



Mitochondrial neurogastrointestinal encephalomyopathy: Clinical and biochemical impact of allogeneic stem cell transplantation in a Greek patient with one novel *TYMP* mutation

A. Paisiou^a, M. Rogalidou^b, R. Pons^c, E. Ioannidou^a, K. Dimakou^b, A. Papadopoulou^b, F. M. Vaz^{d,e,f}, G. Vessalas^a, S.M.I. Goorden^d, J. Roelofsen^d, A. Zoetekouw^d, M.M. Nieman^d, E. Dimitriou^g, M. Moraitou^g, I. Peristeri^a, H. Michelakakis^{g,*}, A.B.P. van Kuilenburg^d

^a Stem Cell Transplant Unit, Agia Sofia Children's Hospital, Athens, Greece

^b Division of Paediatric Gastroenterology & Hepatology, 1st Department of Paediatrics, National and Kapodistrian University of Athens, Agia Sofia Children's Hospital, Athens, Greece

^c Pediatric Neurology Unit, 1st Department of Pediatrics, Agia Sofia Children's Hospital, National and Kapodistrian University of Athens, Athens, Greece

^d Laboratory Genetic Metabolic Diseases, Amsterdam UMC, University of Amsterdam, Department of Clinical Chemistry, Amsterdam Gastroenterology Endocrinology Metabolism, Amsterdam, the Netherlands

^e Department of Pediatrics, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands

^f Core Facility Metabolomics, Amsterdam UMC, the Netherlands

^g Department of Enzymology and Cellular Function, Institute of Child Health, Athens, Greece

ARTICLE INFO

Keywords:

Mitochondrial neurogastrointestinal encephalomyopathy
MNGIE
Mutation analysis
Allogeneic hematopoietic stem cell transplantation, AHSCT

ABSTRACT

We describe the case of a Greek female patient with the Classic form of the ultra-rare and fatal autosomal recessive disorder Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) and the impact of allogeneic hematopoietic stem cell transplantation on the biochemical and clinical aspects of the disease.

The patient presented at the age of 15 years with severe gastrointestinal symptoms, cachexia, peripheral neuropathy and diffuse leukoencephalopathy. The diagnosis of MNGIE disease was established by the increased levels of thymidine and deoxyuridine in plasma and the complete deficiency of thymidine phosphorylase activity. The novel c.[978dup] (p.Ala327Argfs*?) variant and the previously described variant c.[417 + 1G > A] were identified in *TYMP*. The donor for the allogeneic hematopoietic stem cell transplantation was her fully compatible sister, a carrier of the disease. The patient had a completely uneventful post-transplant period and satisfactory PB chimerism levels. A marked and rapid decrease in thymidine and deoxyuridine plasma levels and an increase of the thymidine phosphorylase activity to the levels measured in her donor sister was observed and is still present sixteen months post-transplant. Disease symptoms stabilized and some improvement was also observed both in her neurological and gastrointestinal symptoms. Follow up studies will be essential for determining the long term impact of allogeneic hematopoietic stem cell transplantation in our patient.

1. Introduction

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an ultra-rare autosomal recessive disorder (MIM #603041). It is caused by a deficiency of thymidine phosphorylase (TP; EC2.4.2.4)

which is the result of loss of function mutations in *TYMP*, a nuclear gene located on chromosome 22 [1,2,3]. The disorder was first described by Okamura et al. in 1976 [4]. TP catalyzes the reversible phosphorolysis of the pyrimidine deoxyribonucleosides, thymidine (dThd) and 2'-deoxyuridine (dUrd) to 2-deoxyribose 1-phosphate and their respective bases,

Abbreviations: AHSCT, allogeneic hematopoietic stem cell transplantation;; CSF, cerebrospinal fluid;; dThd, thymidine;; dUrd, 2'-deoxyuridine;; GVHD, Graft Versus Host Disease;; HSCT, hematopoietic stem cell transplantation;; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy;; mtDNA, mitochondrial DNA;; OLT, orthotopic liver transplantation;; TP, thymidine phosphorylase;; TPN, total parenteral nutrition;; *TYMP*, thymidine phosphorylase gene;; PB, peripheral blood;; PLT, platelet;; VLCFA, very long chain fatty acids.

* Corresponding author at: Department of Enzymology and Cellular Function, Institute of Child Health, 11527 Athens, Greece.

E-mail address: ecfdept@ich.gr (H. Michelakakis).

<https://doi.org/10.1016/j.ymgmr.2021.100829>

Received 25 October 2021; Received in revised form 1 December 2021; Accepted 1 December 2021

Available online 3 December 2021

2214-4269/© 2021 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

thymine and uracil. Deficient TP activity results in the accumulation of its substrates in blood and tissues [5,6].

This accumulation of dThd and dUrd disrupts the normal replication of mitochondrial DNA (mtDNA), leading to multiple deletions, somatic point mutations and depletion of mtDNA, and ultimately mitochondrial failure [7,8,9]. Clinically the disease is characterized by severe and progressive gastrointestinal (GI) and neurological manifestations. It is a fatal disease and death is usually the result of severe malnutrition [10].

Recently several therapeutic approaches aiming in the restoration of TP activity have been developed. These approaches include infusion of erythrocyte encapsulated TP (EE-TP) [11], orthotopic liver transplantation (OLT) [12,13] and hematopoietic stem cell transplantation (HSCT) [14]. We describe the case of a Greek female patient diagnosed at the age of 15 years bearing a novel and a previously described variant in *TYMP* and the clinical and laboratory impact of allogeneic hematopoietic stem cell transplantation (AHSCT).

2. Methods and case report

2.1. Methods

TP activity was assayed in leukocytes which were isolated as described before [15]. The reaction mixture contained 35 mM potassium phosphate (pH 7.4), 1 mM dithiothreitol and 2 mM thymidine. Thymine was detected at 265 nm after separation with reversed-phase HPLC, as described before [15].

dThd and dUrd were measured in plasma essentially as described previously [16]. Briefly, 100 μ l of plasma was mixed with internal standard mixture (13 C-dThd and 13 C-dUrd) and deproteinized using a Ultracel-30 K filter. One 1 μ l of 50% (v/v) acetic acid was added 5 μ l of the mixture was injected on a Waters Acquity HSS T3 100 \times 2.1 mm 1.8 μ m particle size equilibrated with eluant A (25 mM ammonium acetate, pH 5.0) and eluted using eluant B (25 ammonium acetate pH 5.0/MeOH (1:1, v/v) in 8 min with a flow rate of 0.4 ml/min. The gradient was (all linear steps): 0–1 min: 100% A, 1–4 min: to 80% B, 4–5 min isocratic 80% B, 5 min: 100% B and 5–8 min 100% B. The column was coupled to a Waters Xevo TQ-S micro mass spectrometer set in the positive ion mode which monitored four MRM's (dThd [243.0 > 127.0], 13 C-dThd [244.0 > 128.0] (for both dwell time: 0.008 s, cone voltage: 16 V, collision energy: 9.0 eV), 13 C-dUrd [231.0 > 115.0] and dUrd [229.0 > 113.0] (for both dwell time: 0.008 s, cone voltage: 18 V, collision energy: 10.0 eV)). Results were processed in Masslynx 4.2. The activities of β -galactocerebrosidase and arylsulphatase A were assayed in leukocyte homogenates isolated from heparinized blood. β -Galactocerebrosidase activity was determined using tritium-labeled [3 H] galactosylceramide (ARC; American Radiolabeled Chemicals, Inc.) [17]. Arylsulphatase A activity was determined as described previously [18]. Very long chain fatty acids (VLCFA) were determined in plasma by gas chromatography (Agilent 7890A) as described previously [19]. Quantitation of amino acids in CSF was performed using the Biochrom 30 aminoacid analyser.

2.2. Case report

The female patient was born to healthy unrelated parents, with normal psychomotor development and an unremarkable medical history. She was asymptomatic until the age of 16 years when she developed diarrhea and abdominal pain. Six months after the onset of her symptoms severe weight loss (~ 25%) was observed despite adequate caloric intake. Laboratory tests showed normal inflammatory markers, severe hypoalbuminemia and hypogammaglobulinemia, iron deficiency and anemia. Furthermore, reduced vitamin B12 (197 pg/ml; normal 211–911 pg/ml), folic acid (4.7 ng/ml; normal >5.38 ng/ml), potassium (2.4 mmol/l; normal 3.5–5.1 mmol/l) and calcium (7.3 mg/dl; normal 9.2–11 mg/dl) were found. Celiac antibodies were negative and a slight elevation of calprotectin was detected. Endoscopy of the upper and lower digestive tract revealed mild inflammatory changes in the

stomach and terminal ileum. Histology showed eosinophilic infiltration throughout the digestive tract. In the ileum, thickening of the mucosal muscle layer and an increase in villous bundles were observed, although no megamitochondria were found. MRI enterography was normal. The patient was treated with steroids (budenofalk), sulfasalazine, lactose and gluten-free diet without improvement of her symptoms.

She suffered a severe headache episode associated with diplopia and decreased level of alertness of 5 min duration. Brain MRI revealed diffuse leukoencephalopathy. She complained of paresthesias, unsteadiness and fatigue. Neurophysiologic studies demonstrated a sensory-motor demyelinating peripheral neuropathy.

Eight months after the initiation of her symptoms she was referred to Agia Sofia Children's Hospital for further investigations. On physical examination she was afebrile, her weight was 37 kg (< 3rd centile) and she appeared cachectic.

Cognitive and psychological status were normal. She showed mild ophthalmoparesis without ptosis. Hearing was intact. Muscle tone and strength in the upper and lower limbs were normal. She had mild thenar and hypothenar atrophy, decreased light touch and vibration sense in a stocking-glove distribution and absent deep tendon reflexes. Her gait was normal though she had mild difficulties with tandem gait testing.

A central catheter was placed and total parenteral nutrition (TPN) was initiated in combination with oral feeding. Simultaneously, courses of albumin and intravenous immunoglobulins were given. The patient gradually showed an improvement of her weight and normalization of biochemical parameters/deficiencies.

The activities of arylsulphatase A and β -galactocerebrosidase were normal excluding metachromatic and Krabbe leukodystrophies. Normal levels of very long chain fatty acids were found in plasma. Elevated lactic acid and protein concentration were found in cerebrospinal fluid (CSF) and quantitation of CSF amino acids revealed increased alanine levels.

The diagnosis of MNGIE disease was established by increased concentrations of dThd (8.7 μ mol/l; normal <0.1) and dUrd (15.1 μ mol/l; normal <0.2) levels in plasma and the undetectable TP activity in white blood cells. Decreased TP activity, compared to the reference values (357–707 nmol/mg/h), was detected in the father (199 nmol/mg/h), the mother (188 nmol/mg/h) and her sister (181 nmol/mg/h) suggesting that all individuals were carriers of the disease. Sequencing of the *TYMP* gene (reference sequence NM_001953.4) revealed the presence of the previously described c.[417 + 1G > A] and the novel variant c.[978dup] (p.Ala327Argfs*?). The c.[417 + 1G > A] variant is located in an invariant splice donor site and most likely affects the process of *TYMP* pre-mRNA splicing [20]. The c.[978dup] (p.Ala327Argfs*?) variant results in a frame shift and thus the synthesis of a nonsense protein. It also eliminates the termination codon with no additional stop codons within the 3'-UTR, resulting in a nonstop mRNA that may be a substrate for a nonstop mediated decay mechanism that degrades nonstop mRNAs [21,22]. The c.417 + 1G > A variant was identified in the father and the c.978dup [p.Ala327Argfs*?] variant in the mother. Her younger sister was found to be a carrier of the c.417 + 1G > A variant. Thus the genetic studies confirmed that both the parents and her sister are carriers of the disease and that the patient was compound heterozygous for two novel pathogenic variants.

Following the diagnosis of TP deficiency, and after considering all available therapeutic options, it was decided to perform AHSCT.

The patient was conditioned with busulfan 12.8 mg/kg, fludarabine 150 mg/m² and antithymocyte globulin 7.5 mg/kg and she received a bone marrow graft with sufficient cellularity (CD34: 2.7 \times 10⁶/kg, CD3: 3.7 \times 10⁷/kg) from her fully compatible sister. Graft versus host prophylaxis consisted of cyclosporine 3 mg/kg and methotrexate 10 mg/m² on days 1, 3, and 6 after the transplantation. The early and late post transplantation period was completely uneventful. The patient did not present Graft Versus Host Disease (GVHD) or other post-transplant complications. Neutrophil engraftment was achieved on day 19 after the transplantation. It should be mentioned that the patient never

reached platelet (PLT) levels $<20,000/\mu\text{l}$ and never received a PLT transfusion. Peripheral blood (PB) chimerism detected by STR-VNTR on peripheral blood one month after transplantation was 89%. Subsequent testing six months after transplantation revealed a decrease in PB chimerism and cyclosporine was discontinued. After cyclosporine discontinuation, an increase in PB chimerism was observed which one year after the transplant was 96.1%.

Further increase in dThd ($53 \mu\text{mol/l}$) and dUrd ($75 \mu\text{mol/l}$) levels in plasma had been observed three weeks prior to the transplantation. These extremely high levels of thymidine and deoxyuridine have not been observed before in other reported MNGIE patients and the underlying reason is unknown. Clear reduction in the levels of both deoxyribonucleosides was observed following HSCT. Six months post transplant dThd levels were $0.9 \mu\text{mol/l}$ and dUrd $2.4 \mu\text{mol/l}$. A further reduction was observed ten months post-transplant (dThd: $0.6 \mu\text{mol/l}$ and dUrd $1.3 \mu\text{mol/l}$) and sixteen months after the operation the levels of both deoxyribonucleosides were $<0.5 \mu\text{mol/l}$. TP activity in leucocytes showed a clear and sustained increase. Ten months after AHSCT the TP activity was 181 nmol/mg/h and at sixteen months 158 nmol/mg/h .

After bone marrow transplantation, attempts to convert her to enteral nutrition were unsuccessful and she continued to receive parenteral nutrition 4 days per week. Seven months post-transplant her diarrhea improved and her weight increased to 48 kg. However two months later she relapsed with 5 episodes of diarrhea per day and a weight loss of 4 kg.

A new endoscopic examination of upper and lower digestive tract was performed with no special findings macroscopically. Histology revealed eosinophilic infiltration in all digestive tract and in the duodenum an increase of smooth muscle fibers of mucosa muckle layer was noted. Sixteen months post-transplant she is still receiving parenteral nutrition, has gained body weight (body weight 46.800 kg) and the episodes of abdomen pain are very rare. Her diarrhea has improved with respect in frequency and consistency.

Her neurological condition remains stable. Sporadically, she suffers from complicated migraines. Her paresthesias, fatigue and imbalance improved. A repeated MRI conducted 10 months after transplantation showed no progression of her encephalopathy.

3. Discussion

We describe the case of a patient with the Early Onset (or Classic) form of MNGIE disease [10], carrying two novel Class 5 variants, and the impact on the biochemical and clinical parameters of AHSCT. GI symptoms were the presenting clinical signs and gradually central and peripheral nervous system involvement became apparent. MNGIE disease is characterized by the dysfunction of mitochondria due to the impaired mitochondrial DNA (mtDNA) replication which is the consequence of the accumulation of deoxyribonucleosides [7,8,9,23]. The first biochemical marker of mitochondrial dysfunction in our patient was the increased lactate and alanine levels found in her CSF. The diagnosis was established by the increased levels of dThd and dUrd in plasma, the total lack of TP activity and the identification of two pathogenic variants in the *TYMP* gene.

Due to the detrimental effect of increased concentrations of deoxyribonucleosides therapeutic approaches have focused in the reduction of their levels. Transient reduction in their levels has been observed in a number of patients by their direct removal through haemodialysis and continuous ambulatory peritoneal dialysis or through the replacement of the missing enzyme by platelet infusions and infusion of erythrocyte encapsulated TP [10,11,24].

On the other hand, therapeutic approaches such as OLT and HSCT offer the potential for the permanent restoration of the biochemical defect [10,12,13,14,24]. HSCT has been shown to restore TP activity and drastically reduce the circulating levels of dThd and dUrd in plasma. Disease progression is generally halted and improvement has also been

reported [10,14,24].

In our patient the latter approach was adopted. Donor selection was a major concern prior to transplantation. The patient did not have a fully compatible unrelated donor. The only matched donor was her sister who was also a carrier of the disease with suboptimal TP activity. In a retrospective study univariate analysis showed that survival was significantly higher in patients transplanted from fully matched donors ($10/10$ vs $<10/10$) [14]. Based on these data we decided to proceed to transplantation using her sister as donor which appeared the safest choice. As already mentioned, the patient had a completely uneventful post-transplant period and satisfactory PB chimerism levels.

In agreement with the findings of the retrospective study of Halter et al. [14], an increase in the level of activity of TP was observed which reached the activity observed in her donor (sister), who is a carrier of the disease. This increase in TP activity was also associated with a profound decline in the levels of dThd and dUrd.

According to the same study [14], although clinical manifestations of MNGIE improved significantly over time, clinical improvement was a slow process and residual disease manifestations persisted for years after HSCT. Although none of the patients surviving more than 1 year showed progression of MNGIE-related manifestations objective improvement was noted at least after two years. Recovery of the GI tract was shown to be slow and at the time of analysis, incomplete. These observations could be explained by the irreversible effects of toxic deoxyribonucleoside levels before the HSCT. Sixteen months post-transplant our patient, in agreement with these observations, showed stabilization of clinical symptoms, no progression of MNGIE-related manifestations and some improvement was also observed both in her neurological and gastrointestinal symptoms.

In conclusion we describe the favorable outcome of AHSCT in a patient with the classic form of MNGIE disease. Follow up studies will be essential in order to evaluate the long term impact of this approach on disease outcome.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author statement

All authors have approved the content of the paper and its submission. The paper has not been published previously and is not under consideration for publication elsewhere.

Individual contribution to the article.

Anna Pasiou, Eleni Ioannidou, Ioulia Peristeri, Georgios Vessalas: all aspects of AHSCT, participated in the writing of the paper.

Maria Rogalidou, Alexandra Papadopoulou and Konstantina Dimakou: diagnosis, follow up and treatment of GI symptoms, participated in the writing of the paper.

Roser Pons: diagnosis and follow up of neurological disease.

Evangelia Dimitriou: assay of the activity of arylsulphatase A- β -galactocerebrosidase and participated in the writing of the paper.

Marina Moraitou: measurement of VLCFA levels, DNA preparation, coordination of laboratory investigations and participated in the writing of the paper.

Monique Nieman, Jeroen Roelofsen, Lida Zoetekouw, Susan M. I. Goorden: Data generation and analysis.

Frederic M. Vaz, Andre van Kuilenburg: design of the laboratory studies, analysis of the data, writing of the paper.

Helen Michelakakis: design and coordination of the laboratory studies, evaluation of the results, amino acid analysis, writing of the paper.

Declaration of Competing Interest

None.

Acknowledgements

We would like to thank Professor Hans Waterham for his extremely valuable contribution to the diagnosis of the patient. The patient and her family for their collaboration.

References

- [1] I. Nishino, A. Spinazzola, M. Hirano, Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder, *Science* 283 (1999) 689–692, <https://doi.org/10.1126/science.283.5402.689>.
- [2] D. Pacitti, M. Levene, C. Garone, N. Nirmalanathan, B.E. Bax, Mitochondrial neurogastrointestinal encephalomyopathy: into the fourth decade, what we have learned so far, *Front. Genet.* 9 (2018) 669, <https://doi.org/10.3389/fgene.2018.00669>.
- [3] M. Hirano, Y. Nishigaki, R. Martí, Mitochondrial neurogastrointestinal encephalomyopathy(MNGIE): a disease of two genomes, *Neurologist* 10 (2004) 8–17, <https://doi.org/10.1097/01.nrl.0000106919.06469.04>.
- [4] K. Okamura, T. Santa, K. Nagae, T. Omae, Congenital oculoskeletal myopathy with abnormal muscle and liver mitochondria, *J. Neurol. Sci.* 27 (1976) 79–91, [https://doi.org/10.1016/0022-510x\(76\)90236-7](https://doi.org/10.1016/0022-510x(76)90236-7).
- [5] A. Spinazzola, R. Martí, I. Nishino, A.L. Andreu, A. Naini, S. Tadesse, I. Pelal, E. Zammarchi, M.A. Donati, J.A. Olive, M. Hirano, Altered thymidine metabolism due to defects of thymidine phosphorylase, *J. Biol. Chem.* 227 (2002) 4128–4133, <https://doi.org/10.1074/jbc.M111028200>.
- [6] M.L. Valentino, R. Martí, S. Tadesse, L.C. Lopez, J.L. Manes, J. Lyzak, A. Hahn, V. Carelli, M. Hirano, Thymidine and deoxyuridine accumulate in tissues of patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), *FEBS Lett.* 581 (2007) 3410–3414, <https://doi.org/10.1016/j.febslet.2007.06.042>.
- [7] E. Gonzalez-Vioque, J. Torres-Torronteras, A.L. Andreu, R. Martí, Limited dCTP availability accounts for mitochondrial DNA depletion in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), *PLoS Genet.* 7 (2011), e1002035, <https://doi.org/10.1371/journal.pgen.1002035>.
- [8] S. Song, L.J. Wheeler, C.K. Mathews, Deoxyribonucleotide pool imbalance stimulates deletions in HeLa cell mitochondrial DNA, *J. Biol. Chem.* 284 (2003) 43893–43896, <https://doi.org/10.1074/jbc.C300401200>.
- [9] C. Giordano, M. Sebastiani, R. De Giorgio, C. Travaglini, A. Tancredi, M. L. Valentino, M. Bellan, A. Cossarizza, M. Hirano, J., d'Amati and V. Carelli, gastrointestinal dysmotility in mitochondrial neurogastrointestinal encephalomyopathy is caused by mitochondrial DNA depletion, *Am. J. Pathol.* 173 (2008) 1120–1128, <https://doi.org/10.2353/ajpath.2008.080252>.
- [10] M. Hirano, V. Carelli, R. De Giorgio, L. Pironi, A. Accarino, G. Cenacchi, R. D'Alessandro, M. Filosto, R. Martí, F. Nonino, A.D. Pinna, E. Baldini, B.E. Bax, A. Bolletta, R. Bolletta, E. Boschetti, M. Cescon, R. D'Angelo, M.T. Dotti, C. Giordano, L.L. Gramegna, M. Levene, R. Lodi, H. Mandel, M.C. Morelli, O. Musumeci, A. Pugliese, M. Scarpelli, A. Siniscalchi, A. Spinazzola, G. Tal, J. Torres-Torronteras, L. Vignatelli, I. Zaidman, H. Zoller, R. Rinaldi, M. Zeviani, Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): position paper on diagnosis, prognosis, and treatment by the MNGIE international network, *J. Inher. Metab. Dis.* 44 (2021) 376–387, <https://doi.org/10.1002/jimd.12300>.
- [11] M. Levene, M.D. Bain, N.F. Moran, N. Nirmalanathan, J. Poulton, M. Scarpelli, M. Filosto, H. Mandel, A.D. MacKinnon, L. Fairbanks, D. Pacitti, B.E. Bax, Safety and efficacy of erythrocyte encapsulated thymidine phosphorylase in mitochondrial neurogastrointestinal encephalomyopathy, *J. Clin. Med.* 8 (2019) 457, <https://doi.org/10.3390/jcm8040457>.
- [12] R. De Giorgio, L. Pironi, R. Rinaldi, E. Boschetti, L. Caporali, M. Capristo, C. Casali, G. Cenacchi, M. Contin, R. D'Angelo, A. D'Errico, L.L. Gramegna, R. Lodi, A. Maresca, S. Mohamed, M.C. Morelli, V. Papa, C. Tonon, V. Tugnoli, V. Carelli, R. D'Alessandro, A.D. Pinna, Liver transplantation for mitochondrial neurogastrointestinal encephalomyopathy, *Ann. Neurol.* 80 (2016) 448–455, <https://doi.org/10.1002/ana.24724>.
- [13] K. Kripps, W. Nakayuenyongsuk, B.J. Shayota, W. Berquist, N. Gomez-Ospina, C. O. Esquivel, W. Concepcion, J.B. Sampson, D.J. Cristin, W.E. Jackson, S. Gilliland, E.A. Pomfret, M.L. Kueht, R.W. Pettit, Y.A. Sherif, L.T. Emrick, S.H. Elsea, R. Himes, M. Hirano, J.L.K. Van Hove, F. Scaglia, G.M. Enns, A.A. Larson, Successful liver transplantation in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), *Mol. Genet. Metab.* 130 (2020) 58–64, <https://doi.org/10.1016/j.ymgme.2020.03.001>.
- [14] J.P. Halter, W. Michael, M. Schupbach, H. Mandel, C. Casali, K. Orchard, M. Collin, D. Valcarcel, A. Rovelli, M. Filosto, M.T. Dotti, G. Marotta, G. Pintos, P. Barba, A. Accarino, C. Ferra, I. Illa, Y. Beguin, J.A. Bakker, J.J. Boelens, I.F. de Co, K. Fay, C.M. Su, D. Nachbaur, H. Zoller, C. Sobreira, B. Pinto Simoes, S. R. Hammans, D. Savage, R. Martí, P.F. Chinnery, R. Elhasid, A. Gratwohl, M. Hirano, Allogeneic haematopoietic stem cell transplantation for mitochondrial neurogastrointestinal encephalomyopathy, *Brain.* 138 (2015) 2847–2858, <https://doi.org/10.1093/brain/awv226>.
- [15] A.B.P. van Kuilenburg, L. Zoetekouw, Determination of thymidine phosphorylase activity by a non-radiochemical assay using reversed-phase high-performance liquid chromatography, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 82 (2005) 271–275, <https://doi.org/10.1016/j.jchromb.2005.04.009>.
- [16] H. van Lenthe, A.B.P. van Kuilenburg, T. Ito, A.H. Bootsma, A. van Cruchten, Y. Wada, A.H. van Gennip, Defects in pyrimidine degradation identified by HPLC-electrospray tandem mass spectrometry of urine specimens or urine-soaked filter paper strips, *Clin. Chem.* 46 (2000) 1916–1922.
- [17] E. Young, J. Wilson, A.D. Patrick, L. Crome, Galactocerebrosidase deficiency in globoid cell leucodystrophy of late onset, *Arch. Dis. Child.* 47 (1972) 49–50, <https://doi.org/10.1136/adc.47.253.449>.
- [18] M. Lee-Vaupel, E. Conzelman, A simple chromogenic assay for arylsulfatase A, *Clin. Chim. Acta* 164 (1987) 171–180, [https://doi.org/10.1016/0009-981\(87\)90068-4](https://doi.org/10.1016/0009-981(87)90068-4).
- [19] R. Santer, A. Clavier, H.D. Oldigs, J. Schaub, R.B. Schutgens, R.J. Wanders, Isolated defect of peroxisomal beta-oxidation in a 16-year-old patient, *Eur. J. Pediatr.* 152 (1993) 339–342, <https://doi.org/10.1007/BF01956749>.
- [20] D. Mingming, Z. Huiyuan, X. Weishuang, A novel TYMP mutation of mitochondrial neurogastrointestinal encephalomyopathy: case report and literature review, *J. Apoplexy Nervous Dis.* 3 (2016) 238–241.
- [21] J.A. Arribere, A.Z. Fire, Nonsense mRNA suppression via nonstop Decay, *eLife.* 7 (2018), e33292, <https://doi.org/10.7554/eLife.33292>.
- [22] K.T. Powers, J.Y.A. Szeto, C. Schaffitzel, New insights into no-go, non-stop and nonsense-mediated mRNA decay complexes, *Curr. Opin. Struct. Biol.* 65 (2020) 110–118, <https://doi.org/10.1016/j.sbi.2020.06.011>.
- [23] A. Papadimitriou, G.P. Comi, G.M. Hadjigeorgiou, A. Bordoni, M. Sciacco, L. Napoli, A. Prella, M. Moggio, G. Fagioli, N. Bresolin, S. Salani, I. Anastopoulos, G. Giassakis, R. Divari, G. Scarlato, Partial depletion and multiple deletions of muscle mtDNA in familial MNGIE syndrome, *Neurology* 51 (1998) 1086–10792, <https://doi.org/10.1212/wnl.51.4.1086>.
- [24] E.B. Bax, Mitochondrial neurogastrointestinal encephalomyopathy: approaches to diagnosis and treatment, *J. Transl. Genet. Genom.* 4 (2020) 1–16, <https://doi.org/10.20517/jtgg.2020.08>.