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Case report

Late-onset MNGIE without peripheral neuropathy due to incomplete loss of thymidine phosphorylase activity

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A B S T R A C T

Mitochondrial NeuroGastroIntestinal Encephalomyopathy (MNGIE) is an autosomal recessive disorder characterized by severe gastrointestinal dysmotility, cachexia, peripheral neuropathy, ptosis, ophthalmoplegia, and leukoencephalopathy with early onset and severe prognosis. Mutations in the TYMP/ECGF1 gene cause a loss of thymidine phosphorylase catalytic activity, disrupting the homeostasis of intramitochondrial nucleotide pool. We report a woman with a very late onset of MNGIE, lacking peripheral neuropathy. Thymidine phosphorylase activity was markedly reduced in cultured fibroblasts, but only mildly reduced inuffy coat, where the defect is usually detected, and plasma thymidine was mildly increased compared to typical MNGIE patients. TYMP/ECGF1 analysis detected two heterozygous mutations, including a novel missense mutation. These findings indicate that a partial loss of thymidine phosphorylase activity may induce a late-onset and incomplete MNGIE phenotype.

1. Introduction

The syndrome of Mitochondrial NeuroGastroIntestinal Encephalomyopathy (MNGIE, MIM #603041) is an autosomal recessive disorder of oxidative phosphorylation caused by mutations in TYMP/ECGF1, the gene encoding thymidine phosphorylase (TP), a crucial enzyme for the homeostasis of intramitochondrial nucleotide pool [1]. Mutations in TYMP/ECGF1 lead to nearly absent TP catalytic activity, producing systemic accumulations of its substrates, thymidine (dThd) and deoxyuridine (dUrd) in body fluids. Toxic levels of dThd and dUrd induce nucleotide pool imbalances that in turn lead to mtDNA abnormalities (point mutations, multiple deletions, and depletion) [2].

The typical clinical features of MNGIE consist in severe gastrointestinal dysmotility, cachexia, peripheral neuropathy (PN), eyelid ptosis and ophthalmoplegia, and are associated with remarkable leukoencephalopathy evidenced at brain MRI [3]. Clinical onset is usually in young adulthood, with a mean of 19 years, and the prognosis is severe, with a mean age at death of 37 years. The clinical diagnosis can be confirmed by measuring TP activity in buffy coat or the plasma levels of dThd and dUrd [4], and it is subsequently corroborated by molecular investigations.

Although MNGIE has a relatively homogeneous biochemical and clinical phenotype, a few patients with milder biochemical alterations and less severe clinical manifestations have been described in association with mutations in TYMP/ECGF1 [5]. Cases have also been reported presenting highly similar clinical features but lacking leukoencephalopathy, and displaying normal enzyme activity. None of these phenocopies presented mutations in TYMP/ECGF1.

We report on a 67-year-old woman presenting, since age 53 years, the typical MNGIE phenotype but lacking PN. TP activity was mildly reduced in buffy coat, but it was markedly reduced in cultured skin fibroblasts. Direct gene sequencing showed pathogenic mutations in TYMP/ECGF1.

2. Patients and methods

2.1. Case description

A 67-year-old woman, the first of two daughters born to unrelated parents, was healthy until the age of 53 when she underwent a first exploratory laparotomy for profound melena due to bleeding from small intestine ulcers. During surgery, colic-peritoneal adhesions were released and some nodules in the small bowel were...
biopsied. Histological examination revealed fibro-adipose transformation with chronic inflammation. In the following years, the patient presented several episodes of melena and recurrent gastrointestinal manifestations such as early satiety, vomiting, irregular bowel movements and borborhymia. At age 63 years, she presented abdominal pain and distension with fever for which she underwent a second laparotomy that disclosed a purulent peritonitis due to perforation of a small bowel diverticulum. A small bowel resection, with a termino-terminal anastomosis was performed at this time. The following year the patient underwent a new resection of the ileus, after presenting abdominal pain and distension. The patient has always been thin, and her weight progressively declined from 48 to 39 kg. Around age 55 years she started complaining of diplopia, associated with a progressive loss of ocular motility and bilateral eyelid ptosis. At age 64 years, she noticed a progressive gait unbalance, a subjective sensation of “light head” and loss of short-term memory, and a pulsating headache. Moreover, she referred of a recent episode of loss of consciousness without a history of epilepsy. When admitted to our department at age 65 years, the patient was cachectic: her weight was 39 kg and her height 161 cm. Neurological examination revealed diffuse muscle atrophy, mild static and dynamic ataxia, bilateral external ophthalmoplegia and ptosis, and a fine head tremor. All other functions, including strength and sensation, were normal. Deep tendon reflexes were uniformly brisk, and plantar reflexes were flexor. There were neither serological nor cerebrospinal fluid (CSF) overt abnormalities, except for moderately increased lactate levels, which were 27.2 mg/dl in serum (n.v. = 4.5–19.8), and 26.7 mg/dl in the CSF (n.v. = 10–22). CSF protein level was 46.4 mg/dl (n.v. = 10–45 mg/dl). EMG studies showed a myopathic pattern in the four limbs, whereas nerve conduction studies (NCS) showed normal distal latencies, amplitude, and nerve conduction velocities of the explored nerves (Table 1). Neuropsychological testing revealed a mild impairment of visuo-spatial memory. A brain MRI showed diffuse leukoencephalopathy of the cerebral hemispheres and ponto-mesencephalic junction (Fig. 1A). Audiometric testing revealed a subclinical, bilateral, sensorineural hearing loss. An EEG showed isolated spikes and/or sharp waves localized in right temporal region and less evident in left temporal region (Fig. 1B). An open muscle biopsy showed mildly increased variation in fiber diameter, several ragged red and succinate dehydrogenase (SDH)-hyper reactive fibers and cytochrome c oxidase (COX)-negative fibers but no neurogenic changes (Fig. 1C). Spectrophotometric determination of respiratory chain complex activities performed in muscle homogenate and cultured skin fibroblasts disclosed a slightly reduced activity of complex I (65% of normal value) but normal complex IV activity.

Family history was only significant for a maternal cousin who had died at age 35 years because of an allegedly severe muscle disease. Inspection of one of her ID photographs showed a cachectic young lady with bilateral eyelid ptosis. No additional clinical notes were produced by her relatives.

2.2. Biochemical and molecular analyses

TP activity was measured using spectrophotometric method as described previously [6]. Briefly, buffy coat obtained from venous blood and cultured fibroblasts, were homogenized in lysis buffer (50 mM Tris–HCl, pH 7.2, containing 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride and 0.02% mercaptoethanol) and then sonicated for 30 s. Samples were centrifuged at 13,000 g for 30 min at 4 °C. Supernatants protein concentration was determined according to the bicinchoninic acid method [7]. The reaction mixture (final volume 100 μl), containing 100 μg of protein, 10 mM dThd in 0.1 M Tris–arsenate, pH 6.5, was incubated at 37 °C for 1 h. The reaction was terminated by the addition of 1 ml of 0.3 N NaOH. In parallel with each sample, a blank was also processed, to which dThd was added after the addition of NaOH. The amount of thymine formed was measured at 300 nm wavelength and determined based on the 3.4 × 10^3 l/mmol.cm difference in the molar extinction coefficient between dThd and thymine at alkaline pH. Enzyme activity was expressed as nmol of thymine formed per hour per milligram of protein.

Nucleoside concentration was measured in plasma by HPLC. Freshly collected plasma (200 μl) was added to 200 μl of ice-cold 0.6 M HClO4 under vortex mixing. Precipitated proteins were spun down by centrifugation (2 min at 12,000g) and clear supernatants were brought to pH 6–7 by adding suitable amounts of ice-cold 3.5 M K2CO3 and discarding precipitated KClO4 by centrifugation. EMGs showed a myopathic pattern in the four limbs, whereas nerve conduction studies (NCS) showed normal distal latencies, amplitude, and nerve conduction velocities of the explored nerves (Table 1). Neuropsychological testing revealed a mild impairment of visuo-spatial memory. A brain MRI showed diffuse leukoencephalopathy of the cerebral hemispheres and ponto-mesencephalic junction (Fig. 1A). Audiometric testing revealed a subclinical, bilateral, sensorineural hearing loss. An EEG showed isolated spikes and/or sharp waves localized in right temporal region and less evident in left temporal region (Fig. 1B). An open muscle biopsy showed mildly increased variation in fiber diameter, several ragged red and succinate dehydrogenase (SDH)-hyper reactive fibers and cytochrome c oxidase (COX)-negative fibers but no neurogenic changes (Fig. 1C). Spectrophotometric determination of respiratory chain complex activities performed in muscle homogenate and cultured skin fibroblasts disclosed a slightly reduced activity of complex I (65% of normal value) but normal complex IV activity.

### Table 1

<table>
<thead>
<tr>
<th>Sensory nerves</th>
<th>Motor nerves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left radial</td>
<td>Right ulnar</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>1.65</td>
</tr>
<tr>
<td>Amplitude (μV)</td>
<td>17.0</td>
</tr>
<tr>
<td>Velocity (m/s)</td>
<td>66.7</td>
</tr>
<tr>
<td>Right sural</td>
<td>Right tibial</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>2.0</td>
</tr>
<tr>
<td>Amplitude (μV)</td>
<td>9.4</td>
</tr>
<tr>
<td>Velocity (m/s)</td>
<td>57.5</td>
</tr>
<tr>
<td>Left sural</td>
<td>Left peroneal</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>2.90</td>
</tr>
<tr>
<td>Amplitude (μV)</td>
<td>7.8</td>
</tr>
<tr>
<td>Velocity (m/s)</td>
<td>51.7</td>
</tr>
</tbody>
</table>

ADM, abductor digiti minimi; Be Elb, below elbow; Ab Elb, above elbow; AHB, abductor hallucis brevis; EDB, extensor digitorum brevis; Be Knee, below knee; Ab Knee, above knee.
tration/area linear plots were developed for quantification. A mixture of all standard solutions was injected daily to check the reliability of separation and any modification of retention times due to the chromatographic system.

Total DNA was extracted from peripheral blood and tissue homogenates following standard procedures. Direct sequencing of the TYMP/ECGF1 gene used methods reported elsewhere [1]. Southern blotting to determine qualitative or quantitative alterations of mtDNA [8] and qPCR determination of the levels of mitochondrial genome in muscle homogenate [9] used described methods.

3. Results

Residual TP activity in the patient’s leukocytes was 132 nmol/h/mg, corresponding to 18% of mean normal value (range = 250.6–1211 nmol/h/mg of proteins; n = 50), whereas TP activity in the patient’s cultured fibroblasts was 5.7 nmol/h/mg, corresponding to 4.75% of mean normal value (range = 40–200 nmol/h/mg; n = 5). Plasma dThd was mildly elevated (2.1 μM/l) in the patient (n.v. = <0.05 μM/l; n = 10), whereas dUrd was undetectable (n.v. = <0.05 μM/l; n = 10).

Direct sequencing of TYMP/ECGF1 identified the c.1160-1G>A mutation affecting the splice acceptor site of intron 8, and already described to lead to skipping of exon 9 [1], in compound heterozygosity with the novel c.1135G>A (p.E379K) mutation which involves a highly conserved residue in TP (Fig. 1D). The former variant was also detected in the healthy mother but not in the sister of the proband, whereas the novel mutation was not detected in 300 control alleles. Southern blotting did not reveal gross deletions nor severe depletion of mtDNA whereas qPCR analyses showed partial loss of mtDNA genomes in muscle and cultured cells (43% and 58% of normal).

4. Discussion

The patient reported here shows a multisystem disease suggestive of a defect of oxidative metabolism and, to the best of our knowledge, she represents the oldest living MNGIE patient reported and the one with the latest onset. Despite a later age at onset, the complex clinical setting reproduces the MNGIE syndrome with a high degree of central nervous system symptoms possibly related to the patient’s leukoencephalopathy, including cerebellar ataxia, tremor and memory loss, associated with EEG focal abnormalities.

Contrary to patients reported so far, however, there was no evidence of PN, which is regarded as a cardinal feature of MNGIE [10] and, at times, mimics the neurophysiological and clinical presentation observed in CIDP or Charcot–Marie–Tooth disease [11,12]. Interestingly, rare cases with incomplete syndrome have been described, including one late-onset patient with normal NCS after 12 years of disease progression [5,13]. Although follow-up NCS may be necessary, it is possible that, in late-onset MNGIE, PN is dispensable. A variable penetrance might be related to different TYMP/ECGF1 mutations and their ability to promote a toxic effect exerted by the accumulation of unprocessed nucleosides upon the turnover of mtDNA.
Attempts to establish genotype–phenotype correlations in severe MNGIE have been generally disappointing and the degree of clinical severity does not appear to be fully influenced by the extent of reduced TP activity. However, there are cases in whom a less severe TP dysfunction in buffy coat (about 15% of control values) and a moderate accumulation of plasma nucleosides appear to correlate well with a later-onset, milder clinical form [5]. This is in part questioned by findings in our patient in whom the moderate reduction of enzyme activity observed in buffy coat and the mild plasma nucleoside accumulation might well explain both an older age at disease manifestations and lack of a full-blown syndrome, whereas the more marked TP deficit determined in cultured cells would be compatible with a more severe phenotype. It remains too speculative to hypothesize that the differential reduction of TP activity among tissues in the same patient would be attributable to the specific mutations identified, including the novel p.E379K variant. Selection in vitro of cells with high concentrations of nucleosides or a higher mtDNA mutation rate or other factors might also be considered. Regardless of these hypotheses, determination of TP activity in buffy coat should be considered as the standard approach, due to the higher cost and time consumption of cell culturing. Molecular studies remain, however, conclusive for a precise diagnosis.

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