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Liver transplantation for mitochondrial neurogastrointestinal encephalomyopathy

Running Head: Liver transplantation as rescue therapy for MNGIE

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Abstract

Mitochondrial neurogastrointestinal encephalomyopathy 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 the 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 mitochondrial neurogastrointestinal encephalomyopathy.

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Introduction

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 a MNGIE patient who underwent orthotopic liver transplantation (OLT) performed as enzyme replacement therapy.

Case report

A 25-year-old male patient reported a six-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 MRI with proton MR 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 (4 nmol/h*mg; n.v. >253) and increased dThd and dUrd levels (16.5 and 16.2 μM, respectively) (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, 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 1st, 2015. Before transplant, liver function tests were normal although an ultrasonography showed a mild-to-

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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 the bowel continuity was restored because of a stoma prolapse. This procedure determined a transient worsening of GI function, recently recovered.

Methods

The study was approved by St.Orsola-Malpighi Hospital Ethic Committee (protocol#31/2013/O/Tess). The patient gave his informed consent.

Clinical, nutritional and biochemical parameters were prospectively monitored since diagnosis. Brain MRI, including 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±5.9 and 31.3±7.3 years, mean±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 H&E, Masson's trichrome, orcein, histoenzymatic staining and electron microscopy using standard protocols.

Long-range PCR and qPCR were performed to screen and quantify mtDNA deletions in skeletal muscle.[17,18] Quantification of mtDNA content *per cell* was performed by qPCR 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 1). 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 two 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 after OLT a self-

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limiting episode of hypertransaminasemia occurred. Liver histology showed changes unrelated to acute rejection, i.e. 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 SD of the mean normal values) (Table 1) and, relevantly, cerebral white matter lactate was reduced (~40%) (Table 1).

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 0.5 μM (Fig 1H-I).

The explanted liver showed a fatty liver disease with severe microsteatosis and up to 20% centrilobular macrovescicular 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. The mtDNA content *per cell* was significantly reduced *vs.* controls (Fig 2A-C).

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 other two 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 *vs.* post-OLT.

Finally, the Masson's trichrome and orcein staining revealed a marked fibrosis in the submucosa of the ileum, which was unchanged eight-month post-OLT *vs.* 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

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days showed a mild improvement of the neurological features and a reduction of cerebral lactate, without clear-cut changes of the 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%) due 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] Since MNGIE patients may develop liver failure[19] and considering that OLT shows a high survival rate (~85-95%),[20] 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 to the toxicity of conditioning therapy in patients undergoing AHSCT, includes those cases with a high Karnofsky score (≥80) and normal liver function (i.e., 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 six months post-OLT. After the ileostomy closure, a transient worsening of the 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 the bowel continuity restoration. Whether the recent improvement of the 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 occurred in post-mitotic tissues of advanced MNGIE cases, such as the GI tract,[7] have only a limited benefit from the 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 the extent of overall recovery that can be expected by a longer post-OLT period, since the permanent tissue damage cannot be reverted. Concerning brain MR, reduced cerebral lactate and cerebellar MD post-OLT indicates a slight

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metabolic and microstructural improvement. The normalization of nucleosides in early disease stages, by timely performing OLT, would probably prevent the irreversible post-mitotic tissue damage. In this respect, the 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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Authors Contributions: RDG, LP, RR, EB and VC conceived and designed the study, analyzed the data and drafted a significant portion of the manuscript, figures and table. LC, MC, GC, MC, RDAn, ADE, LLG, RL, AM, SM, MCM, VP, CT, and VT acquired and analyzed data and drafted a significant portion of the figures and table. CC, RDAl and ADP contributed to study design and follow-up planning. ADP performed the liver transplantation.

Conflict of interest: Nothing to report.

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Figure legends

Figure 1. **Skeletal muscle features and blood changes pre- and at 400 days post-OLT**.
A and B. Representative images of skeletal muscle showing COX and succinate dehydrogenase histoenzymatic staining (calibration bar = 50 μm in both pictures). There are no changes in the amount of COX-depleted fibers in pre- (A) *vs.* post-OLT (B).
C and D. Transmission electron microscopy of skeletal muscle (calibration bar = 1.5 μm in both pictures). Note the myofibrillary re-organization in the post- (D) *vs.* 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 two unrelated patients, n = 3). All MNGIE cases showed decreased mtDNA content *vs.* controls ($P < 0.01$).
F. mtDNA content (mean±SD) of pre- and post-OLT skeletal muscle. Post-OLT tissue showed an increased mtDNA content *vs.* pre-OLT, failing to reach statistical significance ($P = 0.07$).
G, H and I. Biochemical parameters (TP activity, dThd and dUrd levels) evaluated on blood samples in the pre- and post-OLT follow-up. The lower limits of quantification (LOQ: 2.06 μM for dThd, 2.18 μM for dUrd) and detection (LOD: 0.49 μM for dThd, 0.52 μM 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 60-90-135 days, persisted below LOD during the follow-up.

Figure 2. **Liver and intestinal features pre- 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 (H&E staining; calibration bar = 100 μm in A; calibration bar = 20 μm in the inset).
B. Ultrastructural evaluation of hepatocytes displaying mitochondrial hyperplasia with an oncocytic phenotype (calibration bar = 2 μm).
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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 MNGIE patient. The pictures show a dense fibrosis, mainly detectable in the submucosa (**D** and **E**; calibration bar = 100 μm), along with elastic fiber abnormalities (**F** and **G**; calibration bar = 50 μm), which did not improve eight months post-OLT (**E** and **G**) *vs.* pre-OLT (**D** and **F**).

Table 1. Clinical, nutritional, biochemical, neurological and brain MRI results before, at and after OLT.

		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
CLINICAL and NUTRITIONAL							
Karnofsky performance score		70	30	60	50	40	50
Ability to walk		+	-	+	+	+	+
SF36 MCS		//	32	57	44	27	38
SF36 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)		1400	1640	1040	1350	1450	1650
Oral food intake (kcal/day)		796	445	1503	1375	0	1320
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 x 10^{-3} mm^2/s) Cerebral hemispheres (n.v.: 0.86 ± 0.02, mean±SD)		0.96	//	0.96	0.98	//	//
DTI (MD x 10^{-3} mm^2/s) Cerebellar hemispheres (n.v.: 0.81 ± 0.03, mean±SD)		0.98	//	0.87	0.91	//	//
^{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)	//	//

Abbreviations: 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; nd= 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; Sur=Sural nerve; Tib=Tibial nerve; Uln=Ulnar nerve; WM= white matter.

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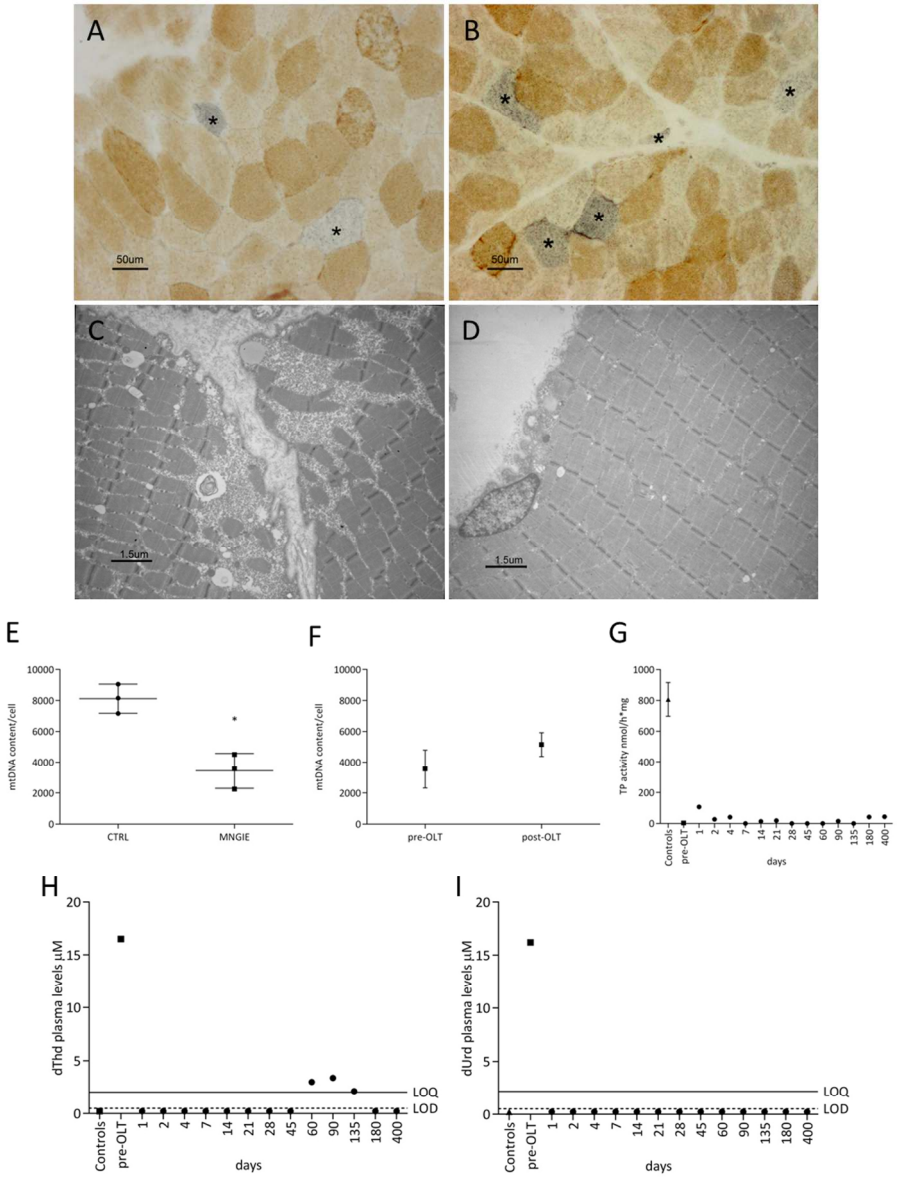

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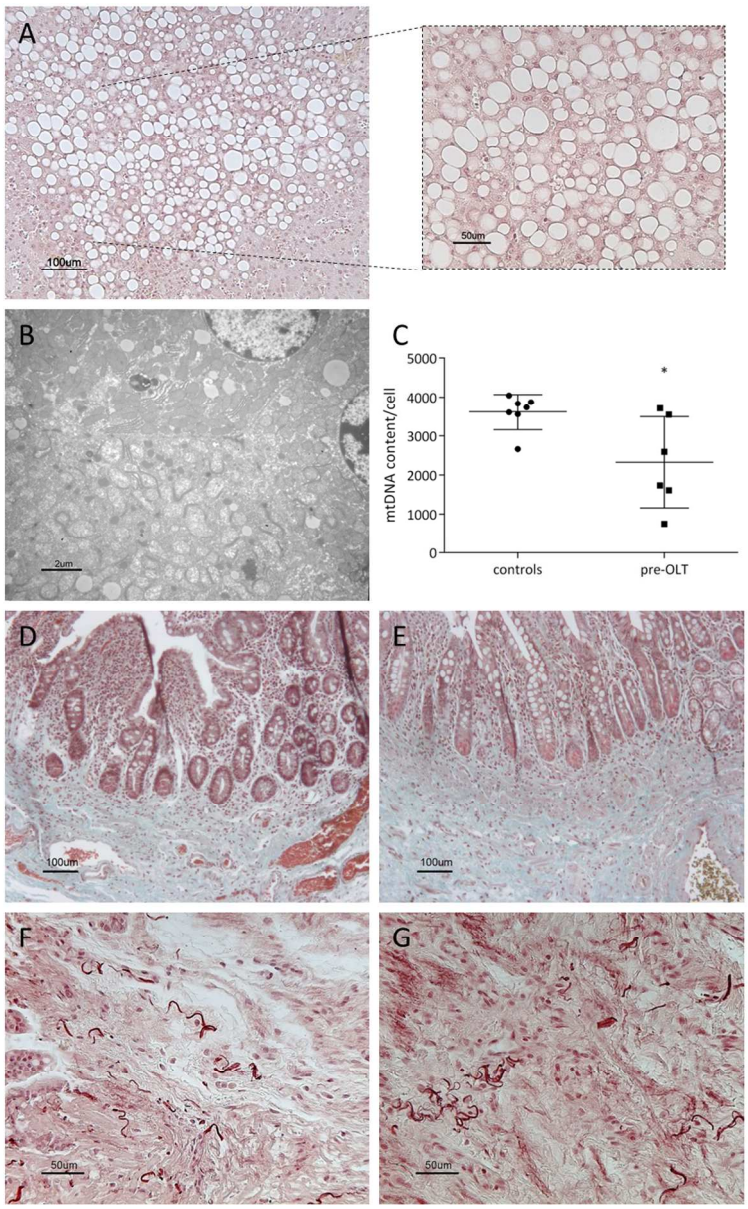

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