue distribution of mutation heteroplasmy, and other genetic modifiers may be responsible for the clinical heterogeneity observed in these two reported patients. The earlier age of onset and more severe clinical course in our proband expands the neurological phenotype associated with this mtDNA mutation.

Conflict of Interest

The authors declare that they do not have any conflict of interests to report.

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