Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) in a Malaysian patient with a novel mutation in thymidine phosphorylase gene: A case report

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Abstract

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a rare neurodegenerative multisystem disorder inherited in an autosomal recessive manner and characterized clinically by gastrointestinal dysmotility, cachexia, ophthalmoparesis and/or ptosis, peripheral neuropathy and leukoencephalopathy. Heterogenous causative mutations in the thymidine phosphorylase (TP) gene located on chromosome 22q13 have been identified. This is the first reported case of a 25-year-old Malaysian patient, of indigenous Bajau ethnicity who presented with recurrent abdominal pain before developing other clinical features of classical MNGIE. Biochemical correlates include elevated plasma levels of thymidine, deoxyuridine and lactate. The brain MRI showed diffuse leucoencephalopathy while nerve conduction studies were consistent with demyelinating polyneuropathy. Direct DNA sequencing of the nine coding exons of the TP gene showed both a novel and a previously described mutation. The former is a point mutation in exon 5 (NG_011860.1:g.7387C>T) at amino acid position 179, resulting in a stop codon and premature truncation of thymidine phosphorylase (TP) protein while the latter mutation occurred at exon 10 (NG_011860.1:g.9279C>T) resulting in a missense homozogous mutation at amino acid position 471. Definite diagnosis was based on clinical features, plasma and urinary nucleosides and the identification of mutations in the TP gene. This case report adds to the knowledge of genotype-phenotype relationship of TP mutations and its occurrence among ethnic groups worldwide.

INTRODUCTION

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is the result of mutations in the thymidine phosphorylase (TP) gene and a defective TP enzyme which catalyzes the phosphorolysis of thymidine to thymine and deoxyribose-1-phosphate. The disease is clinically defined by a multisystem syndrome affecting the central and peripheral nervous system, muscle and the gastrointestinal tract. The clinical onset is variable but is typically between the second and fifth decades of life. This is the first reported case of a Malaysian patient with this disorder. Molecular analysis confirmed a novel mutation.

CASE REPORT

A 25-year-old Malaysian man of ethnic Bajau descent was admitted for recurrent right iliac fossa pain and associated increased frequency of bowel motion and weight loss of 22 kg over a period of 7 years. The stool was normal in appearance, consistency and volume. Previous investigations were performed for coeliac disease and Marfan’s Syndrome three years ago but had defaulted further clinical follow-up. Family history was significant. He was the fifth out of eight siblings. He was the product of a consanguineous marriage as his parents were distant cousins. The pregnancy was uneventful and he was born via normal vaginal delivery. His developmental milestones were normal. His second brother had a similar illness and passed away at the age of 25 several years ago. Both his sisters had histories of recurrent abdominal pain. On physical examination, he was cachetic with a body mass index (BMI) of 11.1 kg/m². His cognitive function was intact. There
were no cranial nerve palsies except for bilateral ptosis and a complex ophthalmoplegia. The fundi were normal. There was wasting in all limbs. The muscle tone was reduced. Both the proximal and distal muscle power was equal at grade 4. The distal deep tendon reflexes were absent. There was no objective sensory loss.

Further laboratory studies showed elevated serum lactate at 3 mmol/L (normal 0.6-2.2 mmol/L). A lumbar puncture revealed elevated CSF lactate (3.4 mmol/L; normal < 2.8 mmol/L). There was hypoalbuminemia (28; normal 34-50g/L). The calcium and phosphate were 2.22 (normal 2.12-2.52 mmol/L) and 1.17 (normal: 0.81-1.58 mmol/L), respectively. The serum ferritin was 28.3 ng/ mL (normal: 22-322). The thyroid function test and the creatine kinase level were both normal. The anti-endomysial antibody and the anti-tissue transglutaminase antibody were both negative. Electrocardiogram showed normal sinus rhythm without conduction block. The audiometry test showed bilateral sensorineural hearing loss. The nerve conduction studies revealed demyelinating sensorimotor neuropathy. Both muscle biopsy and electromyography were not performed. The T2-weighted MRI and flair sequence of the brain revealed bilateral leukodystrophy, sparing the corpus callosum (Figure 1 and Figure 2). CT scan of the abdomen was normal. Duodenal biopsies were performed and showed normal villous appearance and nonspecific duodenitis. Biopsies of the terminal ileum and colon were normal.

Elevated urinary deoxyuridine (256 μmol/ mmol creatinine; normal < 1) and urinary thymidine (298 μmol/mmol creatinine; normal < 1) (Figure 3) were detected. There was also elevated plasma deoxyuridine (16 μmol/L; normal < 1 μmol/L) (Figure 4). Thymidine phosphorylase activity was not quantified.

The Institutional Review Board of the University of Malaya Medical Centre, Kuala Lumpur gave approval for the protocol of genetic studies for this patient. Written informed consent for blood samples and for the use of medical data for publications were obtained from all the family members including the proband. Blood was collected in ethylenediamine tetraacetic acid (EDTA) tubes. For the screening of mutations, DNA was isolated from peripheral blood leukocytes of the proband using standard methods. The whole blood sample was extracted using the protocol as per manufacturer’s instructions from the QIAamp® DNA Blood Mni Kit (Qiagen GmbH, Hilden, Germany). The TP gene was amplified using previously published primers.3,4 PCR amplification conditions can be made available upon request. Direct sequencing of both sense

Figure 1. T2-weighted MRI brain showing bilateral leukodystrophy of the proband
and antisense strands of the amplified products was performed using dye terminator chemistry, and the fragments were loaded on an automated DNA sequencer. The nine coding exons of the TP gene (exons 2–10) were amplified using nine sets of intronic primers, as previously described based on modified protocols. The patient harboured two mutations in exons 5 and 10, respectively. There was a novel point mutation in exon 5 (NG_011860.1:g.7387C>T) at amino acid position 179, resulting in a stop codon and premature truncation of thymidine phosphorylase (TP) protein. The latter mutation occurred at exon 10 (NG_011860.1:g.9279C>T) resulting in a missense homozygous mutation at amino acid position 471 and this mutation had previously been described.5 5 normal healthy controls were selected. They were also age and sex matched. Exon 5 from these subjects were sequenced and no evidence of a similar mutation was found. The partial electropherogram and protein translation encompassing these mutations are shown in Figure 5A and Figure 5B.

While awaiting further evaluation in hospital, the patient developed aspiration pneumonia and died despite treatment with multiple antibiotics.

**DISCUSSION**

The diagnostic difficulties were well illustrated in this case by the long latency required before all the appropriate diagnostic tests were obtained despite a positive family history. It has also been well recognised that the majority of patients with

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**Figure 2.** FLAIR sequence MRI brain showing subcortical leukodystrophy sparing the corpus callosum

**Figure 3.** Urine chromatogram showing elevated deoxyuridine and thymidine
MNGIE present with non-specific gastrointestinal symptoms with initial diagnoses entertained as primary gastrointestinal or psychiatric disorders. Although the clinical manifestations eventually proved to be classical in our patient, the age of onset and disease progression of this condition was unpredictable.

The unique aspect of our case was the ethnic origin of the proband. His family descended from the indigenous Bajau tribe of East Malaysia which loosely described an ethnic group that originated many generations ago from the Southern Philippines. The Bajau is the second largest indigenous ethnic group in the state of
Sabah, making up 13.4% of the total population of 3.12 million inhabitants (2010 census). MNGIE was first described in 1976 by Okamura et al. as “congenital oculoskeletal myopathy with abnormal muscle and liver mitochondria”. The mutations in the TP gene have been mapped to the tip of the long arm of chromosome 22 (22q13.32-qter). More than thirty mutations have been identified in nine of the ten exons in the TP gene. The different mutations that have been described were missense, splice-site, microdeletions, frameshift, microduplications and single nucleotide insertions. MNGIE has been recognized to be more prevalent in Western populations with disproportionately large numbers of case reports from populations which include Ashkenazi and Persian Jews, Mexican, Spanish, Greek, Italian, English and Canadian patients. This observation may be related to greater awareness of the condition and adequate laboratory resources for diagnosis. In Asia, Japanese and Thai patients have been described but this is the first reported case from the multi-ethnic Malaysian population, a country located in South East Asia with a total population of 28.6 million inhabitants. However, the prevalence of this condition is not known in the Malaysian population.

In our patient, we did not perform TP activity as the biochemical results and clinical features were of adequate diagnostic certainty to proceed to DNA sequencing. Nevertheless, TP function is the main determinant of disease in MNGIE and is expressed in the gastrointestinal system, peripheral nerve, brain, spleen, bladder and lung. This tissue distribution is consistent with the main clinical features. Paradoxically, skeletal muscles lack TP activity but the pathological changes in MNGIE were noted by the observation of ragged-red fibres, COX deficiency and mtDNA alterations in the muscle tissue. The relatively late-onset of MNGIE compared with other mitochondrial diseases that typically present in infancy or childhood is postulated to be due to the progressive accumulation of mtDNA defects induced by toxic levels of thymidine and deoxyuridine. Once the proportion of defective mtDNA reaches a critical threshold, tissue-specific mitochondrial dysfunction manifests clinically. While TP is not expressed in all tissues, cellular and plasma thymidine and deoxyuridine levels appear to be in equilibrium among all body compartments and it is postulated that the pathogenesis of this disease is mediated through this mechanism.

Interestingly, no correlations have been demonstrated between the severity of the clinical features and specific mutations. An important clinical observation in MNGIE patients was that overall prognosis and length of survival was linked to the degree of gastrointestinal involvement. Death usually occurs at a mean age of 37 as a result of cachexia, peritonitis, intestinal rupture, chronic intestinal pseudo-obstruction, oesophageal bleeding secondary to cirrhosis or aspiration pneumonia as observed in our patient. No treatment modality has been proven but possible options include allogeneic bone marrow transplant, exogenous TP enzyme replacement or measures to reduce plasma thymidine and deoxyuridine.

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Written informed consent was obtained from the patient and subsequently from the immediate family for the publication of this case report including any accompanying images and illustrations.

DISCLOSURE

Conflicts of interest: None

REFERENCES


