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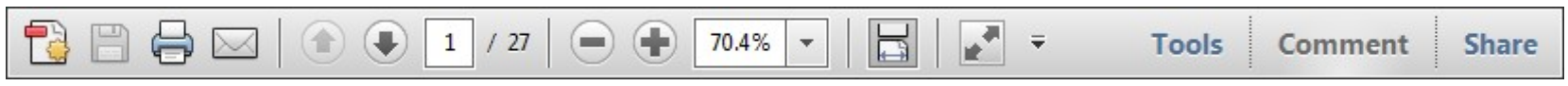

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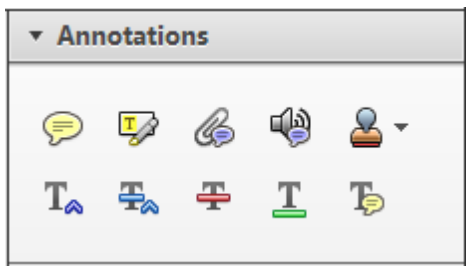

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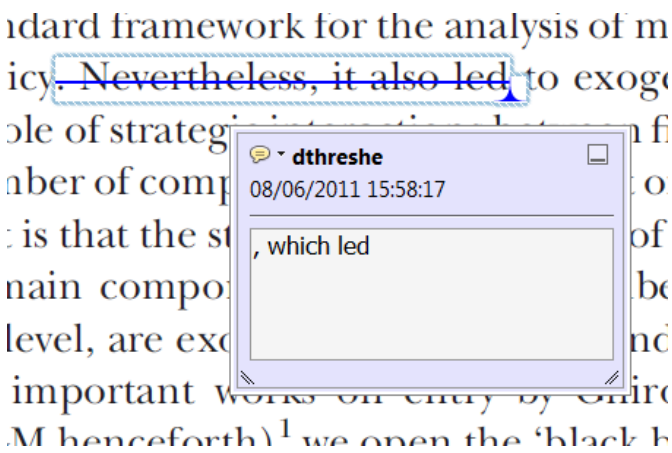

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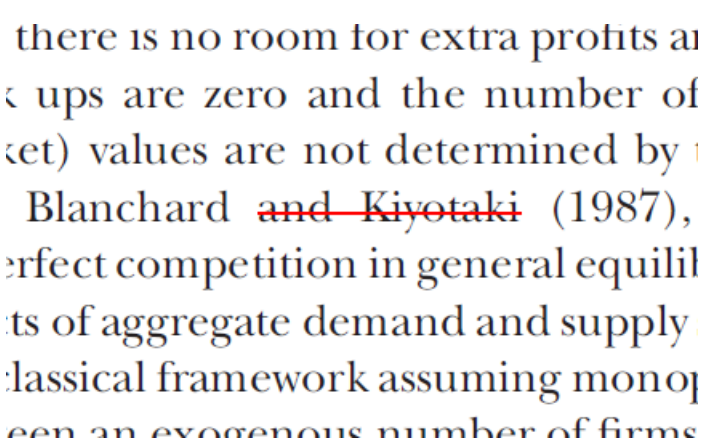

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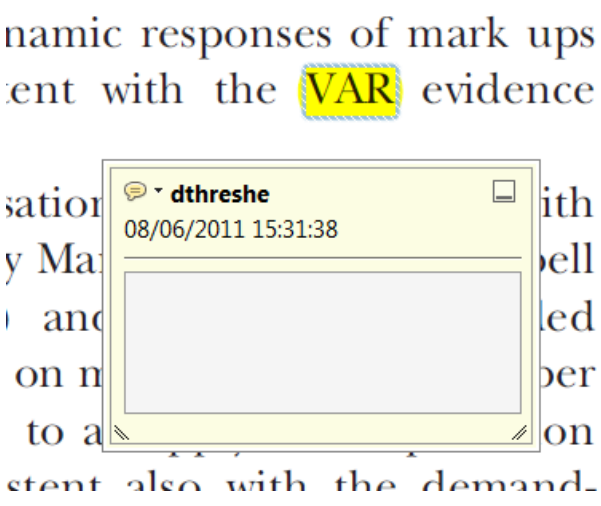

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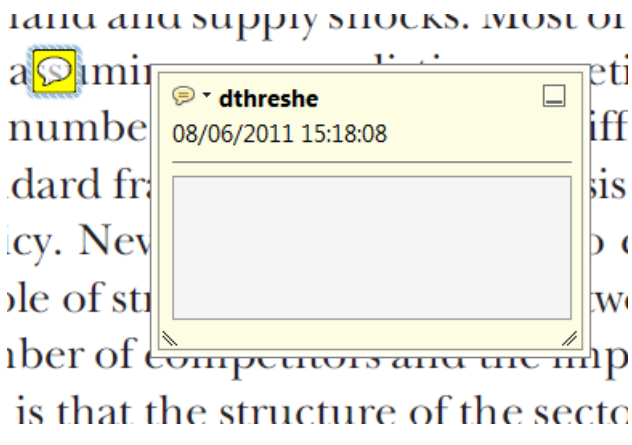

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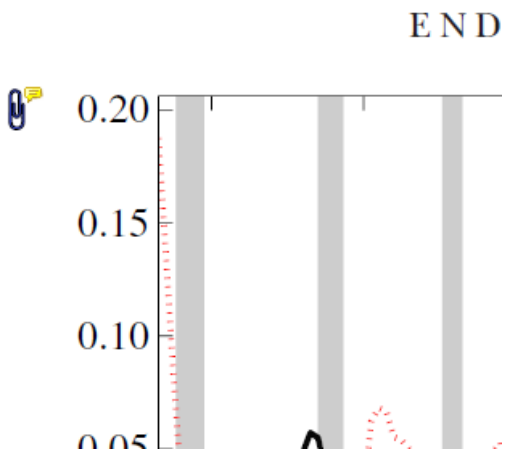

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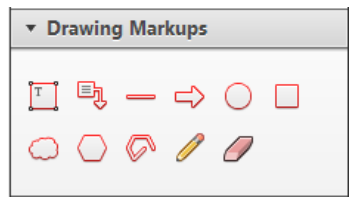

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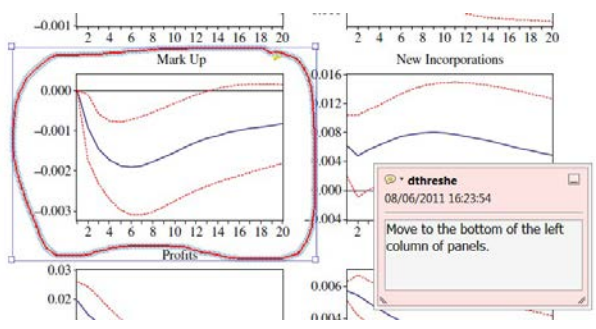

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Liver Transplantation for Mitochondrial Neurogastrointestinal Encephalomyopathy

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Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a fatal, recessive disease caused by mutations in the gene encoding thymidine phosphorylase, leading to reduced enzymatic activity, toxic nucleoside accumulation, and secondary mitochondrial DNA damage. Thymidine phosphorylase replacement has been achieved by allogeneic hematopoietic stem cell transplantation, a procedure hampered by high mortality. Based on high thymidine phosphorylase expression in the liver, a 25-year-old severely affected patient underwent liver transplantation. Serum levels of toxic nucleosides rapidly normalized. At 400 days of follow-up, the patient's clinical conditions are stable. We propose liver transplantation as a new therapy for MNGIE.

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Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a rare, autosomal-recessive disease characterized by severe gastrointestinal (GI) and neurological dysfunctions.[1] MNGIE is caused by mutations in the nuclear *TYMP* gene, leading to a marked reduction or absence of thymidine phosphorylase (TP) activity.[2] This results in a systemic toxic accumulation of nucleosides thymidine (dThd) and deoxyuridine (dUrd),[3,4] which induces mitochondrial DNA (mtDNA) depletion, multiple deletions, and point mutations.[5–7]

TP replacement has been achieved to date by allogeneic hematopoietic stem cell transplantation (AHSCT), a procedure hampered by high mortality.[8–11] We recently documented that the liver can be a tissue source of TP.[12] Herein, we report the biochemical and clinical results of an MNGIE patient who underwent orthotopic liver transplantation (OLT) performed as enzyme replacement therapy.

Case Report

A 25-year-old male patient reported a 6-year history of recurrent arthritis, watery diarrhea, and abdominal pain misdiagnosed as Crohn's disease. Despite the immunosuppressive treatment, GI symptoms worsened and a progressive weight loss required parenteral nutrition. Therefore, he was re-evaluated with a number of tests, including a neurological examination. This showed mild hyporeflexia and imbalance, without ptosis, ophthalmoparesis, or segmental hyposthenia. Electromyography showed demyelinating sensory-motor polyneuropathy. Brain magnetic resonance imaging (MRI) with proton magnetic resonance spectroscopy (^{1}H-MRS) revealed moderate-to-severe hyperintense cerebral and cerebellar white matter, along with brain lactate increase. These findings indicated MNGIE. Muscle biopsy revealed cytochrome-c-oxidase (COX) negative (Fig 1A), rare "ragged-red" fibers, and ultrastructural abnormalities (Fig 1C). Biochemical profiling showed markedly reduced plasma TP activity (4nmol/h*mg; n.v. >253) and increased dThd and dUrd levels (16.5 and 16.2μM, respectively; F1 AQ3

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AQ1

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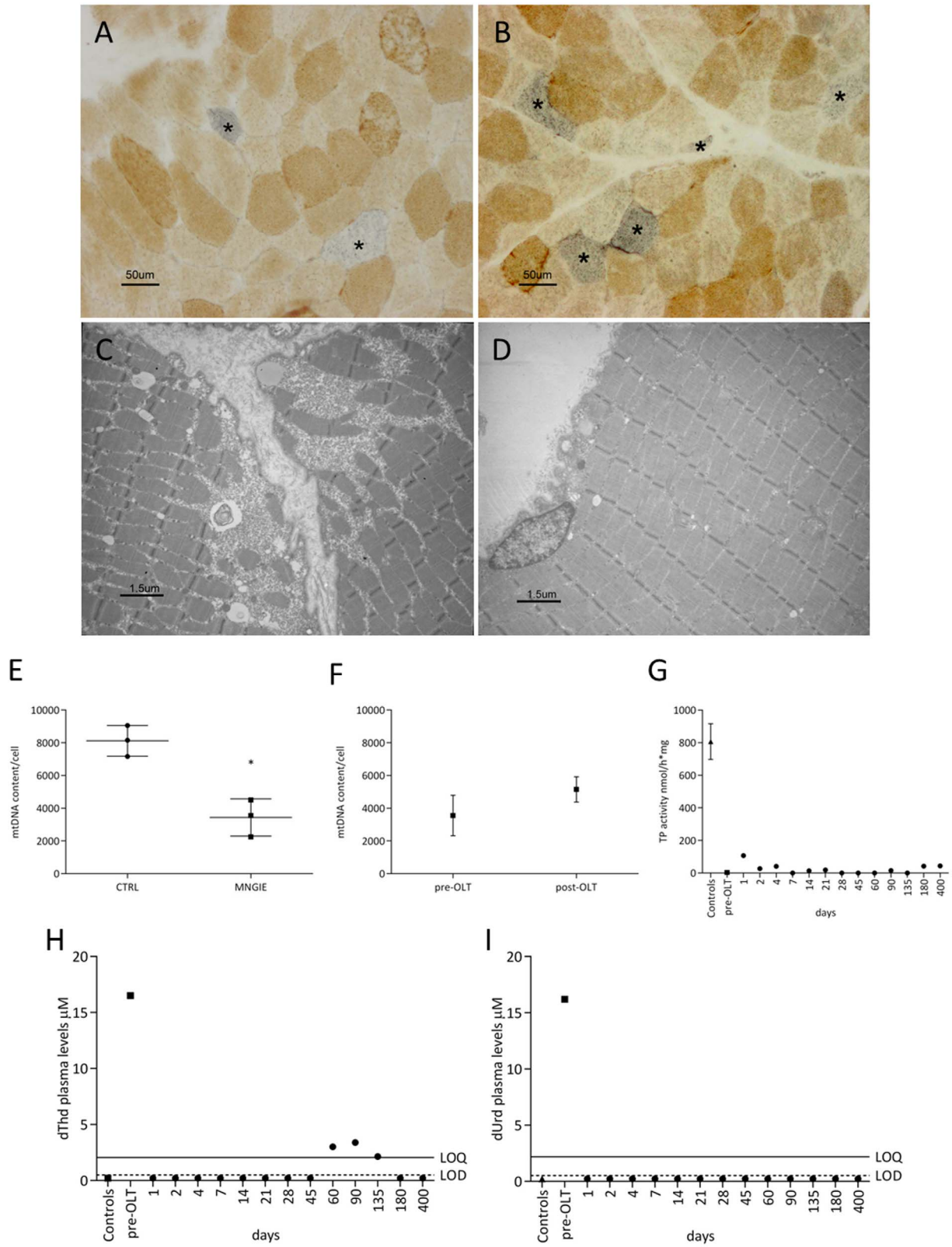
Fig 1G–I). The *TYMP* gene sequence revealed a homozygous c.1160-1G>A mutation (RefSeq NM_001113755.2), establishing the diagnosis of MNGIE.

Lacking ad-hoc donors for AHSCT and after a further deterioration of clinical conditions (relapsing fever, daily vomiting, abdominal pain, severe malnutrition,



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inability to walk, bed restriction with lower-limb hyposthenia, ophthalmoparesis, and nerve conduction worsening) over a 6-month period, he was considered for OLT, performed on March 1, 2015. Before transplant, liver function tests were normal, although an ultrasonography showed a mild-to-moderate liver steatosis with normal portal vein flow. During surgery, decompressive gastrostomy and ileostomy were performed to reduce subocclusion and intestinal bacterial translocation episodes. Tacrolimus and prednisone were started as standard post-transplant treatment. The immediate post-OLT period was uneventful. Eight months post-OLT, the ileostomy was closed and bowel continuity was restored because of a stoma prolapse. This procedure determined a transient worsening of GI function, recently recovered.

Materials and Methods

The study was approved by the St. Orsola-Malpighi Hospital Ethic Committee (protocol no. 31/2013/O/Tess). The patient gave his informed consent.

Clinical, nutritional, and biochemical parameters were prospectively monitored postdiagnosis. Brain MRI, including diffusion tensor imaging (DTI) and (^{1}H-MRS) localized in the parietal white matter, was performed. Mean diffusivity (MD) values derived from the cerebral and cerebellar histograms[13] and absolute and relative metabolite concentrations were calculated[14] and compared to values obtained in two distinct groups of 10 healthy males (30.7 $\pm$ 5.9 and 31.3 $\pm$ 7.3 years, mean $\pm$ standard deviation [SD], respectively).

Blood samples were obtained up to 400 days post-OLT. dThd and dUrd concentrations were assessed in plasma fraction.[15] TP activity was measured on buffy coat.[16]

Skeletal muscle, explanted liver, and ileal full-thickness biopsies were processed for hematoxylin and eosin, Masson's trichrome, orcein, histoenzymatic staining, and electron microscopy using standard protocols.

Long-range polymerase chain reaction (PCR) and quantitative PCR were performed to screen and quantify mtDNA deletions in skeletal muscle.[17,18] Quantification of mtDNA content *per cell* was performed by quantitative PCR in the liver and skeletal muscle.[18]

Results

After OLT and until the ileostomy closure, Karnofsky and SF36 scores, ability to walk, oral food intake and nutritional parameters improved (Table). After restoration of bowel continuity, GI function transiently worsened with increased fluid output from the decompressive gastrostomy, associated with reduced oral feeding and recurrent episodes of fever likely caused by bacterial translocation. In the last 2 months of follow-up, an improvement of the GI function was observed, with a decrease of gastrostomy output, spontaneous bowel movements, and restoration of oral feeding. Immediately post-OLT, a self-limiting episode of hypertransaminasemia occurred. Liver histology showed changes unrelated to acute rejection, that is, severe macrovescicular steatosis, centrilobular spotty necrosis with neutrophils, and Councilman bodies. Although the cause of this episode was unclear, the reduction of energy content in the parenteral nutrition led to the persistent normalization of liver enzymes. Neurological examination revealed improvement of lower-limb strength associated with a partial recovery of neurophysiological findings. Compared to pre-OLT, there were no major changes detectable at conventional brain MRI at 3 and 6 months post-OLT, whereas MD of cerebellar hemispheres was slightly reduced (>2 SDs of the mean normal values; Table) and, relevantly, cerebral white matter lactate was reduced (~40%; Table).

Buffy coat TP activity remained unchanged. A persistent reduction of plasma dThd and dUrd levels was observed 24 hours post-OLT, apart from a transient and slight increase of dThd at 60, 90, and 135 days. At 400 days of follow-up, these levels were below 0.5μM (Fig 1H,I).

The explanted liver showed a fatty liver disease with severe microsteatosis and up to 20% centrilobular

FIGURE 1: Skeletal muscle features and blood changes pre-OLT and at 400 days post-OLT. (A and B) Representative images of skeletal muscle showing COX and succinate dehydrogenase histoenzymatic staining (calibration bar = 50μm in both pictures). There are no changes in the amount of COX-depleted fibers in pre-OLT (A) versus post-OLT (B). (C and D) Transmission electron microscopy of skeletal muscle (calibration bar = 1.5μm in both pictures). Note the myofibrillary reorganization in the post-OLT (D) versus pre-OLT (C). (E) Scatter-plot of mtDNA content *per cell* (mean ± SD) in skeletal muscle of controls (n = 3) and MNGIE cases (proband and 2 unrelated patients; n = 3). All MNGIE cases showed decreased mtDNA content versus controls ($p < 0.01$). (F) mtDNA content (mean ± SD) of pre-OLT and post-OLT skeletal muscle. Post-OLT tissue showed an increased mtDNA content versus pre-OLT, failing to reach statistical significance ($p = 0.07$). (G–) Biochemical parameters (TP activity and dThd and dUrd levels) evaluated on blood samples in the pre-OLT and post-OLT follow-up. LOQ (2.06μM for dThd and 2.18μM for dUrd) and LOD (0.49μM for dThd and 0.52μM for dUrd) of nucleosides are indicated by solid and dash lines, respectively (H and I). As expected, TP activity profile in the buffy coat did not change in the post-OLT (G). The markedly high nucleoside dThd (H) and dUrd (I) plasma concentrations in the pre-OLT period dropped right after OLT and, apart from a transient and slight increase of dThd at 60 to 90 to 135 days, persisted below LOD during the follow-up. COX = cytochrome-c-oxidase; CTRL = control; dThd = thymidine; dUrd = deoxyuridine; LOD = limit of detection; LOQ = limit of quantification; MNGIE = mitochondrial neurogastrointestinal encephalomyopathy; mtDNA = mitochondrial DNA; OLT = orthotopic liver transplantation; SD = standard deviation; TP = thymidine phosphorylase.

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TABLE. Clinical, Nutritional, Biochemical, Neurological, and Brain MRI Results Before, At, and After OLT

		PEG and Ileostomy ↓		Ileostomy Closure and Bowel Continuity Restoration ↓		
	At Diagnosis	At OLT	90 Days Post-OLT	180 Days Post-OLT	300 Days Post-OLT	400 Days Post-OLT
Clinical and nutritional						
Karnofsky performance score	70	30	60	50	40	50
Ability to walk	+	–	+	+	+	+
SF-36 MCS	//	32	57	44	27	38
SF-36 PCS	//	22	41	31	29	31
Body weight (kg)	41.1	38.6	39.0	39.7	37.6	37.2
BMI (kg/m^2) (n.v. >18.5)	13.4	12.6	12.7	13.0	12.3	12.1
PN support (kcal/day)	1,400	1,640	1,040	1,350	1,450	1,650
Oral food intake (kcal/day)	796	445	1,503	1,375	0	1,320
Biochemical						
Serum albumin g/l (n.v. >35)	39	24	42	40	29.8	28.3
CRP mg/dL (n.v. < 0.8)	0.38	17.25	0.79	0.85	1.53	1.31
Serum lactate mg/dl (n.v. <19)	20	49	//	18	//	//
AST U/L (n.v. <38), ALT U/l (n.v. <41)	18 / 18	16 / 16	22 / 37	24 / 31	11/6	25/39
Direct bilirubin mg/dl (n.v. <0.30)	0.15	0.35	0.1	0.1	0.1	0.1
ENG data						
MCV (m/s) L Uln/R Tib	29/25	28/n.d.	//	32/24	30/22	//
CMAP amplitude (mV) L Uln/R Tib	9.1/5.7	8.0/n.d.	//	8.9/0.1	6.4/0.1	//
SCV (m/s) L Uln/L Sur	48/40	42/42	//	44/40	45/40	//
SAP amplitude (μV) L Uln/L Sur	4.7/1.2	6.1/1.0	//	6.8/0.9	5.3/1.3	//
Brain MRI						
Structural imaging	Diffuse T2-weighted WM hyperintensity	//	Unchanged	Unchanged	//	//
DTI (MD $\times$ 10^{-3} mm^2/s) Cerebral hemispheres (n.v.: 0.86 $\pm$ 0.02, mean $\pm$ SD)	0.96	//	0.96	0.98	//	//
DTI (MD $\times$ 10^{-3} mm^2/s) Cerebellar hemispheres (n.v.: 0.81 $\pm$ 0.03, mean $\pm$ SD)	0.98	//	0.87	0.91	//	//

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TABLE: Continued

	At Diagnosis	PEG and Ileostomy ↓ At OLT	90 Days Post-OLT	Ileostomy Closure and Bowel Continuity Restoration ↓ 180 Days Post-OLT	300 Days Post-OLT	400 Days Post-OLT
^{1}H-MRS- PWM [NAA] (mM) (n.v.: 10.03 ± 1.02, mean ± SD)	7.17	//	6.32	7.32	//	//
^{1}H-MRS- PWM Lac/Cr (Lac AU) (n.v.: absent lactate)	0.36 (830 AU)	//	0.21 (529 AU)	0.21 (501 AU)	//	//

ALT = alanine aminostransferase; AST = aspartate aminotransferase; AU = arbitrary units; BMI = body mass index; CMAP = compound motor action potential; Cr = creatine; CRP = C-reactive protein; DTI = diffusion tensor imaging; ENG = electroneurography; ^{1}H-MRS = proton magnetic resonance spectroscopy; Lac = lactate; L = left; MCS = Mental Component Summary; MCV = motor conduction velocity; MD = mean diffusivity; [NAA] = N-acetyl-aspartate concentration; n.d. = not detectable; PEG = percutaneous endoscopic gastrostomy; PCS = Physical Component Summary; PN = Parental Nutrition; PWM = parietal white matter; R = right; SAP = sensory action potential; SCV = sensory conduction velocity; SD = standard deviation; SF-36 = 36-Item Short-Form Health Survey; Sur = sural nerve; Tib = tibial nerve; Uln = ulnar nerve; WM = white matter.

AQ4

macrovesicular steatosis along with grade 3 siderosis. Histoenzymatic staining revealed a patchy reduction of COX activity in the liver. The ultrastructural evaluation demonstrated hepatocytes with an oncocytic-like mitochondrial hyperplasia. mtDNA content *per cell* was significantly reduced versus controls (Fig 2A–C).

F2

Compared to pre-OLT, the percentage of COX-negative muscle fibers remained similar in the 6-month post-OLT biopsy (0.73% vs 1.37%). However, the deranged appearance of myofibrils at electron microscopy improved in the post-OLT specimen (Fig 1A–D).

The mtDNA copy number in muscle biopsies from pre-OLT and the other 2 MNGIE patients was significantly lower than controls ($p < 0.01$; Fig 1E). Compared to pre-OLT, mtDNA content increased in the post-OLT muscle biopsy without reaching statistical significance (Fig 1F). Quantitative assessment of mtDNA multiple deletions revealed low amounts of deleted molecules (0 ± 5%), with no differences in the pre-OLT muscle biopsy versus post-OLT.

Finally, Masson's trichrome and orcein staining revealed a marked fibrosis in the submucosa of the ileum, which was unchanged 8 months post-OLT versus pre-OLT specimens (Fig 2D–G).

Discussion

The present case highlights that OLT is feasible in advanced MNGIE and reverses the severe biochemical imbalance of the disease. Besides this remarkable result, the follow-up of 400 days showed a mild improvement of the neurological features and a reduction of cerebral lactate, without clear-cut changes of GI function.

MNGIE is a very severe, fatal disease with no established therapeutic options. Permanent tissue replacement of TP is currently considered the best treatment to recover TP activity and reduce nucleoside imbalance. AHSCT produced biochemical and clinical improvement,[8,9,11] although associated with a high mortality rate (~70%) attributed to a number of factors, including the difficulty of finding an optimal donor and the need of aggressive conditioning and immunosuppressive chemotherapy. Also, the "end-stage" illness of patients who underwent AHSCT represented a risk factor to a poor outcome.[10]

Solid organ transplantation has also been hypothesized as an alternative option for MNGIE, having demonstrated *TYMP* expression in the human liver.[12] Given that MNGIE patients may develop liver failure[19] and considering that OLT shows a high survival rate (~85–95%),[20] the liver appeared to be the ideal organ for transplantation aimed at stably rescuing TP activity.

In the herein reported case, at 400 days post-OLT follow-up, we documented the normalization of dThd and dUrd levels. These findings indicated that TP activity in the donor liver is as effective as that exhibited by the grafted bone marrow.[11] Remarkably, the high mortality rate, related

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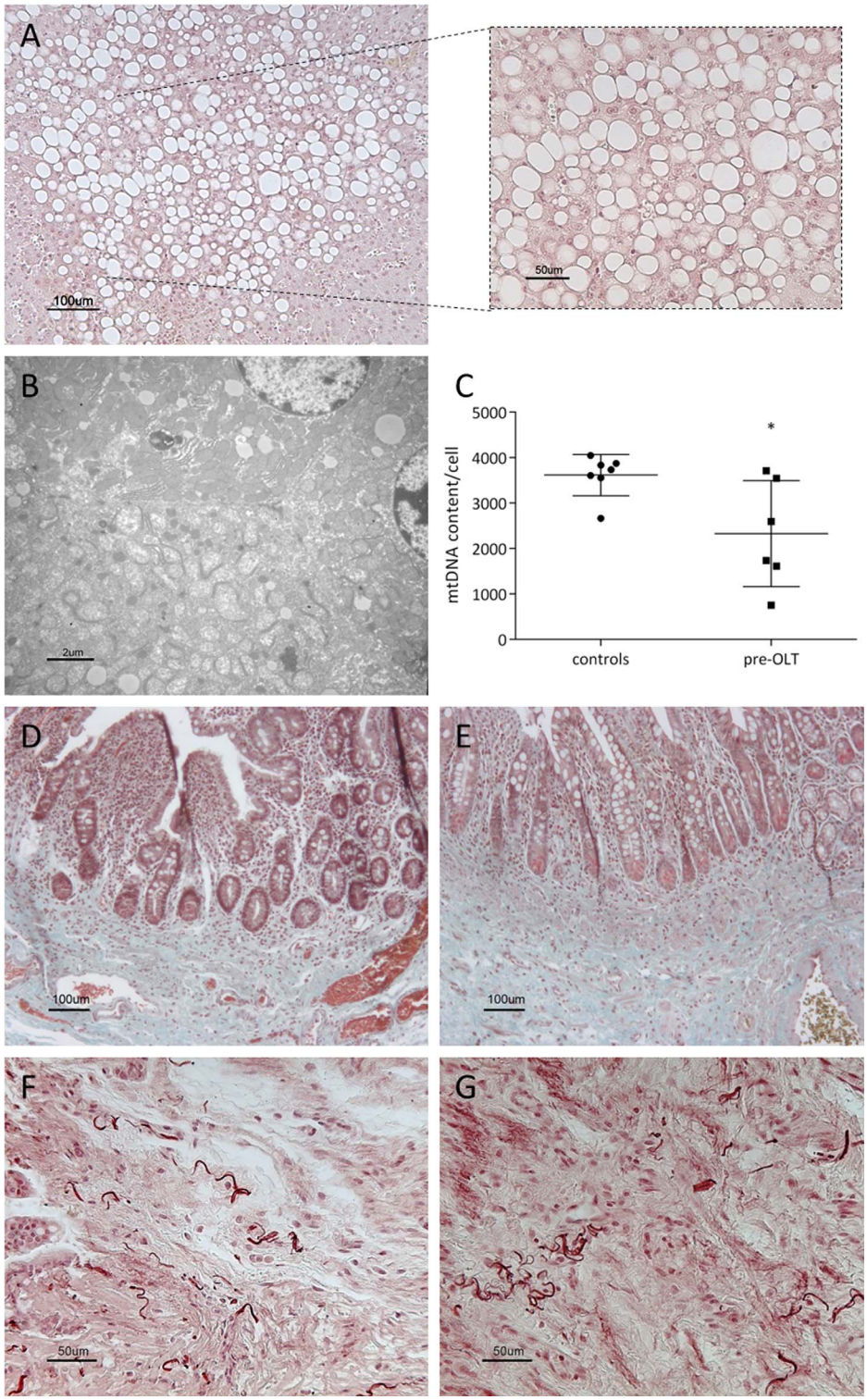

FIGURE 2: **Liver and intestinal features pre-OLT and post-OLT. (A) Representative picture illustrating the explanted liver tissue with clear features of steatohepatitis; a more readily detectable view is shown in the high-magnification inset (hematoxylin and eosin staining; calibration bar = 100μm in A; calibration bar = 20μm in the inset). (B) Ultrastructural evaluation of hepatocytes displaying mitochondrial hyperplasia with an oncocytic phenotype (calibration bar = 2μm). (C) Scatter plot of mtDNA content *per cell* (mean ± standard deviation) in the explanted liver showing a decreased mtDNA content versus (n = 5) controls ($p < 0.05$). (D–G) Representative pictures showing the histochemical analysis (Masson's trichrome in D and E and orcein in F and G) in the ileum of the mitochondrial neurogastrointestinal encephalomyopathy patient. The pictures show a dense fibrosis, mainly detectable in the submucosa (D and E; calibration bar = 100μm), along with elastic fiber abnormalities (F and G; calibration bar = 50μm), which did not improve 8 months post-OLT (E and G) versus pre-OLT (D and F). mtDNA = mitochondrial DNA; OLT = orthotopic liver transplantation.**

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to the toxicity of conditioning therapy in patients undergoing AHSCT, includes those cases with a high Karnofsky score (≥80) and normal liver function (ie, 50% mortality in this subset of MNGIE patients).[11] This significant drawback is overcome by OLT, in which a conditioning treatment is not required. Karnofsky and quality-of-life scores, neurological features, digestive symptoms, and nutritional parameters improved during the first 6 months post-OLT. After the ileostomy closure, a transient worsening of GI function occurred. This would imply that the venting ileostomy contributed to relieve the GI dysmotility-related symptoms that relapsed in the early period after bowel continuity restoration. Whether the recent improvement of GI function represents a consequence of the OLT-related nucleoside clearance or just a late adaptation to the ileostomy closure remains unsettled. Likely, it could be that the consolidated degenerative damage that occurred in postmitotic tissues of advanced MNGIE cases, such as the GI tract[7] has only a limited benefit from nucleoside clearance. Concerning the skeletal muscle, it may take time to correct the mtDNA defects, as documented by the positive trend observed in recovering mtDNA copy number. Thus, it remains uncertain as to the extent of overall recovery that can be expected by a longer post-OLT period, given that the permanent tissue damage cannot be reverted. Concerning brain magnetic resonance, reduced cerebral lactate and cerebellar MD post-OLT indicates a slight metabolic and microstructural improvement. Normalization of nucleosides in early disease stages, by timely performing OLT, would probably prevent irreversible postmitotic tissue damage. In this respect, GI tract abnormalities would represent an important criterion for the timing of patient referral to OLT.

In conclusion, this report describes a successful tissue enzyme replacement strategy casting hope for MNGIE patients. The appropriate timing for OLT and long-term outcome are eagerly awaited.

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Author Contributions

R.D.G., L.P., R.R., E.B., and V.C. conceived and designed the study, analyzed the data, and drafted a significant portion of the manuscript, figures, and table. L.C., M.C., G.C., M.C., R.D.An., A.D.E., L.L.G., R.L., A.M., S.M., M.C.M., V.P., C.T., and V.T. acquired and analyzed data and drafted a significant portion of the figures and table. C.C., R.D.Al., and A.D.P. contributed to study design and follow-up planning. A.D.P. performed the liver transplantation. R.D.G., L.P., and R.R. share co-first authorship. V.C., R.D.Al., and A.D.P. share co-last authorship.

Potential Conflicts of Interest

Nothing to report.

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