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NEW HORIZONS IN THE TREATMENT OF MITOCHONDRIAL NEUROGASTROINTESTINAL ENCEPHALOMYOPATHY (MNGIE): FROM PATHOGENESIS TO THERAPIES

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Abstract. Mitochondrial neurogastrointestinal encephalomyopathy syndrome (MNGIE), also known as mitochondrial gastrointestinal encephalopathy, is an extremely rare hereditary metabolic disorder caused by mutations in the nuclear gene *TYMP*, which encodes the enzyme thymidine phosphorylase (TP). The subsequent systemic accumulation of deoxyribonucleosides leads to mutations in the mitochondrial genetic material and ultimately to the failure of the organelle itself. The degenerative nature of the disease and the intricate nature of its clinical symptoms effectively hinder proper diagnosis, which, with the life expectancy of MNGIE patients estimated at 37 years, significantly complicates its treatment. Currently, available therapeutic approaches, based on symptomatic treatment, are slowly giving way to newly developed experimental therapies. *In vitro* and *in vivo* models have played a key role in this topic, contributing to the deepening and understanding of the disease mechanisms over the years, thus providing an important foundation for further research and potential therapies. This review provides a comprehensive overview of the current state of knowledge on MNGIE, with a focus on current therapeutic options, available disease models, and diagnostic procedures. This review aims to increase clinical awareness and support the development of more effective treatments and diagnostics, as well as to provide valuable information that can improve the quality of life and care of patients with MNGIE.

Key words: MNGIE, mitochondrial neurogastrointestinal encephalomyopathy syndrome, thymidine phosphorylase, *TYMP*, metabolic disorder, mitochondrial disease.

INTRODUCTION

Mitochondrial diseases are characterized by an extraordinary diversity in terms of associated clinical manifestations and underlying genetic causes, which have so far been linked to pathogenic variants found in over 300 disease-related genes. It is worth noting that this number includes not only mutations in mitochondrial genetic material (mtDNA), as pathogenic variants have been identified in all 37 genes within the mtDNA itself. The nuclear genome (nDNA) also plays a significant role, specifically in 296 genes encoded by it, where such

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variants occur (Thompson et al. 2020). Due to this dual genetic control, these disorders can be transmitted according to any pattern of inheritance, making mitochondrial diseases one of the most common inherited diseases, with an estimated prevalence of 4.7 per 100,000 in children and nearly three times higher in adults. The high number of pathogenic variants, combined with the presence of this organelle in all nucleated cell types, results in the potential for disease development at any time, as well as in any organ or tissue. Furthermore, since these conditions represent a clinically and genetically heterogeneous group of disorders affecting the synthesis and function of mitochondrial proteins, they can impact both single and multiple organ systems (Scarpelli et al. 2013; Yadak et al. 2017; Thompson et al. 2020; Mavraki et al. 2023).

One example of such a pathogenic variant is a mutation in the nuclear gene *TYMP*, resulting in a deficiency of thymidine phosphorylase, which has been associated with MNGIE. First described by Okamura 48 years ago, this extremely rare and fatal disease remains incompletely characterized (Okamura et al. 1976; Hirano et al. 2021). Its progressive nature, combined with severe and heterogeneous neurological and gastrointestinal symptoms, significantly complicates accurate diagnosis. Diagnostic delays and the lack of approved therapies are just some of the many obstacles faced by patients with MNGIE. The limited information on diagnostic and therapeutic options is attributed to the lack of controlled studies with adequate follow-up periods. Nevertheless, various experimental approaches are being developed, with promising results. Gene therapy, which has shown preclinical efficacy, holds great promise, although therapeutic options aimed at temporarily or permanently restoring TP activity are also being considered. For MNGIE patients, the timing of the treatment is crucial, as the severity of symptoms significantly impacts treatment success. Therefore, it is essential to deepen our understanding of symptomatology, diagnostic procedures, disease models, and available treatment methods to facilitate access to those most in need (Pacitti et al. 2018; Bax 2020; Hirano et al. 2021).

MOLECULAR ETIOLOGY OF MNGIE: FUNCTIONAL CONSEQUENCES OF MUTATIONS

Located near the end of the long arm of chromosome 22, in region 13.33 – 22q13.33 (OMIM – Online Mendelian Inheritance in Man, #603041), the *TYMP* gene consists of 10 exons spread over an area encompassing 4.334 kb (exact location – Chr22: 50525752...50530085) and encodes the cytosolic enzyme thymidine phosphorylase (TP, EC 2.4.2.4) (Hagiwara et al. 1991; Hirano and Díaz 2016; HGNC:3148). It is the deficiency of TP that has been linked to the accumulation of qualitative (deletions and duplications) and quantitative (depletion) defects in mitochondrial DNA (Pacitti et al. 2018; OMIM *131222). Since the identification of the *TYMP* gene, 116 different mutations have been reported in the Human Gene Mutation Database (HGMD Professional, access date: 23.05.2024), which have been classified as benign or pathogenic variants. These mutations have been mapped to both exonic and intronic regions and include the following mutations: small deletions and insertions, missense mutations, small indels, and splice-site mutations. The causative factor in the pathogenesis of MNGIE is the impairment of TP function resulting from the aforementioned mutations in the *TYMP* gene, more specifically, a severe reduction or complete loss of enzymatic activity (Yadak et al. 2017; Kripps et al. 2020).

Thymidine phosphorylase, also known as platelet-derived endothelial cell growth factor (PD-ECGF) or gliostatin, is a homodimer composed of two identical subunits, with a monomer molecular weight of 50 kDa. This enzyme is located in the cytosol and is responsible for cat-

alysing the first step in the degradation of pyrimidine deoxyribonucleosides – it catalyses the reversible phosphorylation of thymidine (deoxythymidine – dThd) and deoxyuridine (dUrd) to their respective bases: thymine and uracil, and 2-deoxy-D-ribose-1-phosphate. TP deficiency leads to an imbalance in the mitochondrial deoxyribonucleoside pool and the accumulation of dThd and dUrd within the cell (Fig. 1). Although this reaction is reversible, the enzyme's most important metabolic function is catabolism (Kamatani et al. 2013; Pacitti et al. 2018).

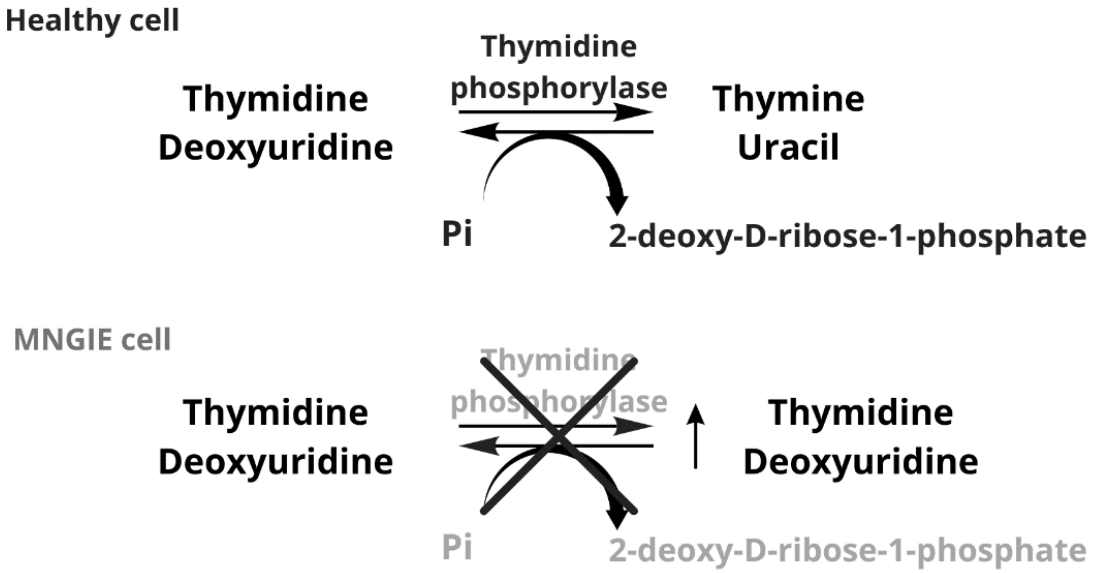

Fig. 1. The reaction catalysed by the enzyme thymidine phosphorylase (TP) in the context of MNGIE pathophysiology

Moreover, TP exhibits deoxyribosyltransferase activity, which is responsible for transferring the deoxyribosyl moiety from a pyrimidine nucleoside to another pyrimidine base (Kamatani et al. 2013). Through this mechanism, by regulating the availability of thymidine for DNA biosynthesis, this enzyme plays a crucial role in the nucleoside salvage pathway and participates in the recycling of pyrimidine bases, indirectly affecting mitochondrial genome replication and expression (Nishino et al. 2000; Kamatani et al. 2013; Hirano and Díaz 2016; Ronchi et al. 2020).

It is important to emphasize that mtDNA replication is not confined to the S phase of the cell cycle, as mtDNA undergoes continuous replication, as evidenced by the occurrence of this process in the mitochondria of postmitotic cells. However, for this process to be feasible, a constant supply of deoxyribonucleotide triphosphates (dNTPs) is required (Hubert and Sutton 2017). Within mitochondria, *de novo* synthesis of dNTPs does not occur, instead, they are either imported from the cytosol, where their primary source is the cytoplasmic *de novo* pathway, or they are generated *via* the mitochondrial salvage pathway, which is governed by TP (Rötig and Poulton 2009; Hubert and Sutton 2017). Since the mitochondrial dNTP pool is largely dependent on the cytoplasmic pool, which in turn is regulated by ribonucleotide reductase (RNR) responsible for their synthesis, resting cells, and non-proliferating tissues (such as nervous and muscle tissue) that have reduced nucleotide demand pose a problem (Hubert and Sutton 2017). The accompanying reduction in RNR activity leads to the down-

regulation of the cytoplasmic *de novo* pathway, directly resulting in a marked decrease in the overall cytosolic dNTP pool. At this point, this pathway can no longer provide the nucleotides required for mtDNA replication, and its role is taken over by the mitochondrial salvage pathway, where TP plays a key role. In the event of TP loss of function, thymidine kinase 2 (TK2) takes over its role in thymidine salvage, converting nucleosides into the corresponding monophosphate nucleotides, which contributes to an imbalance in the composition of mitochondrial deoxyribonucleotides (Rötig and Poulton 2009; Garcia-Diaz et al. 2014; Elamin et al. 2016; Ronchi et al. 2020).

The limited capacity for mtDNA repair, compared to nuclear DNA, makes it particularly susceptible to the resulting imbalance (Pacitti et al. 2018). Dysfunction of thymidine phosphorylase leads to the gradual accumulation of mutations in mitochondrial genetic material, ultimately resulting in pathogenic secondary mtDNA instability, which manifests as multiple deletions, mtDNA depletion, and site-specific point mutations (Garcia-Diaz et al. 2014). Consequently, all these changes may account for the gradual loss of the ability to repair and replicate mtDNA, ultimately leading to the overall failure of this organelle (Nishigaki et al. 2003).

APPLICATION OF *IN VITRO* AND *IN VIVO* MODELS IN MNGIE PATHOGENESIS RESEARCH

Research on MNGIE relies on various experimental models, both *in vivo* and *in vitro*, allowing for a thorough understanding of the mechanisms of pathogenesis and the development of new therapies. Despite significant progress in understanding the fundamental mechanisms of MNGIE, the need for specific models that reflect the full range of this disease's pathophysiology remains unmet.

Most of the *in vitro* models developed to date have contributed valuable information on the impact of deoxyribonucleoside imbalance on cellular function, but they mainly focused on fibroblasts, which do not always reflect the specificity of the organ systems crucial in the context of MNGIE. Pioneering studies by Spinazzola et al. (2002) and Nishigaki et al. (2003) were based on these models, where the authors focused on analysing the impact of deoxyribonucleoside pool imbalance on mtDNA, revealing mechanisms related to thymidine phosphorylase dysfunction and their impact on mtDNA mutation accumulation (Spinazzola et al. 2002; Nishigaki et al. 2003). Subsequent studies, including those by Song et al. (2003), Ferraro et al. (2005), and Pontarin et al. (2006), expanded the understanding of the impact of excess thymidine on dNTP pools and the resulting mtDNA damage, also highlighting mechanisms leading to mtDNA depletion (Song et al. 2003; Ferraro et al. 2005; Pontarin et al. 2006). Building on this information, the findings of González-Vioque et al. (2011) revealed that mtDNA depletion is the result of limited dNTP availability, not just an imbalance in nucleotide pools (González-Vioque et al. 2011). In recent years, new research possibilities have been provided by an innovative approach by Pacitti and Bax (2018), in which brain organoids were created using induced pluripotent stem cells (iPSC). This approach provides new insights into the pathophysiology of MNGIE in the context of the central nervous system (Pacitti and Bax 2018). These are just some of the valuable insights offered by *the in vitro* models developed so far. To better illustrate their diversity and application in MNGIE research, Table 1 presents a brief summary of the findings and contributions of individual *in vitro* models to the characterization of the biochemical and molecular mechanisms of the disease.

Table 1. MNGIE in vitro models

Cell type	Key results	Significance of the model and conclusions
	Spinazzola et al. (2002)	
Fibroblasts of healthy patients and patients with MNGIE	MNGIE fibroblasts, unlike controls, are unable to catabolize thymidine. Instead, they release it, leading to thymidine accumulation in the culture medium.	This model confirms changes in thymidine metabolism in MNGIE patients. Loss of TP activity in MNGIE leads to thymidine accumulation in the blood, which disrupts the balance of mitochondrial nucleotides leading to abnormalities in mtDNA.
	Nishigaki et al. (2003)	
Fibroblasts of patients with MNGIE	Thirty-six point mutations in mtDNA were identified (most of them site-specific, mainly the T-to-C transition), which have been linked to increased levels of dThd and dUrd. Increased production of reactive oxygen species was also noted.	The model suggests that the next nucleotide effect and dislocation mutagenesis may contribute to the mutations. The results suggest that mutations in mtDNA result from a generated imbalance in the mitochondrial nucleoside pool due to TP deficiency.
	Song et al. (2003)	
HeLa cell line	Cells cultured in thymidine medium showed multiple deletions in mtDNA and changes in the balance of the mitochondrial dNTP pool – expansion of the dTTP and dGTP pools and loss of the dCTP and dATP pools.	The model showed that increased thymidine levels lead to an imbalance of the dNTP pool, resulting in mutations in mtDNA. The results obtained are consistent with a mutagenic mechanism based on the mispairing of TG and the subsequent effect of the next nucleotide.
	Ferraro et al. (2005)	
Dormant skin and lung fibroblasts (healthy), CCD cell line 34Lu	In thymidine-supplemented dormant cells, there was a moderate increase in dTTP and dGTP and depletion of dCTP, which could interfere with normal mtDNA replication and result in damage.	This model highlights the importance of the dNTP rescue pathway in postmitotic cells and suggests that diseases associated with mtDNA depletion are dormant cell diseases.
	Pontarin et al. (2006)	
Dormant skin and lung fibroblasts (healthy), CCD cell line 34Lu	There was an increase in the dNTTP pool, and long-term thymine exposure led to partial depletion of mtDNA, but no deletions or point mutations were noted.	The results point to a possible regulatory mechanism in which the substrate cycle protects against overexpansion of dTTP (via dephosphorylation), at the expense of increased ATP consumption. This model suggests that an increase in the dTTP pool may lead to mtDNA depletion in dormant cells, reflecting MNGIE-related mechanisms.
	González-Vioque et al. (2011)	
Mitochondria isolated from murine hepatocytes	Excess dTTP led to a decrease in mtDNA replication through secondary depletion of dCTP. This decrease was not due to inhibition of dCTP transport by excess dTTP, but to competition with it.	The research model proved that secondary depletion of dCTP, resulting from an excess of dTTP, is responsible for delayed mtDNA replication, not the excess itself. The rate of mtDNA replication is limited by the lowest available dNTP, not by an excess of either dNTP.
	Pacitti and Bax (2018)	
iPSC of healthy patients and patients with MNGIE	The developed brain organoids show the presence of differentiated cell types (neurons, astroglial cells, oligodendrocytes) and features of interest for the MNGIE study – the expression of astrocytes, oligodendrocytes and myelin markers. Myelination patterns in MNGIE organoids did not differ from those of controls.	The model offers a unique opportunity to study the impact of MNGIE in the context of its specific changes, particularly in the central nervous system (CNS).

In vivo models are also a crucial source of information. The first such mouse model of MNGIE was developed by Haraguchi et al. (2002), using a double knockout (KO) of thymidine phosphorylase (*Tymp* −/−) and uridine phosphorylase (*Upp* −/−), but it exhibited only partial disease features and did not fully reflect the clinical spectrum of MNGIE. This double gene knockout was due to differences in thymidine metabolism, which in mice was phosphorylated not only by thymidine phosphorylase, as in humans, but also by uridine phosphorylase 1 and 2 (Haraguchi et al. 2002). A later model developed by López et al. (2009) also failed to fully reflect the disease, with MNGIE pathology limited mainly to the brain, without involving gastrointestinal tissues or skeletal muscles, which may be explained by several factors, including differences in the lifespan of mice and humans. The short lifespan of mice limits the possibility of mtDNA damage accumulation, which may affect the full development of the disease phenotype (López et al. 2009). In response to these limitations, Garcia-Diaz et al. (2014) introduced a model with exogenous thymidine and deoxyuridine supplementation, leading to significant biochemical changes in the brain and small intestine, as well as a more complete representation of MNGIE clinical features such as weight loss, muscle pathology, and leukoencephalopathy. Unfortunately, this model also had its limitations, as evidenced by differences in mtDNA levels in the brain and muscles (Garcia-Diaz et al. 2014). A summary of the described mouse models is provided in Table 2.

Table 2. MNGIE in vivo model

Cell type	Key results	Significance of the model and conclusions
Haraguchi et al. (2002)		
Murine KO *Tymp* $^{-/-}$ *Upp* $^{-/-}$	Despite complete inhibition of TP activity, the mice did not manifest the MNGIE phenotype, even after thymidine supplementation. Hyperintense brain MRI changes and abnormalities in myelin structures were noted. No abnormalities in mtDNA were noted.	This model suggests that other genetic factors (e.g., the neighbouring *SCO2* or *TK2* genes) may contribute to the full development of MNGIE. The developed model may aid in elucidating the physiological role of TP activity and serve as a useful tool in analysing the correlation between MNGIE pathogenesis and abnormal thymidine metabolism. However, it has significant limitations.
López et al. (2009)		
Murine KO *Tymp* $^{-/-}$ *Upp* $^{-/-}$	The depletion of mtDNA in the mouse brain intensified with age. Decreased activity of mitochondrial respiratory chain complexes was observed, along with late-onset vacuolar leukoencephalopathy in the white matter and cerebellum, which was not associated with demyelination.	The model explains the pathogenic mechanism of MNGIE and highlights the presence of structural changes in the brain, including leukoencephalopathy, further confirming the impact of mitochondrial dysfunction on brain function.
Garcia-Diaz et al. (2014)		
Murine KO *Tymp* $^{-/-}$ *Upp* $^{-/-}$	Exogenous administration of thymidine and deoxyuridine reduced survival and exacerbated symptoms such as weight loss, muscle weakness, leukoencephalopathy and depletion of mtDNA in the brain and small intestine.	The model reflects the pathogenic mechanisms of MNGIE, emphasizing the role of thymidine and deoxyuridine accumulation in its pathogenesis – exogenous pyrimidine nucleoside stress exacerbates the MNGIE phenotype. This model serves as a valuable tool for studying disease mechanisms and testing novel therapies.

Both *in vitro* and *in vivo* models have played a crucial role in studying this rare mitochondrial disease, but each encountered limitations due to biological and metabolic differences between rodents and humans (*in vivo* models) or the inability to fully replicate the complexity of the organism (*in vitro* models). In the case of *in vitro* models, despite their ability to mimic some aspects of disease pathology, they cannot fully replicate the complexity and dynamics of human tissues, nor the full range of clinical symptoms observed in MNGIE patients. Similarly, *in vivo* models cannot fully replicate the clinical picture of the disease, a challenge compounded by the relatively short lifespan of mice. All these limitations highlight the need for further development and refinement of MNGIE models to accurately reflect its pathology.

GENETIC PREDISPOSITIONS AND EPIDEMIOLOGICAL ANALYSIS OF MNGIE

Mitochondrial neurogastrointestinal encephalomyopathy is an exceptionally rare inherited metabolic disorder that ultimately leads to death. MNGIE does not exhibit genetic or ethnic predispositions, as the occurrence of this disease has been reported in widely dispersed and ethnically diverse populations, including Hispanics, Ashkenazi Jews, Europeans, Jamaicans, and Americans. However, the mode of inheritance increases the risk of its occurrence in populations with a high degree of consanguinity (Yadak et al. 2017; Pacitti et al. 2018; Jamalipour Soufi et al. 2024). Analysis of patient distribution indicates a relatively high prevalence of the disease within the European population. In contrast, the distribution of mutations suggests founder effects for specific mutations, such as c.866A>G in Europe and c.518T>G in the Dominican Republic, which could inform the focus of genetic studies in these regions (Garone et al. 2011). Since MNGIE is inherited in an autosomal recessive manner, this means that the risk of disease manifestation in offspring of parents who are carriers is 25%. For asymptomatic carriers, this risk increases to 50% (Pacitti et al. 2018). In terms of mutations in the *TYMP* gene, patients are either homozygous or compound heterozygous, which means that the diagnosis is based on the presence of biallelic pathogenic variants in this gene (Hirano and Díaz 2016). When the level of mutated mtDNA reaches a critical threshold, which typically occurs when 80–90% of all mitochondria are affected, a biochemical phenotype is produced, leading to the development of phenotypic symptoms (Pacitti et al. 2018). The heteroplasmic nature of mitochondria, characterized by the existence of two or more mitochondrial genotypes within the same cell, combined with the threshold effect, may account for the extended latency period and contribute to the observed heterogeneity of phenotypes (Nishigaki et al. 2003).

Typically, symptoms of MNGIE appear between the first and second decades of life and progress over time. The average life expectancy for a patient is estimated to be 37 years, with the mean age of onset being 18 years, however, this age may not be accurate, as several cases have been reported that deviate from these data, with the earliest recorded onset at 5 months, and several other cases reported after the third decade of life. Nonetheless, for most patients, the first symptoms appear during childhood (Garone et al. 2011; Kripps et al. 2020). The prevalence of MNGIE is estimated to be between 1–9 per 1 000 000 worldwide, however, these data are entirely dependent on information presented in the literature (Orphanet 2024). A report from 2012 confirmed 140 historical cases of MNGIE. Due to the variable clinical presentation and the ease of misdiagnosis or overlooking the condition, there is uncertainty about the actual frequency of this disorder and the overall accuracy of the reported data (Kripps et al. 2020).

SYMPTOMATOLOGY AND CLINICAL MANIFESTATIONS OF MNGIE

Clinically, MNGIE is a multisystemic, autosomal recessive disorder classically characterized by multiple organ dysfunction. It presents with a broad spectrum of symptoms, which typically manifest within the first three decades of a patient's life. The primary clinical features defining this syndrome and enabling its diagnosis include severe gastrointestinal dysmotility leading to cachexia, peripheral neuropathy, ocular symptoms (ptosis), and leukoencephalopathy. Additional symptoms involving certain neurological, muscular, cardiac, or endocrine features constitute secondary criteria (Zimmer et al. 2014; Filosto et al. 2018).

Despite considerable variability in the age of onset and the order in which the organs are affected, half of the patients initially report gastrointestinal issues. This observation aligns with the fact that gastrointestinal dysmotility is one of the most common features of MNGIE, encompassing conditions such as intestinal obstruction, gastroesophageal reflux, early satiety, nausea, small intestinal bacterial overgrowth (SIBO), or episodic abdominal pain and/or bloating (Garone et al. 2011; Barisic et al. 2022). These dysfunctions ultimately lead to significant weight loss (an average of 15 kg), resulting in severe malnutrition and cachexia. Consequently, individuals with MNGIE typically exhibit significantly reduced muscle mass and a frail, slender physique. Although the primary cause of gastrointestinal involvement is unknown, several hypotheses are under consideration. One possibility is damage to the enteric nervous system or dysfunction of the intestinal smooth muscles induced by mitochondrial defects (Nishino et al. 1999; Pacitti et al. 2018). These pathological changes may result from mtDNA depletion in the muscularis propria of the small intestine, particularly in its outer layer and small vessels. MtDNA deletions leading to depletion of mitochondrial genetic material have been observed to a mild degree in the stomach and upper esophagus (Giordano et al. 2008; Filosto et al. 2018). In each case, as the disease progresses, gastrointestinal symptoms intensify, leading to death from inadequate nutritional status (Pacitti et al. 2018). The marked gastrointestinal dysmotility is characteristic of chronic intestinal pseudo-obstruction (CIPO), which, due to its high similarity to the aforementioned clinical presentation, is becoming an increasingly recognized feature of various mitochondrial encephalomyopathies (Filosto et al. 2018).

This relentlessly progressive and degenerative disease also affects the nervous system, causing neuropathies that manifest as numbness, tingling sensations (paraesthesia), or limb weakness. As in muscle tissue, the uneven distribution of mtDNA mutations in nerves contributes to the clinical aspects of acquired neuropathy. In most cases, neuropathies exhibit a demyelinating nature; however, mixed cases with axonal neuropathy have also been reported (resulting in axonal-demyelinating neuropathy). These neuropathies are characterized by reduced motor and sensory nerve conduction velocities and partial conduction block. This phenomenon may be caused by the uneven distribution of mtDNA abnormalities along the nerves. Neuropathies can manifest as mild sensory neuropathies or progressive sensorimotor neuropathies, which clinically and electrophysiologically resemble other conditions (such as chronic inflammatory demyelinating polyneuropathy – CIDP or Charcot–Marie–Tooth disease) (Pacitti et al. 2018; Hirano et al. 2021).

Neurological symptoms commonly observed in MNGIE patients also include ocular manifestations. Progressive, bilateral, and relatively symmetrical ptosis and ophthalmoplegia or oculomotor paresis are among the most frequent symptoms, though mild myopia and glaucomatous features have also been reported. The preferential involvement of extraocular muscles does not appear coincidental, as these muscles have a high energy demand,

making them more susceptible to the effects of mitochondrial dysfunction (Filosto et al. 2018; Pacitti et al. 2018).

A distinctive feature of MNGIE is the typically asymptomatic involvement of the central nervous system in the disease's clinical presentation. Notable signal changes in magnetic resonance imaging (MRI) corresponding to white matter alterations may suggest progressive leukoencephalopathy. As the disease progresses, the encephalopathy becomes more pronounced and tends to coalesce and spread. The characteristic diffuse leukoencephalopathy of this disorder, combined with the previously described symptoms, significantly narrows the diagnostic possibilities, suggesting the potential occurrence of MNGIE in the patient (Garone et al. 2011; Scarpelli et al. 2013; Pacitti et al. 2018).

A review of the literature indicates that there are additional minor clinical criteria suggestive of this fatal metabolic disorder. These include sensorineural hearing loss, metabolic and endocrine disorders (diabetes, lactic acidosis), cardiac complications (typically asymptomatic, associated with left ventricular hypertrophy and bundle branch block), or changes in the systemic adaptive immune response (resulting from gut microbiome dysbiosis) (Pacitti et al. 2018; Bax 2020).

Despite the characterization of the main clinical features of MNGIE, the biggest challenge remains the lack of correlation between specific *TYMP* mutations and distinct phenotypes. As a result, predicting the age of onset or severity of the disease based on mutations becomes impossible (Kamatani et al. 2013). There are known cases in which the same *TYMP* mutation did not lead to the same phenotype, which could be related to heterozygosity among patients. On the other hand, there are patients who exhibit phenotypes with clear involvement of one organ system but understanding why one is more or less affected than another remains unknown (Pacitti et al. 2018).

DIAGNOSTIC METHODS FOR MNGIE

Throughout their lives, MNGIE patients frequently encounter misdiagnoses, leading to diagnostic delays ranging from 5 to 10 years. The average life expectancy of patients (37 years) significantly underscores the need for early diagnosis of this disease (Nishino et al. 2000; Taanman et al. 2009). However, due to the variability of its symptoms and the possibility of confusing them with other conditions, accurate diagnosis, especially in the early stages, remains challenging. Several key features appear to be critical in resolving diagnostic uncertainty, including the presence of sensorimotor neuropathy, leukoencephalopathy, gastrointestinal disturbances, ophthalmoplegia (weakness of the extraocular muscles), and ptosis. The combination of all these clinical features greatly facilitates an accurate diagnosis, but difficulties arise in patients who present with only one of the previously mentioned symptoms. As a result, some MNGIE patients undergo various unnecessary diagnostic and surgical interventions, such as laparotomy. This procedure is often performed because gastrointestinal disturbances, which are almost always present, are the primary cause of morbidity and mortality in MNGIE (Nishino et al. 2000; Garone et al. 2011; Bax 2020). These disturbances are also frequently misdiagnosed as anorexia nervosa. Another significant symptom, which affects nearly all patients with few exceptions, is peripheral neuropathy, whose asymptomatic course can be detected through nerve conduction studies. In terms of diagnostic tools, brain MRI has proven to be particularly valuable, almost always revealing diffuse symmetrical leukoencephalopathy with confluent T2 hyperintensity in the white matter of MNGIE patients (Garone et al. 2011; Jamalipour Soufi et al. 2024).

In addition to these methods, the gold standard in MNGIE diagnosis has become the significantly elevated levels of thymidine and deoxyuridine in plasma (dThd rises to 3 μmol/L and dUrd to 5 μmol/L, compared to undetectable levels in healthy individuals) (urine analysis is unreliable) and the severely reduced or nearly absent thymidine phosphorylase activity in leukocytes of the buffy coat (<8% of the average reference TP value) (Martí et al. 2004; Pacitti et al. 2018; Hirano et al. 2021). According to studies, typical MNGIE is caused by less than 10% of normal enzyme activity. Residual activity between 10–20% is associated with milder late-onset phenotypes. Conversely, a reduction in TP activity by 70% or less is insufficient to be classified as pathogenic. This is due to the observation that heterozygous carriers of *TYMP* mutations show residual enzyme activity above 30%, which does not lead to overt clinical symptoms (plasma dThd and dUrd levels are undetectable). However, there are exceptions to this rule. Cases of late-onset or milder phenotypes have been reported in the literature in patients with significantly reduced or nearly undetectable TP activity (Filosto et al. 2018; Pacitti et al. 2018).

Another important factor in making an accurate diagnosis is conducting a detailed interview with the patient and their family, which can confirm autosomal recessive inheritance. This information may prompt the specialist to refer the patient for nDNA screening for mutations in the *TYMP* gene. Unfortunately, due to the fact that these tests are considered specialized, their availability is limited, and they may require a significant amount of time and money from the patient. Therefore, many specialists wait to order such tests until there is a strong suspicion of MNGIE. Nevertheless, conducting the above-presented biochemical analyses and clinical examinations reduces the risk of missing a diagnosis when mutation sites are not identified or when variants of uncertain significance or wild-type sequences are detected. It is often the loss of TP activity and nucleoside accumulation that prompts targeted gene testing for primary mutations in the *TYMP* gene or comprehensive whole-genome analyses to search for secondary mtDNA mutations. This can be achieved through Sanger sequencing or Next-Generation Sequencing (NGS) (Nishino et al. 2000; Pacitti et al. 2018; Barisic et al. 2022).

Additionally, MNGIE patients frequently exhibit metabolic abnormalities such as lactic acidosis, elevated protein levels in cerebrospinal fluid, or deficiencies in mitochondrial respiratory chain enzymes. To confirm or exclude other disorders, auxiliary tests are conducted, including skeletal muscle biopsies, which in rare cases may reveal ragged red fibers, as well as histology of the small intestine mucosa, swallowing tests, and gastric emptying studies (Hirano 1993; Hirano et al. 2021).

Almost a decade has elapsed since the diagnostic algorithm for MNGIE developed by Zimmer and colleagues was introduced. According to this algorithm, patients presenting with clinical signs suggestive of MNGIE, such as gastrointestinal dysmotility, external ophthalmoplegia/ptosis, significant weight loss, and peripheral neuropathy, are advised to undergo brain MRI. If the imaging reveals the presence of leukoencephalopathy, the subsequent step involves biochemical analysis of bodily fluids to detect the excess of specific metabolites. If increased levels of thymidine and deoxyuridine are identified, genetic sequencing of the *TYMP* gene is advised (Zimmer et al. 2014). This algorithm addresses the issue of frequent misdiagnoses in MNGIE cases, a common issue that often results in patients being ineligible for experimental treatments by the time an accurate diagnosis is finally made due to the advanced state of their condition. In numerous cases, a correct diagnosis is only made after the death of one or two family members exhibiting similar symptoms. Therefore, early recognition of MNGIE increases the patient's chances of meeting therapy criteria and achieving a positive outcome (Garone et al. 2011; Pacitti et al. 2018; Jamalipour Soufi et al. 2024).

SYMPTOMATIC AND THERAPEUTIC TREATMENT: CURRENT STANDARDS IN THE FIGHT AGAINST MNGIE

Currently, there is no specific therapy for patients with MNGIE that has been confirmed to be effective in clinical trials. The current treatment guidelines are symptomatic and rely on supportive care combined with a coordinated approach from multiple clinicians. Given that this metabolic abnormality is associated with systemic imbalances in nucleosides, most clinical interventions focus on combating the excess of metabolites and striving to restore balance in the mtdNTP pool (Halter et al. 2011).

Gastrointestinal issues, resulting from gastrointestinal dysmotility, are treated with various medications: analgesics, prokinetics, antiemetics, as well as antibiotics for treating SIBO (Oztas et al. 2010). However, it is crucial to avoid medications that disrupt mitochondrial function when treating specific symptoms of MNGIE (e.g., valproate, phenytoin, linezolid) (Halter et al. 2011; Pacitti et al. 2018). For patients resistant to opioid treatment or experiencing persistent pain, more advanced techniques such as celiac plexus block are considered. In malnourished patients, various forms of parenteral nutrition are often employed. Unfortunately, due to serious parenteral complications, such as the development of liver steatosis and cholestasis, this form of treatment should not be prolonged. Additionally, there is controversy over the risk of underfeeding associated with parenteral nutrition, which may lead to further mitochondrial toxicity. Neurological aspects of MNGIE typically require physical therapy and occupational therapy (Bax 2020; Hirano et al. 2021). Limb pain caused by polyneuropathy is alleviated with centrally acting agents (e.g., amitriptyline, gabapentin (Finsterer and Frank 2017). In the absence of targeted treatment, MNGIE patients require comprehensive care based on collaboration among various specialists (Pacitti et al. 2018).

Despite its rarity, MNGIE has garnered significant interest due to the fact that it is one of the few mitochondrial diseases amenable to treatment that can potentially save patients' lives. The excitement surrounding this field, as well as mitochondrial diseases in general, is justified, as the most significant therapeutic progress to date has been made in the treatment of MNGIE (Filosto et al. 2018). These studies include a range of experimental therapeutic approaches aimed at overcoming the mitochondrial defect. Currently, two main treatment strategies are distinguished. The first, focusing on directly reducing elevated dNTP levels, includes hemodialysis (HMD) and continuous ambulatory peritoneal dialysis (CAPD). The second strategy involves supplementing the missing enzyme through platelet transfusion, allogeneic hematopoietic stem cell transplantation (AHSCT), orthotopic liver transplantation (OLT), and enzyme replacement therapy. What unites all of these therapeutic options is the goal of reducing or eliminating pathological nucleoside concentrations, thereby alleviating intracellular disturbances resulting from their imbalance. This approach prevents further mtDNA damage and translates into stabilization or improvement of the patient's condition (Halter et al. 2011; Bax 2020; Barisic et al. 2022).

As we approach the fifth decade of MNGIE research, we know that its effective treatment remains a challenge. Research conducted over the years has validated the aforementioned strategies and provided valuable insights into their effectiveness and safety. The first attempt to restore biochemical balance in the form of hemodialysis proved insufficient in the long term. As indicated by the results, HMD transiently lowers thymidine and deoxyuridine levels in serum and urine – returning to baseline levels within 24 hours after the procedure. The lack of sustained reduction of these metabolites in cerebrospinal fluid may explain the lack of neurological benefits and the overall lack of impact on the clinical progression of the disease.

To be effective, such a procedure would need to be performed three to four times a week. Such a high frequency carries the risk of complications and poses a significant burden on the patient (Spinazzola et al. 2002; Röeben et al. 2017).

The second approach considered in this strategy is continuous ambulatory peritoneal dialysis, which involves filling the peritoneal cavity with a dialysis solution, allowing the diffusion of harmful metabolites from the blood, through the peritoneal membrane, into the waste dialysate, which is replaced every 4–8 hours. Clinically, patients experienced improvement in gastrointestinal symptoms, weight gain (3.5–13 kg), and enhanced motor function. These results suggest that CAPD may hold potential as a symptom-targeted approach for gastrointestinal issues. However, this strategy also carried the risk of complications such as catheter infections, mild peritonitis, peritoneal sclerosis, and issues related to glucose in the dialysis solution and bowel perforation during catheterization (Yavuz et al. 2007; Ariaudo et al. 2015; Sivadasan et al. 2016).

Platelet infusion, the first attempt to replace TP deficiency with donor-derived platelets, also showed limited efficacy. The mechanism of this strategy is based on the diffusion of 2'-deoxyuridine along with thymine through the membrane of donor platelets into cells, where they are metabolized by cytosolic thymidine phosphorylase into thymine and uracil. Platelets were chosen deliberately, as they are the main source of this enzyme in humans. Results showed that such an infusion provided transient thymidine phosphorylase activity and reduced dThd and dUrd concentrations in the patients' plasma. Although all obtained results were reversible and short-lived, they pointed to the potential therapeutic nature of therapies based on the sustained restoration of circulating TP (Lara et al. 2006; Röeben et al. 2017; Barisic et al. 2022).

One of the two well-defined treatment options for MNGIE that offers the possibility of permanent TP correction is allogeneic hematopoietic stem cell transplantation. However, this treatment is limited by the availability of a suitable donor and the poor clinical condition of patients. Additionally, it carries an increased risk of complications and mortality. The over 60% mortality rate presented in one study, among a group of 24 patients, was attributed to transplant failure or infections associated with the patients' impaired ability to tolerate aggressive conditioning chemotherapy. Nevertheless, the available data confirm the possibility of restoring TP function and improving the clinical picture following AHSCT. However, certain conditions must be met regarding the patient's health and the potential donor. Due to the high complication rate, this strategy is recommended for patients in a stable clinical condition with an optimal donor. Given the frequency of misdiagnoses, applying the "early transplant" principle in MNGIE seems challenging to implement routinely (Hirano et al. 2006; Halter et al. 2011; Bax 2020; Ramón et al. 2021).

The second long-term strategy for restoring TP enzymatic activity and halting disease progression is liver transplantation. This organ is characterized by elevated thymidine phosphorylase expression, and the success rate of the procedure is estimated at 90%. To date, six patients with MNGIE have successfully undergone OLT. Case studies have shown near normalization of plasma thymidine levels and clinical improvement in patients. The frequent hepatopathies in MNGIE patients, combined with the high survival rate of OLT and simultaneous improvement in TP activity, make this therapeutic approach seem ideal. Unfortunately, the procedure is limited by the availability of a suitable donor and carries the risk of transplant-related complications and long-term immunosuppressive therapy (De Giorgio et al. 2016; D'Angelo et al. 2017).

Among the treatment strategies for MNGIE, there is also room for a new enzyme replacement therapy using autologous thymidine phosphorylase encapsulated in erythrocytes (EETP – erythrocyte encapsulated thymidine phosphorylase). Encapsulating the enzyme within the

patient's erythrocytes ex vivo, and then reintroducing them into the patient's bloodstream, is intended to replace the deficiency. This is where the "magic" of EETP happens, based on the free diffusion of thymidine and deoxyuridine through the erythrocyte membrane into the cell, where the encapsulated enzyme awaits to catalyse their metabolism. The resulting products can then freely move into the plasma for further metabolism. Interestingly, such enzyme encapsulation carries pharmacological benefits associated with prolonging the enzyme's half-life. EETP has an encouraging safety profile, associated with lower invasiveness, and while it reduces plasma thymidine levels, preliminary data show that this therapy is not as effective as AHSCT or OLT (Godfrin et al. 2012; Levene et al. 2019; Bax 2020).

For these reasons, both EETP and the previously discussed CAPD are unlikely to serve as final treatment options but are considered promising bridging therapies, supporting patients awaiting liver or stem cell transplantation. By stabilizing the patient's condition, they can increase the likelihood of qualifying for these procedures (Pacitti et al. 2018).

EXPERIMENTAL THERAPIES: NEW APPROACHES TO MNGIE TREATMENT

MNGIE is a severe disease that continues to pose numerous challenges for clinicians and researchers. Currently, available therapies, such as AHSCT and OLT, offer significant potential, but their effectiveness is constrained by the availability of compatible donors and the risks associated with complex medical procedures. Gene therapy appears to be a promising alternative that is gaining increasing interest in solving these problems. The mouse models presented in Table 2 play a critical role in the study of the pathogenesis of MNGIE and provide a solid basis for the development of new therapies, including the gene therapy mentioned above, which shows promising results in preclinical studies. A mouse model with *Tymp* $^{-/-}$ *Upp* $^{-/-}$ knockout was used to recreate the metabolic imbalances, on which two promising gene therapy approaches were studied. In the case of MNGIE, the expected outcome is the systemic removal of dThd and dUrd accumulation rather than targeting a specific tissue. Consequently, any organ or cell available in the bloodstream could be a potential target for TP transduction. Hematopoietic tissue (HSC), one of the richest sources of thymidine phosphorylase in humans (but not in mice), and liver tissue, which also shows high enzyme activity in humans (but not in mice), were selected as targets (Pacitti et al. 2018; Bax 2020).

The proposed hematopoietic stem cell gene therapy based on a lentiviral vector (HSCGT-LV) is an innovative approach that uses LV to carry a functional copy of the human *TYMP* gene into the patient's autologous hematopoietic cells. This therapy involves isolating HSCs from MNGIE patients, transducing them *ex vivo* with vectors carrying the functional *TYMP* gene, and then returning them to the patient. This strategy enables the restoration of stable TP expression, which reduces the excess of nucleosides. The existence of a mouse disease model allowed for preclinical testing of this approach, demonstrating its feasibility. Studies indicate that the animal model exhibited long-term biochemical correction, with stable TP activity and subsequent reduction of dThd and dUrd levels to nearly undetectable levels (Torres-Torronteras et al. 2011; Yadak et al. 2018). However, the research did not stop there, as another study was conducted to further demonstrate the feasibility of HSCGT-LV, this time using peripheral blood mononuclear cells from two MNGIE patients. In this case, stable expression of functional TP was also achieved, which catabolized the excess nucleosides present in the extracellular environment (Torres-Torronteras et al. 2011).

Another promising alternative is liver-targeted gene therapy using adeno-associated virus (AAV) vectors. To better assess the effectiveness of this therapy, a murine model was used,

in which the disease phenotype was exacerbated by oral administration of dThd and dUrd. Subsequently, these mice were intravenously administered a specially designed AAV vector carrying the human *TYMP* gene, with targeted liver expression. Studies have shown that even low doses of the AAV vector were effective in consistently reducing nucleoside levels to values close to normal. In particular, the AAV vector with the AAT promoter proved to be the most effective, not only normalizing biochemical markers but also improving neurological function. These findings confirm the feasibility of the proposed gene therapy (Torres-Torronteras et al. 2011; Vila-Julià et al. 2020).

In the context of gene therapy, the selection of an appropriate vector depends on the patient's health status and the specifics of the therapy. Both lentiviral (LV) and adeno-associated virus (AAV) vectors are characterized by favourable safety profiles. The lentiviral vector used in HSCGT therapy is associated with a lower oncogenic risk compared to *γ-retroviruses*, making it a preferred choice for specific applications. In contrast, AAV vectors are particularly valued for their episomal nature, which presents a low risk of random insertion into the nuclear genome. They appear to be more suitable for patients in terminal stages (Yadak et al. 2017). Ultimately, regardless of the viral vector used, the chosen therapeutic option should ensure long-term expression of the gene of interest without causing adverse effects. It is crucial to accurately determine the vector dose sufficient to correct the biochemical imbalance without potential side effects (Yadak et al. 2017; Ramón et al. 2021).

An experimental approach to enzyme replacement therapy, drawing on EETP, has also been developed, involving the use of polymeric nanoreactors, which offer a promising alternative to traditional delivery systems. The designed PMOXA-PDMS-PMOXA polymeric nanoparticles were intended to encapsulate TP. By reconstructing nucleoside-specific Tsx porins, the authors ensured permeability, allowing for the transport of substrates and products. The studies revealed that these "nanoreactors" were stable and enzymatically active in mouse blood serum at 37°C, without protein leakage and without inducing an inflammatory response. However, after three days of incubation, TP activity decreased by half, necessitating further research to fully assess their long-term stability and biocompatibility in a clinical context. Nonetheless, this is a promising alternative to traditional enzyme replacement therapies and could serve as a valuable addition to the current therapeutic repertoire (De Vocht et al. 2009).

CONCLUSION

MNGIE is a fatal metabolic disorder that, since its initial description, has seen significant advancements in both diagnostic capabilities and understanding of its pathogenic mechanisms. Nevertheless, the development of therapies for this rare and complex disease still faces significant obstacles. The difficulties associated with diagnosing and monitoring the disease's progression underscore the need for further research. Although promising preclinical and experimental results provide hope, there remains a lack of clinical indicators that unequivocally correlate with patient improvement. The promising outcomes of preliminary preclinical therapies offer optimism but require further validation and safety evaluation. In this context, while mouse models of MNGIE do not fully replicate the clinical picture of the disease in humans, they remain a key research tool. By enabling detailed studies and monitoring therapeutic effects, they contribute to the development of new treatment strategies. Currently, MNGIE remains a disease that requires an interdisciplinary approach and long-term research commitment. New therapeutic strategies, the development of biomarkers, and a

better understanding of the molecular mechanisms of this disease may significantly improve patient quality of life and prognosis in the future. Unfortunately, early diagnosis and prompt initiation of therapy remain crucial, highlighting the need to raise awareness among physicians and patients. Thus, the topic of MNGIE treatment remains open, encouraging scientists to continue research and explore new therapeutic paths. Ultimately, understanding the cellular and genetic mechanisms of MNGIE is key to developing new therapeutic approaches that can provide lasting benefits to patients.

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NOWE SPOJRZENIE NA LECZENIE MITOCHONDRIALNEJ NEUROJELITOWEJ ENCEFALOMIOPATII (MNGIE): OD PATOGENEZY DO TERAPII

Streszczenie. Mitochondrialna neurojelitowa encefalomiopatia (ang. *mitochondrial neurogastrointestinal encephalomyopathy syndrome*, MNGIE), znana również jako mitochondrialna encefalopatia żołądkowo-jelitowa, to niezwykle rzadkie dziedziczne zaburzenie metaboliczne spowodowane mutacjami w genie jądrowym *TYMP*, odpowiedzialnym za kodowanie enzymu fosforylazy tymidynowej (TP). Idąca za tym systemowa akumulacja deoksyrybonukleozydów prowadzi do mutacji w mitochondrialnym materiale genetycznym, a w ostateczności do niewydolności samego organellum. Zwyrodnieniowy charakter choroby i złożoność cechujących ją objawów klinicznych skutecznie utrudnia prawidłowe

zdiagnozowanie, co przy szacowanej na 37 lat średniej długości życia chorych na MNGIE znacząco utrudnia leczenie. Obecnie dostępne podejścia terapeutyczne, bazujące na leczeniu objawowym, powoli ustępują miejsca nowo opracowywanym terapiom eksperymentalnym. Kluczową rolę w tym temacie odegrały modele *in vitro* i *in vivo*, które na przestrzeni lat przyczyniły się do pogłębienia i zrozumienia mechanizmów choroby, stanowiąc tym samym istotny fundament dla dalszych badań i potencjalnych terapii. Niniejszy przegląd obejmuje kompleksowe zestawienie aktualnego stanu wiedzy na temat MNGIE, ze szczególnym uwzględnieniem obecnych opcji terapeutycznych, dostępnych modeli choroby oraz procedur diagnostycznych. Jego celem jest zwiększenie świadomości klinicznej, wspieranie rozwoju skuteczniejszych metod leczenia i diagnostyki, jak również dostarczenie cennych informacji, które mogą przyczynić się do poprawy jakości życia i opieki chorych na MNGIE.

Słowa kluczowe: MNGIE, mitochondrialna encefalomiopatia neurojelitowa, fosforylaza tymidynowa, *TYMP*, choroba metaboliczna, choroba mitochondrialna.