

# Biochemical abnormalities in a patient with thymidine phosphorylase deficiency with fatal outcome

Jaap A. Bakker · Patrick Schlessler ·  
Hubert J. M. Smeets · Baudouin Francois ·  
Jürgen Bierau

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**Abstract** Deficiency of the cytosolic enzyme thymidine phosphorylase (TP) causes a multisystem disorder called mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) syndrome. Clinical symptoms are gastrointestinal dysfunction, muscle involvement and neurological deterioration. TP deficiency is biochemically characterised by accumulation of thymidine and deoxyuridine in body fluids and compromised mitochondrial deoxyribose nucleic acid (mtDNA) integrity (depletion and multiple deletions). In this report we describe a patient with the clinical and biochemical features related to the end stage of the disease. Home parenteral nutrition had started to improve the clinical condition and preparations were initiated for stem

cell transplantation (SCT) as a last resort treatment. Unfortunately, the patient died during the induction phase of SCT. This report shows that TP deficiency is a severe clinical condition with a broad spectrum of affected tissues. TP deficiency can be easily determined by the measurement of pyrimidine metabolites in body fluids and TP activity in peripheral blood leucocytes. Early detection and treatment may prevent the progress of the clinical symptoms and, therefore, should be considered for inclusion in newborn screening programmes.

## Abbreviations

BMI	body mass index
CIPO	chronic intestinal pseudo-obstruction
DPD	dihydropyrimidine dehydrogenase
EMA	ethylmalonic acid
ESI	electrospray ionisation
GI	gastrointestinal
HLA	human leucocyte antigen
MNGIE	mitochondrial neurogastrointestinal encephalomyopathy
MRM	multiple reaction mode
mtDNA	mitochondrial deoxyribose nucleic acid
OXPPOS	oxidative phosphorylation
SCT	stem cell transplantation
TP	thymidine phosphorylase
WBC	white blood cell

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## References to electronic databases:

thymidine phosphorylase: EC 2.4.2.4.  
dihydropyrimidine dehydrogenase : EC 1.3.1.2.  
MNGIE syndrome: OMIM 603041  
dihydropyrimidine dehydrogenase deficiency: OMIM 274270  
TYMP: GenBank no. NM\_001953

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J. A. Bakker (✉) · J. Bierau  
Department of Clinical Genetics, Laboratory for Biochemical Genetics, Maastricht University Medical Centre,  
P. Debyelaan 25,  
6229 HX Maastricht, The Netherlands  
e-mail: jaap.bakker@gen.unimaas.nl

P. Schlessler · B. Francois  
Department of Paediatrics, CHC Clinique de l'Espérance,  
Montegnée-Liege, Belgium

H. J. M. Smeets  
Department of Clinical Genetics, Clinical Genomics Unit,  
Maastricht University Medical Centre,  
Maastricht, The Netherlands

## Introduction

In the 1990s thymidine phosphorylase (TP; EC 2.4.2.4) deficiency was identified as the most common cause of the clinical syndrome called mitochondrial neurogastrointestinal encephalomyopathy (MNGIE; OMIM 603041) (Nishino et al.

2000). As indicated in this acronym, TP deficiency affects several tissues (Table 1) (Nishino et al. 2001).

Although TP deficiency is a multisystem disease, the gastrointestinal problems are the most prominent and, in the majority of cases, are the presenting symptoms. Visceral manifestations are caused by mitochondrial dysfunction of the intestinal smooth muscle, and the mitochondrial abnormalities in oesophageal and small intestine biopsies were identical to the aberrations observed in skeletal muscle of patients with other mitochondrial diseases (Blondon et al. 2005; Giordano et al. 2008). Neurological symptoms are often mild, although, initially, magnetic resonance imaging (MRI) of the brain does not always reveal a leucoencephalopathy; ultimately, all patients develop leucoencephalopathy. The clinical picture develops gradually in the course of life, and mild cases can easily be misdiagnosed, especially when the symptoms are not recognised as part of a syndrome (Teitelbaum et al. 2002).

When a mitochondrial disease is being considered, biochemical investigations often include measurement of the respiratory chain capacity in a muscle biopsy. A reduction in the overall oxidative phosphorylation (OXPHOS) capacity of the mitochondria can be observed in MNGIE, as well as decreased activity of the enzymes of the respiratory chain having mitochondrial deoxyribose nucleic acid (mtDNA)-encoded subunits (Marti et al. 2003). At the level of mtDNA, a decreased amount (depletion) and a loss of integrity (multiple mtDNA deletions) are observed, indicating a defect in mtDNA maintenance. Additional laboratory investigations show elevated concentrations of thymidine and deoxyuridine in all body fluids (Marti et al. 2003). The TP activity in MNGIE patients with homozygous or compound heterozygous TP mutations is normally barely detectable, whilst the activity is approximately 30% of control values in heterozy-

gous carriers (Marti et al. 2004). Today, over 30 different mutations have been identified in the gene encoding TP, *TYMP* (GenBank no. NM\_001953) (Laforce et al. 2009; Marti et al. 2005; Nishino et al. 2000).

### Clinical history

At the age of 16 the patient was investigated because of frequent episodes of diarrhoea. At that time no gastrointestinal (GI) abnormalities were noted. Ten years later his GI symptoms had worsened and his overall clinical condition was poor. His body weight was 58 kg, resulting in a body mass index (BMI) of 16.6. During the following years frequent episodes of (micro)perforation of the intestinal tract resulted in resection of a part of the proximal jejunum. Furthermore, perforation of the sigmoid was diagnosed and was followed by a new resection of the affected part of the intestinal tract.

Neurological investigations showed generalised muscle areflexia, bilateral ptosis and the beginnings of ophthalmoplegia. At that time MRI revealed massive supra- and infratentorial demyelination, although the leucoencephalopathy was asymptomatic. Investigations of a muscle biopsy showed a predominance of type 1 fibres; type 2 fibres were scarcely visible. Aberrations in the type 1 fibres (accumulation of lipid, glycogen depletion, myophagia), peripheral location of the mitochondria and excessive succinate dehydrogenase (SDH) activity in most fibres supported the diagnosis of a mitochondriopathy. Mutation analysis for TP was initiated and revealed a homozygous A to C transition in exon 7 (A3371C) (Nishino et al. 2000).

In the following years the gastrointestinal problems worsened, resulting in chronic intestinal pseudo-obstruction (CIPO) with gastroparesia. Because of a further decrease in the BMI to 14.3, home parenteral nutrition was started. MRI

**Table 1** Clinical features of our patient compared with those in the literature

Clinical symptom	Proband	Literature (Nishino et al. 2001)
Cachexia	+	+
<i>Gastrointestinal manifestations</i>	+	+
Borborygmi	+	+
Abdominal pain	+	+
Diarrhoea	+	+
Diverticulosis	+	+
Pseudo-obstruction	+	+
<i>Neurological manifestations</i>	+	+
Ptosis	+	+
Ophthalmoplegia	+	+
Peripheral neuropathy	+	+
Hearing loss	+	+
Depression	+	Unknown

showed cerebral ventriculomegaly, when compared to MRI images made 5 years earlier. Ptosis was still prominent, with decreased eye movements (ophthalmoplegia). Only at this time did the myopathy become clinically relevant. The family history revealed a sister with a clinical history of anorexia, denutrition, neurological problems and depression, who died in her thirties. Most probably this was a case of TP deficiency too. Other family members reported no clinical problems related to TP deficiency.

To slow down the progression of the disease, stem cell transplantation (SCT) was considered. Family members were screened for human leucocyte antigen (HLA) genotype, TP activity and the genetic defect in the *TYMP* gene: both siblings were heterozygous carriers of the *TYMP* mutation and had TP activities in the heterozygous carrier range, but only one had an HLA genotype compatible with that of the patient. The patient was prepared for SCT, but unfortunately died during the induction phase of the chemotherapy.

### Biochemical, enzymological and DNA investigations

Purine and pyrimidine metabolites in body fluids were analysed with liquid chromatography–tandem mass spectrometry (LC-MSMS) (Waters Micromass Quattro Micro). Purines and pyrimidines were detected in both electrospray ionisation (ESI)-positive mode and ESI-negative mode using multiple reaction monitoring (MRM). Thymidine phosphorylase activity was measured in isolated peripheral blood leucocytes, as described before (van Kuilenburg and Zoetekouw 2005). Briefly, lysed leucocytes (2.5–30 µg protein) were incubated with 2 mM thymidine at 37°C, pH 7.4 for 60 min. After termination of the reaction with perchloric acid, the samples were centrifuged, and the supernatant was neutralised and analysed by high-performance liquid chromatography (HPLC). Thymine concentrations were calculated against standard concentrations of thymine. Dihydropyrimidine dehydrogenase (DPD, EC 1.3.1.2.) activity was determined using incubation with <sup>14</sup>C-labelled thymine as described

before (Van Kuilenburg et al. 1997). Reference values for TP and DPD activity and pyrimidine metabolites in body fluids were established using samples originating from patients without MNGIE ( $n=25$ ).

Screening for the familial mutation in exon 7 of the *TYMP* gene in the family members was done as described before (Nishino et al. 2000), with the exception that a different set of primers was used: forward primer TGGCAACCCA GGGTGCAGCA and reverse primer GGCGGGGACGGG TCTTAG.

### Results

Thymidine and deoxyuridine concentrations in the urine and plasma of the patient were strongly elevated (Table 2). In addition, analysis of purine and pyrimidine metabolites revealed an elevated excretion of thymine and uracil: 23 µmol/mmol creatinine and 51 µmol/mmol creatinine, respectively (reference values <1 µmol/mmol creatinine and 1.0–14 µmol/mmol creatinine). Dihydropyrimidines were not detectable. In plasma, uracil and thymine were undetectable. Thymidine and deoxyuridine were not detectable in the plasma of the parents and the siblings.

Amino acid analysis in the patient showed a mild generalised hyperaminoaciduria; furthermore, the excretion of ethylmalonic acid (EMA) and creatine was slightly elevated (data not shown).

TP activity in peripheral white blood cells (WBCs) of the patient was consistent with the diagnosis MNGIE (Table 2). TP activity in the parents and siblings of the patient were in the heterozygous carrier range.

The presence of thymine and uracil urged us to measure DPD activity. The specific DPD activity in the patient was in the normal range, at 16.04 nmol/mg protein per hour [reference values ( $n=24$ ) 16.54±4.57 nmol/mg protein per hour] in lymphocytes. Sequencing of the *TYMP* gene revealed that the proband was homozygous for a *c.866A>C* mutation in exon 7, resulting in the substitution of glutamic acid by

**Table 2** TP activities in leucocytes and metabolite concentrations in body fluids of the proband and family members

Subject	TP activity (nmol/mg protein per hour)	Thymidine in plasma (µmol/l)	Deoxyuridine in plasma (µmol/l)	Thymidine in urine (µmol/mmol creatinine)	Deoxyuridine in urine (µmol/mmol creatinine)
Proband	10	10	20	29	51
Father	200	n.d.	n.d.		
Mother	320	n.d.	n.d.		
Sister	300	n.d.	n.d.		
Brother	260	n.d.	n.d.		
Reference	360–830	<0.1	<0.1	<5	<1

n.d., not detectable

alanine at position 289 of the protein (p.E289A). The siblings and both parents were heterozygous carriers for this mutation.

## Discussion

Deficiency of thymidine phosphorylase consistently results in elevated concentrations of thymidine and deoxyuridine in body fluids (Marti et al. 2004). The excess availability of, especially, thymidine causes an intracellular (intra-mitochondrial) imbalance in deoxynucleotide triphosphates, leading to disturbances in mtDNA replication and maintenance. This results in reduced mtDNA copy number (depletion) and multiple deletions in the mtDNA, which affect the enzymes involved in oxidative phosphorylation (Lopez et al. 2009; Pontarin et al. 2006). Investigations of muscle confirmed the presence of a mitochondriopathy. Elevated EMA excretion is suggestive of malfunction of the mitochondrial electron transport system. Proximal tubulopathy, often associated with mitochondrial disease, may be the cause of the mild hyperaminoaciduria in our patient.

We observed an increased excretion of thymine and uracil in our patient, which has been described before (Fairbanks et al. 2002). Because of the normal activity of DPD and the absence of dihydropyrimidines and *N*-carbamoyl amino acids, a defect in pyrimidine degradation does not seem plausible. The bi-directionality of TP might partly explain the elevated excretion of thymine and uracil (Temmink et al. 2007).

Therapy for TP deficiency is still not well established. Dialysis and platelet infusions have been used, both unsuccessful in the attempts to lower the circulating concentrations of thymidine and deoxyuridine permanently (la Marca et al. 2006; Lara et al. 2006). Allogenic stem cell transplantation (SCT) has been more successful in correcting the metabolic derangements: restoration of TP activity was observed, and concentrations of thymidine and deoxyuridine in plasma decreased to undetectable levels (Hirano et al. 2006). SCT was the primary goal in the treatment of our patient, despite the poor clinical condition. Both siblings were considered as stem cell donors. Although TP activities were in the heterozygous range, we reasoned that this would be sufficient to correct the metabolic derangements, as individuals with >8% residual activity do not develop MNGIE syndrome (Marti et al. 2005). However, before the SCT procedure could be initiated, the patient died.

The underlying mechanism as to why TP deficiency presents in adulthood in most cases remains unclear. The genetic defect and the subsequent metabolic derangement are obviously present from the start of life. In vitro studies with cultured skin fibroblasts of TP patients have shown

reduced cytochrome C oxidase (COX) activity (Nishigaki et al. 2003). Immunocytochemical staining for the mtDNA-encoded COX subunit I in fibroblasts of TP patients showed a mosaic expression pattern, in contrast to that of control fibroblasts, where the staining was uniform (Taanman et al. 2009). Furthermore, site-specific mtDNA point mutations were detected in cultured skin fibroblasts of TP patients. Multiple deletions and depletions were not detectable (Nishigaki et al. 2003; Taanman et al. 2009). Only when fibroblast were exposed to an excess of thymidine and deoxyuridine over a longer period did the mtDNA content decrease (Pontarin et al. 2006). When the nucleosides were withdrawn, the mtDNA content returned to normal. This phenomenon suggests a dose-accumulation effect in TP deficiency before clinical symptoms occur, caused by depletion or multiple deletions of the mtDNA.

## Conclusion

TP deficiency is a multisystem, and ultimately fatal, disease. The most promising cure is SCT and has to be considered for all patients with TP deficiency. Transplantation with stem cells from heterozygous siblings can, in principle, correct the metabolic defect (Hirano et al. 2006). It can be expected that all the reversible clinical symptoms will be resolved, because, so far, no patients with MNGIE-like symptoms and heterozygous TP activities have been described. Early detection of TP deficiency is therefore of utmost importance, and we advocate the inclusion of screening for elevated thymidine and deoxyuridine concentrations as markers for TP deficiency in neonatal screening programmes.

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