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Mitochondrial neurogastrointestinal encephalopathy in an Indian family with possible manifesting carriers of heterozygous *TYMP* mutation

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ABSTRACT

Background: Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a distinctive autosomal recessive disorder with mitochondrial alterations due to mutations *TYMP* gene encoding thymidine phosphorylase.
Materials and methods: Study of clinical and biochemical characteristics of a family with MNGIE.
Results: Index case was a 32 year old man presenting with recurrent vomiting, early satiety and progressive weight loss. He had ptosis, restricted eye movements, generalized muscle wasting, and absent tendon reflexes. Lactate levels were elevated in venous blood and CSF lactate. MRI brain showed diffuse leucoencephalopathy. Barium swallow showed near total obstruction at mid portion of vertical limb of duodenum with ileus. Esophageal manometry suggested myopathy. Muscle biopsy revealed moderate numbers of ragged blue and ragged red fibers as well as cytochrome *c* oxidase deficient fibers. An elder brother had similar symptoms and expired after a surgical procedure and a 28 year old brother has similar illness. The father had asymptomatic bilateral ptosis with mild ophthalmoparesis. The paternal grandfather and paternal aunt also had bilateral ptosis. Clinical diagnosis of MNGIE was confirmed in the two living brothers by demonstrating severe defects of thymidine phosphorylase activity in buffy coat, elevated thymidine and deoxyuridine in plasma, and a homozygous *TYMP* c.893 G>A mutation.
Conclusions: This family with biochemically and genetically confirmed mitochondrial neurogastrointestinal encephalopathy syndrome uncharacteristically included heterozygous *TYMP* mutation carriers manifesting extra-ocular weakness. It is important to identify MNGIE patients early because therapeutic options are emerging.

1. Introduction

Mitochondrial diseases due to defects of intergenomic communication are caused by primary nuclear gene defects which cause pathogenic secondary alterations of mitochondrial DNA (mtDNA). Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is characterized by extraocular muscle weakness, gastrointestinal dysmotility, cachexia, peripheral neuropathy, leukoencephalopathy and mitochondrial DNA abnormalities [1–5]. MNGIE is caused by mutations in the *TYMP* gene encoding thymidine phosphorylase (TP) resulting in a severe or total abolition of TP activity [6]. In this report we present the clinical, biochemical, histopathological, and genetic features of a family with MNGIE.

2. Case report

Three brothers, with non-consanguineous parents, were affected with classical MNGIE syndrome. (Fig. 1) The proband was a 32-year-old man who presented with one year history of recurrent vomiting, early satiety and weight loss. After consuming small quantities of food he felt bloated and abdominal discomfort, and belched. The symptoms progressively worsened for 6 months and he was repeatedly admitted to a hospital for recurrent severe vomiting due to intestinal pseudo-obstruction. He lost 18 kg over one year. In retrospect, he recalled being thin and short and having bilateral ptosis with restricted ocular movements since early childhood No symptoms of cognitive dysfunction, hearing loss, peripheral neuropathy, or muscle weakness. Examination revealed an extremely slender constitution; height was 152 cm and weight was 32 kg (Fig. 2A). General physical examination was notable for scaphoid abdomen with prominent bowel sounds. Neurological examination revealed normal intellectual functions, vision, and fundi, but he had bilateral partial ptosis with gross restriction of eye movements in all directions (Fig. 2B), and mild bifacial weakness. Although muscles were generally thin and mildly

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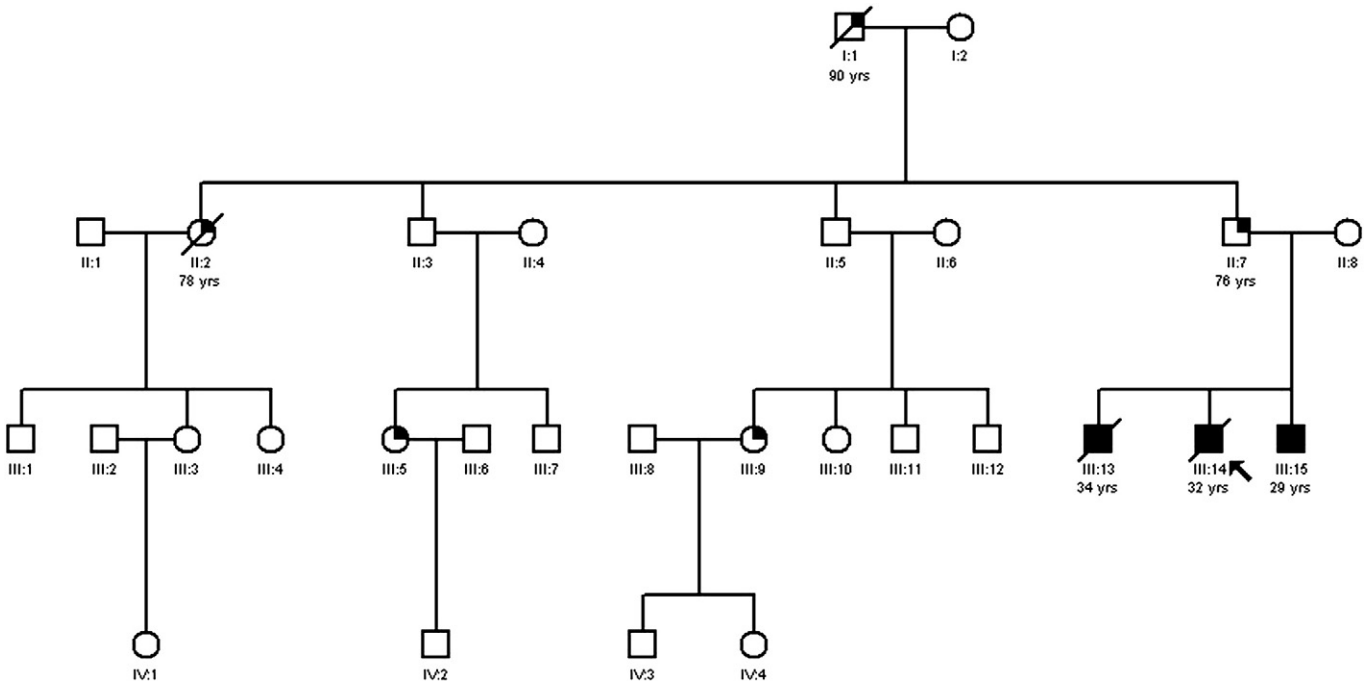

Fig. 1. Pedigree diagram of the family. Solid Symbols: Affected with classical MNGIE. Semisolid symbols: individuals manifesting only bilateral ptosis. Case II.7 examined. Has only ptosis. Case III.4 and Case III.8. Reported to have ptosis. Not examined.

hypotonic limb strength was normal. Sensory examination was normal. Tendon reflexes were absent. Over the next two years, the gastrointestinal manifestations progressed with recurrent episodes of pseudo-obstruction culminating in a fatal bout of pseudo-obstruction one and a-half years after presentation.

Electromyography revealed features of myopathy and nerve conduction studies showed demyelinating sensorimotor neuropathy. Barium swallow and barium meal studies performed during an episode of intestinal pseudo-obstruction showed normal esophageal emptying, gastric dilatation and delayed emptying, dilatation of the duodenal cap and segment up to the level of papilla, but near total pseudo-obstruction at mid portion of vertical limb of duodenum. The remainder of the small bowel loops were normal. Endoscopy revealed normal esophagus, fluid residue in the stomach, enlarged duodenal bulb, grossly dilated and tortuous second segment of the duodenum with effaced folds and residue. Esophageal manometry showed decreased lower esophageal sphincter pressure, low amplitude esophageal contractions suggestive of myopathy. Lactate levels were

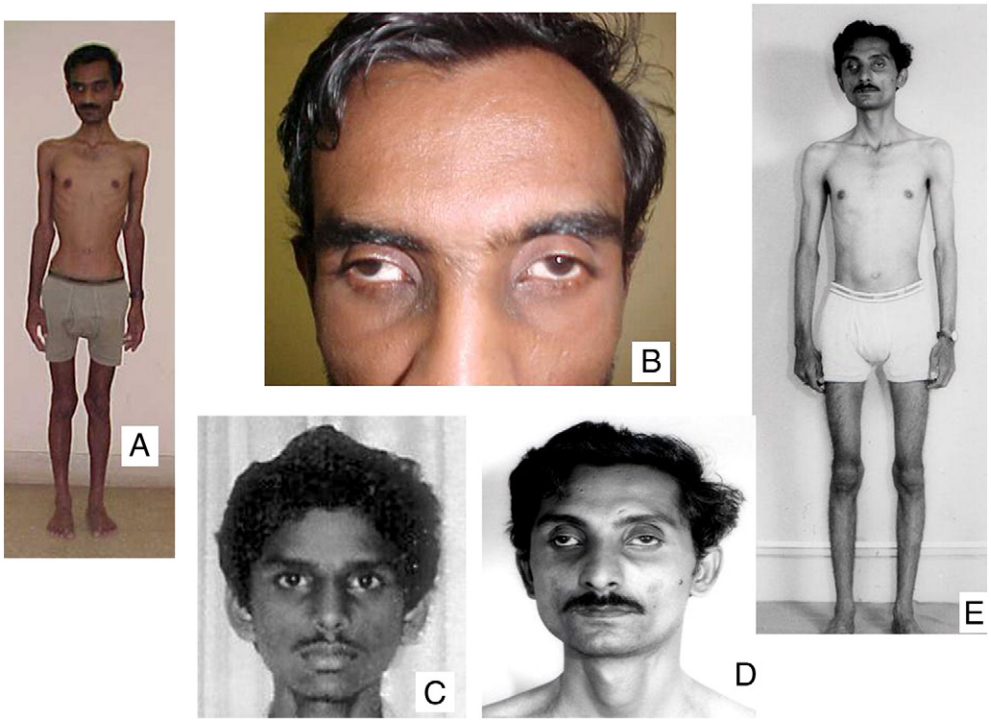

Fig. 2. A–E. 2A,B. Proband with typical slender habitus, ptosis and ophthalmoparesis 2C. Proband's elder sibling had no ocular symptoms 2D,E. Proband's younger brother with slender habitus, ptosis, and ophthalmoparesis.

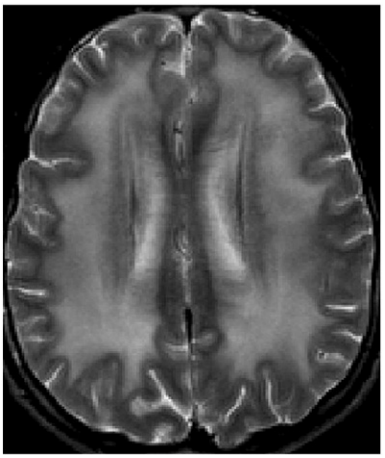

Fig. 3. T2 W MRI brain image showing diffuse cerebral leukoencephalopathy.

elevated in venous blood 30 mg/dl (normal 4.5–20) and cerebrospinal fluid (CSF) 41.5 mg/dl. CSF also showed elevated protein 123 mg/dl (normal <45), no cells and normal glucose. Creatine kinase was normal. Electrocardiography was normal and echocardiogram showed mitral valve prolapse. Ophthalmic evaluation and audiometry were normal. MRI of brain showed leukoencephalopathy with diffusely increased T2signal in the cerebral white matter (Fig. 3).

The biceps muscle biopsy showed several 'ragged blue' fibers by succinate dehydrogenase (SDH) histochemistry and 'ragged red' fibers with modified Gomori trichrome (MGT) stain. This accounted for 20% of all the fibres. On serial sections, these abnormal fibers demonstrated marked reduction or complete absence of cytochrome *c*- oxidase (COX) histochemical stain. In addition, some non-ragged red/blue fibers showed COX deficiency (Fig. 4). The percentage of COX deficient/COX negative fibres on COX-SDH staining was 30%.

The elder brother was also thin and had bilateral ptosis and ophthalmoparesis since childhood (Fig. 2C). At age 31, he developed gastrointestinal symptoms with frequent episodes of pseudo-obstruction, recurrent diarrhea and weight loss and died at age 34 during an episode of acute intestinal pseudo-obstruction. The 30-year-old brother was thin (height 153 cm and weight 34 kg) and had bilateral ptosis and restricted eye movements, but no gastrointestinal symptoms. (Fig. 2D,E). Muscle strength, sensory examination, and tendon reflexes were normal. He refused to undergo any investigations. The 76-year-old father had drooping eyelids from childhood with no other systemic or neurological symptoms. On examination, he had bilateral ptosis (Fig. 5C.) The paternal grandfather of the patients lived to 90 years of age and was noticed to have bilateral drooping of eyelids since adolescence (Fig. 5A). He had no symptoms of gastrointestinal or other neurological symptoms. Proband's

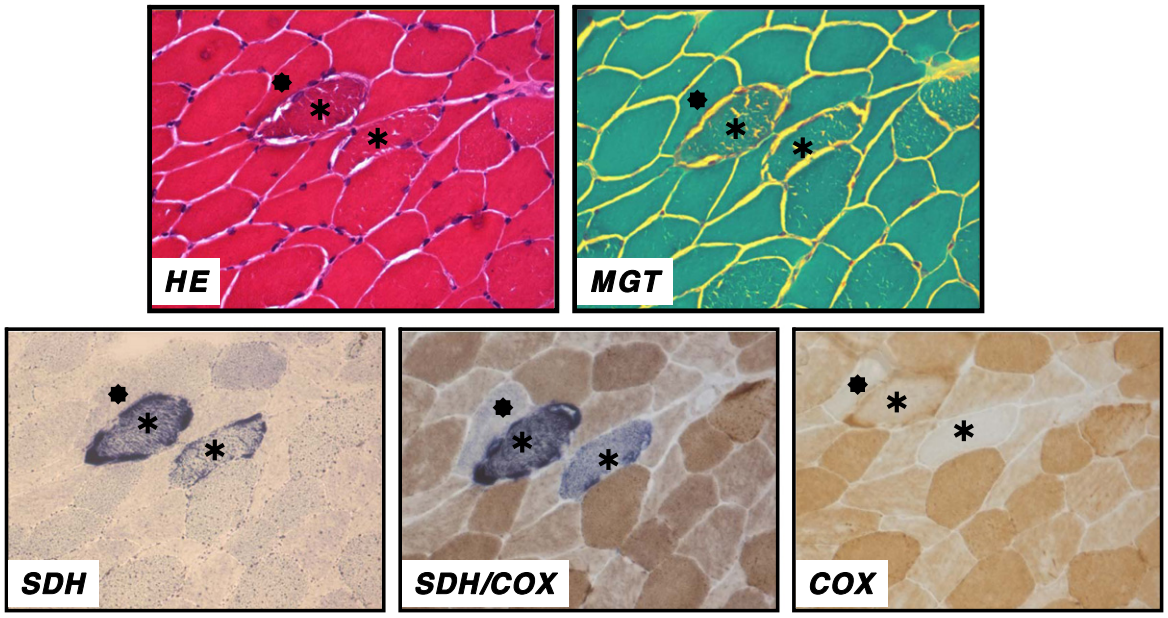

Fig. 4. Muscle histology showing ragged red fibers with modified Gomori trichrome (MGT) stain and ragged-blue fibers with succinate dehydrogenase (SDH) histochemistry (*) and non-ragged fibers(□). Serial sections showing ragged-red/blue and one non-ragged red/blue fibers with absent or reduced COX activity.

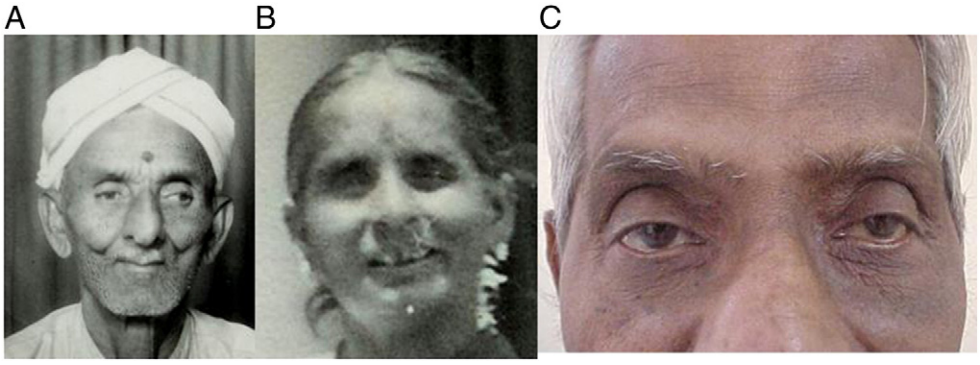

Fig. 5. A–C. 5A. Proband's paternal grandfather with ptosis and left exotropia 5B. Proband's paternal aunt with ptosis 5C. Proband's father with ptosis and no ophthalmoparesis.

paternal aunt lived up to 78 years and had symptoms of bilateral ptosis (Fig. 5B) and no other complaints. Both brothers tested demonstrated severe defects of thymidine phosphorylase activity in buffy coat and elevated thymidine and deoxyuridine in plasma. (Proband: TP activity = 0 [normal 634 ± 212 nmol/h/mg-protein], plasma thymidine 1.3 μmol/L [normal <0.05] and deoxyuridine = 6 micromole/L [normal <0.05]. Younger brother: TP activity = 55 nmol/h/mg-protein, plasma thymidine = 4.5 μmol/L and deoxyuridine = 12 μmol/L). Both brothers have a novel homozygous *TYMP* c.893 G>A mutation (p.G298D). The father showed partial thymidine phosphorylase enzyme activity (104 nmol/h/mg-protein) but no detectable thymidine or deoxyuridine in plasma. He is heterozygous for the *TYMP* mutation and sequencing of all exons and flanking introns did not reveal any other potential mutations.

3. Discussion

MNGIE is a multisystem disorder, with a clinically recognizable and stereotypic phenotype. A review of 52 MNGIE patients with *TYMP* gene mutations revealed that the major clinical manifestations, seen in all patients, are: gastrointestinal with diarrhea, early satiety, vomiting and gastroparesis; neurological with leukoencephalopathy and peripheral neuropathy; thin habitus; and weight loss [2]. The age-at-onset varies from infancy to middle age, but commonly in adolescence or early adulthood and almost all die by age of 58 years [2]. The most prominent and debilitating symptom is gastrointestinal dysmotility, which can affect any portion of the entire enteric system [1,7]. Decreased small intestine motility and delayed gastric emptying are the most common forms of dysmotility [1,2,4]. Our index case and elder brother also had predominant gastrointestinal manifestations with repeated admissions under the gastroenterologist.

Our proband had the six obligate clinical criteria that defines MNGIE syndrome: severe gastrointestinal dysmotility, cachexia, ptosis with progressive external ophthalmoparesis, peripheral neuropathy, leukoencephalopathy and evidence of mitochondrial dysfunction (elevated lactate in blood and CSF and abnormal mitochondrial morphology in muscle biopsy). Peripheral neuropathy in our patient was subclinical. Although thin throughout life, he developed progressive weight loss coincident with worsening gastrointestinal symptoms. The gastrointestinal manifestations were related to dysmotility with early satiety, recurrent vomiting, and intestinal pseudoobstruction as reported by multiple authors [1,2,4,8,9]. Histological studies suggest that the gastrointestinal dysmotility of MNGIE is due to visceral myopathy, particularly the external muscular layer of the intestine [7,10] and the esophageal manometry indicated visceral myopathy in the proband. Skeletal muscle histopathology showed ragged red/blue fibers and COX-deficient and negative fibers, which are hallmarks of mitochondrial myopathies and often observed in MNGIE [1,2,4,8,9]. Brain MRI showed the classical diffuse white matter T2 hyperintensity characteristic of the leukoencephalopathy of MNGIE [11]. Despite the severe leukoencephalopathy, our patients showed normal intellectual function, which is typical of MNGIE, although rare cases have manifested cognitive dysfunction [12,13]. Sparing of cognitive functions in MNGIE can be explained by the observation that the leukoencephalopathy in MNGIE seems to be due to impaired blood-brain barrier causing edema rather than demyelination [14].

In contrast to the gastrointestinal problem, the neuromuscular features may be mild. Ptosis and ophthalmoparesis may be asymptomatic. Our patients also had asymptomatic ptosis and ophthalmoparesis, which were overlooked until noted by a physician. The neuropathy was also subclinical in our proband.

MNGIE, is caused by mutations in the *TYMP* gene encoding thymidine phosphorylase (TP) resulting in a severe or total abolition of TP activity [6]. Loss of TP activity leads to dramatic elevations of thymidine and deoxyuridine in plasma and tissue of MNGIE patients and the excess nucleosides are thought to cause unbalanced deoxynucleotide triphosphates (dNTPs) that, in turn, cause secondary mtDNA alterations [15,16]. The biochemical and molecular genetic defects of MNGIE are readily testable in blood and allow definitive confirmation of the diagnosis, particularly in individuals in early stages of the disease before the full-syndrome manifests. It is important to identify these patients at an early stage of the illness as therapeutic options are emerging [17–20].

All studies on genetic inheritance of MNGIE have reported an autosomal recessive pattern of inheritance. There are no reports on MNGIE with possible autosomal dominant inheritance pattern. Consistent with autosomal recessive inheritance, all three brothers had classical MNGIE; however, intriguingly, the proband's father aged 76 years and the paternal aunt aged 78 years and paternal grandfather had childhood onset bilateral ptosis without ophthalmoparesis, gastrointestinal or myopathic symptoms.

Biochemical and molecular genetic studies were performed only in the father. This family history suggests that heterozygous *TYMP* mutation carriers may manifest extra-ocular muscle weakness. It is possible that genetic or environmental modifiers may be contributing to the ptosis in the father and other relatives who may be TYMP mutation carriers.

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