Biallelic variants in *COX4I1* Associated with A Novel Phenotype Resembling Leigh Syndrome, Developmental Regression, Intellectual Disability and Seizures

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Abstract:

Autosomal recessive COX4I1 deficiency has been previously reported in a single individual who presented with short stature, poor weight gain, dysmorphic features, and features of Fanconi anemia caused by a homozygous pathogenic variant in *COX4I1*. *COX4I1* encodes the subunit 4, isoform 1 of cytochrome c oxidase. Cytochrome c oxidase is a respiratory chain enzyme that plays an important role in mitochondrial electron transport and reduces molecular oxygen to water leading to the formation of ATP. Defective production of cytochrome c oxidase leads to variable phenotypic spectrum ranging from isolated myopathy to Leigh syndrome. Here, we describe siblings, born to consanguineous parents, who presented with encephalopathy, developmental regression, hypotonia, pathognomonic brain imaging findings resembling Leigh syndrome and a novel homozygous variant on *COX4AI1*, expanding the known clinical phenotype associated with pathogenic variants in *COX4I1*.

Key words: *COX4I1*; cytochrome c oxidase; mitochondrial disease; Leigh syndrome

1. INTRODUCTION

Biallelic variants in *COX4I1* (OMIM: 123864) have been previously described in a patient who presented with short stature, poor weight gain, dysmorphic features and features of Fanconi anemia (Abu-libdeh et al., 2017). *COX4I1*, located at *16q24.1,* encodes the subunit IV isoform 1, the principal isoform for COX-IV subunit of cytochrome c oxidase (COX) or Complex IV in human beings and other vertebrates. COX plays an important role in oxidative phosphorylation by transferring electrons from cytochrome c to molecular oxygen and contributes to a proton electrochemical gradient across the inner mitochondrial membrane necessary for ATP formation (Li et al., 2006). Complex IV consists of 14 different subunits, including three encoded by mitochondrial DNA (COX subunits I-III) that form an important catalytic core of the enzyme. The remaining 11 subunits encoded by nuclear DNA (COX subunits IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc, VIII and NDUFA4) are found to be tightly bound to subunits I-III (Sinkler et al., 2017). COX IV exists in two isoforms in humans and other mammals, COX subunit IV isoform 1 (COXIV- I1) and COX subunit IV isoform 2 (COX IV-I2). COX4I1 is ubiquitously expressed in mammals (Sinkler et al., 2017). Mitochondrial diseases resulting in COX deficiency (OMIM: 220110) present with marked clinical heterogeneity ranging from fatal neonatal lactic acidosis to adult myopathy. Here, we describe a novel *COX4I1* variant in two siblings who present with developmental regression, seizures and pathognomonic changes in brain imaging resembling a Leigh syndrome phenotype.

2. CLINICAL REPORT

2.1 Patient 1 is a three-year-old male who was born to consanguineous Iraqi parents at 37 weeks of gestation via C-section. His birth weight was 3.316 kilograms (kg). He had normal growth and development throughout the first eight months of life. Developmental regression of motor skills

became evident at eight months of age when he stopped crawling and standing up with support. An extensive biochemical work up, including acylcarnitine profile, plasma amino acids, urine organic acids analyses, and creatine kinase (CK), provided unremarkable results. Lactate was slightly elevated at 3.0 mmol/L (Reference range: 0.2-2.0 mmol/L). Brain MRI showed mild to moderate generalized cerebral/cerebellar atrophy and evidence of bilateral hypertrophic olivary degeneration with conspicuous non-enhancing lesions along the medullary/pyramid and subtle signal changes along the bilateral basal ganglia and cerebellar fossa (Figure 1a). These findings were concerning for mitochondrial encephalopathy, which prompted a referral to Genetics Clinic at Texas Children's Hospital.

Upon evaluation at Genetics Clinic at 13 months of age, he had further regression of his motor skills. He was unable to sit without support and did not have head control. He had profound hypotonia and required gastrostomy tube placement for feedings. Family history was significant for a nine-year-old male sibling with a similar clinical presentation. At the time of the initial visit, his weight was 8.82 kg (13^{th} percentile), his height was 74 cm (8^{th} percentile) and his head circumference was 44.5 cm (7^{th} percentile). Physical examination did not show any dysmorphic features except bilateral hypoplasia of the distal phalanges of the 2^{nd}-5^{th} toes (Figure 1b). He also had axial and appendicular hypotonia and patellar hyperreflexia. He developed seizures at two years of age, described as epileptic spasms without hypsarrhythmia, as captured on electroencephalogram (EEG). Currently, his antiepileptic treatment includes zonisamide and levetiracetam. Untargeted metabolomics analysis of his plasma and CSF demonstrated elevated lactate and fumarate (Table 1). His chromosomal microarray (oligonucleotide + SNP) did not show any copy number variants but detected long contiguous stretches with absence of heterozygosity (AOH) encompassing 137 Mb in total consistent with the history of parental

consanguinity. Exome sequencing revealed a homozygous variant in *COX4I1* (Table 1). Coenzyme Q_{10} (Ubiquinol) therapy was initiated at 8 mg/kg/day following confirmation of molecular diagnosis. Four months after initiation of therapy with Coenzyme Q_{10}, parents reported improvement in head control and tone.

2.2 Patient 2 is the older sibling of Patient 1 and is an 11 years old male. He was born full term in Iraq. He met all developmental milestones at appropriate ages until 11 months of life. Regression of motor skills was noted around 11 months of age when he started to have frequent falls and was unable to stand up. Infantile spasms associated with hypsarrhythmia on EEG started at 12 months for which he was treated with ACTH. His seizures were also treated with topiramate and valproic acid. Motor regression continued through the 1st year of life. Clonazepam was initiated to treat myoclonic jerks. He had a normal comprehensive work up including CK, plasma amino acids, urine organic acid analysis, carbohydrate deficient transferrin, lactate and pyruvate. MRI of the brain showed gliosis of bilateral basal ganglia, thalami, cerebellum and periventricular white matter with diffuse cerebral and cerebellar volume loss, and congenital hypoplasia of the inferior vermis (Figure 1c). His electromyogram (EMG) was normal. He had multiple genetic tests, including *SCN1A* deletion-duplication and sequencing and comprehensive epilepsy next generation sequencing panel, which were normal. A chromosomal microarray was done, which showed multiple areas of AOH consistent with the history of parental consanguinity.

Upon confirmation of the sibling's diagnosis through exome sequencing, Patient 2 had known familial mutation testing for *COX4I1,* which identified homozygosity for the same variant (Table 1). At the time of initial evaluation at the Genetics Clinic, he was 14 years of age. He had profound hypotonia with poor head control, inability to sit unsupported and was wheelchair

bound. Like his brother, he also required gastrostomy tube for feedings. He had failure to thrive as evidenced by weight, height, and head circumference at 2^{nd}, $< 1^{st}$, $< 2^{nd}$ percentiles, respectively. He did not have any dysmorphic features. He had notable hypotonia with strength greater in left side than the right. EEG evolved into multifocal spikes with the pattern of epileptic encephalopathy. Skeletal muscle analysis after muscle biopsy at eight years of age revealed mild type II myofiber atrophy. A mitochondrial respiratory chain enzyme analysis on the muscle biopsy specimen showed that complex IV activity was reduced (Supplementary Table 1). Coenzyme Q_{10} therapy (Ubiquinol) was initiated at 8.5 mg/kg/day following confirmation of molecular diagnosis. Parents reported improvement in visual focus four months after initiation of Coenzyme Q_{10} therapy.

3. RESULTS

A homozygous c.454C>A (p.P152T) variant in *COX4I1* was detected by trio exome sequencing in Patient 1 and confirmed by familial mutation testing in Patient 2. This amino acid is highly conserved across multiple vertebrate species from zebrafish to humans, and this variant is absent in public databases such as ExAC or gnomAD (Lek et al., 2016). *In silico* analyses for conservation suggests that this variant is evolutionarily conserved and constrained (phastCons score=1, phyloP=7.461). Furthermore, Ensembl predictors (that combine multiple *in silico* algorithms as features to reach a prediction) support the variant to be likely damaging to the COX4I1 protein product (REVEL score: 0.839 (threshold >0.75 implies damaging). The identified homozygous *COX4I1* variant resides in one of the AOH blocks spanning approximately 5.3 Mb located on chromosome 16q23.1–16q24.1. Both Patient 1 and Patient 2 had normal chromosomal breakage studies.

Untargeted metabolomics profiling on plasma and CSF in Patient 1 showed elevated fumarate (Z-score 2.9) and lactate (Z-score 3.4; Table 1). The mitochondrial respiratory chain enzyme analysis done on muscle biopsy specimen of Patient 2 showed that cytochrome c oxidase activity was reduced to 16% residual activity compared to control values, meeting a major modified Walker criterion (Supplementary Table 1).

4. DISCUSSION

Only recently a human autosomal recessive disorder linked to *COX4I1* has been identified. Abu-Libdeh et al described a novel mitochondrial disease associated with a homozygous variant in *COX4I1* in a 3.5-year-old female who presented with Fanconi anemia, short stature, poor weight gain, mild dysmorphic features, and normal brain MRI without features indicative of mitochondrial disease (Abu-libdeh et al., 2017). The siblings presented herein share some phenotypic features including short stature, failure to thrive and microcephaly (Table 1) in the presence of a novel homozygous variant (p.P152T) in *COX4I1*. While both siblings did not have evidence of Fanconi anemia as mentioned previously, they had additional manifestations including developmental regression, intellectual disability, seizures and pathognomonic changes in brain imaging resembling Leigh syndrome that were not present in the first reported case. These additional features suggest a phenotypic expansion of COX4I1 deficiency. To our knowledge, this is the second clinical report involving a variant in *COX4I1* and the first report describing a Leigh-like syndrome association with COX4I1 deficiency.

The most common presentation of mitochondrial disease in the pediatric population is in the form of Leigh syndrome (subacute necrotizing encephalomyelopathy) (Lake et al., 2015). The clinical features, including but not limited to developmental delay and regression, dystonia, ataxia and ophthalmoplegia, are often seen in conjunction with imaging findings such as bilateral

symmetric T2 hyperintensitities in basal ganglia and/or brain stem with MR spectroscopy revealing elevated brain lactate levels (Bonfante et al., 2016; Cavanagh & Harding, 1994; Rahman et al., 1996; Sofou et al., 2014).

Even though both siblings described here did not have hematological features and abnormal chromosome breakage studies consistent with Fanconi anemia, other hematological disorders such as sideroblastic anemia has been previously well described in multisystemic mitochondrial disorders such as Pearson syndrome, MLASA syndrome (mitochondrial myopathy, lactic acidosis, and SA) and complex I deficiency associated with a hemizygous change in *NDUFB11* (Falcon & Howard, 2017; Lichtenstein et al., 2016; Rileyet al., 2010; Tesarova et al., 2019). However, it was not until recently that defective oxidative metabolism and mitochondrial localization along with spontaneous mitochondrial fragmentation have been described in Fanconi anemia cells (Bottega et al., 2018; Cappelli et al., 2017; Pagano et al., 2014). Imbalance of NAD^+/NADH in COX deficiency has been postulated as the underlying mechanism of DNA instability and increased double-stranded DNA breaks (Douiev & Saada, 2018). Further follow-up in our patients is required in order to establish whether hematological features are a consistent finding that would be associated with COX4I1 deficiency.

The first variant described by Abu-Libdeh et al in *COX4I1* was found to be in a conserved residue in the transmembrane helix domain that interacts with COX I and COX II leading to decreased mRNA expression and COX activity in the patient's fibroblasts (Abu-libdeh et al., 2017). Similarly, the variant observed in the siblings presented herein is conserved across many species, and ETC analysis on the muscle biopsy specimen from patient 2 revealed reduction in COX activity, further supporting the pathogenicity of this variant in our patients.

Interestingly, untargeted metabolomics profiling (Kennedy et al., 2017; Miller et al., 2015) performed on patient 1 revealed elevated lactate and fumarate, which indicate perturbation in energy metabolism secondary to mitochondrial dysfunction further providing functional evidence for the pathogenicity of the variant identified in *COX4I1*. Evidence of mitochondrial dysfunction by the use of untargeted metabolomic analysis in CSF and plasma may provide functional validation for variants of unknown significance observed in nuclear genes associated with mitochondrial disease, providing semi-quantitative values for TCA cycle intermediates and altered lipid metabolism as a consequence of abnormal mitochondrial function (Esterhuizen et al., 2017; Shayota et al., 2019; Tam et al., 2019).

Treatment with Coenzyme Q_{10} in mitochondrial disorders is well established in Coenzyme Q_{10} deficiency (Duncan et al., 2009; Rötig et al., 2000). In disorders where mitochondrial respiratory chain is affected, Coenzyme Q_{10}, in addition to restoring electron flow in the mitochondrial respiratory chain, also acts as an antioxidant and helps reduce the oxidative stress and has been recommended despite lack of proven efficacy (Hargreaves, 2014; Parikh et al., 2014). Given these facts, Coenzyme Q_{10} was started in both patients herein presented. The treatment has provided minor clinical improvements four months following initiation of therapy. We hypothesize that therapeutic trial with Coenzyme Q_{10} in patients with COX4I1 deficiency may help stabilize the course of the disease.

In summary, the clinical features of short stature, failure to thrive, microcephaly, developmental regression, intellectual disability, seizures and pathognomonic finding of Leigh-like syndrome on brain imaging along with untargeted metabolomics findings and the reduced COX activity proven by muscle biopsy provide functional evidence for the pathogenicity of the

variants described here and further suggest expansion of the clinical phenotype linked to autosomal recessive COX4I1 deficiency.

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INFORMED CONSENT

Genetic analysis was performed after obtaining a written informed consent from both parents. Consent to publish clinical data of the patients was obtained from both parents of the minors.

CONFLICT OF INTEREST:

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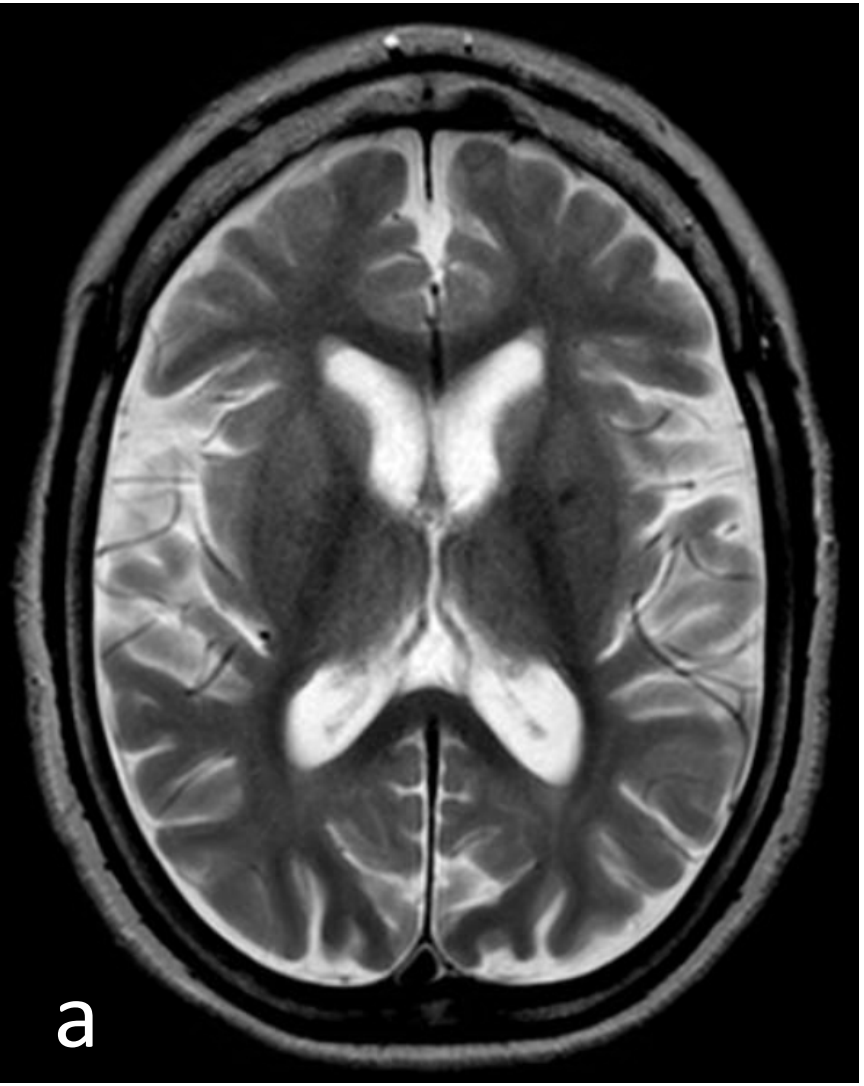
Clinical features	Abu-Libdeh et al., 2017	Our cases	
		Sibling 1	Sibling2
COX4I1 genotype	Homozygous c.412G>A (p.Glu138Lys) hg19 (GRCh27)	Homozygous c.454C>A (p.P152T) hg19 (GRCh37)	Homozygous c.454C>A (p.P152T) hg19 (GRCh37)
Short stature	+	+	+
Weight <3rd centile	+	+	+
Microcephaly	+	+	+
Dysmorphic features	Prominent nasal bridge, fifth finger clinodactyly, frontal bossing	-	-
Developmental regression	-	+	+
Seizure	-	+	+
MRI brain	Normal	Hypertrophic olivary degeneration, cerebellar volume loss	T2 prolongation in basal ganglia and thalami, volume loss of thalami
Elevated serum lactate	-	+	-
Chromosome breakage studies	+	-	-
Metabolomic profile Plasma	N/A	Fumarate + 2.9 Lactate +1.14	N/A
Metabolomic profile CSF	N/A	Lactate + 3.4	N/A

Table 1. Comparison of the clinical and laboratory features of *COX4I1* deficiency. Comparison between the first case presented by Abu-Libdeh et al., 2017 and our patients. Clinical, molecular, brain imaging and laboratory features are presented. In addition, metabolomic data for Case 1 is also presented showing elevated fumarate and lactate in plasma and CSF samples respectively. Numerical values represent Z-scores of different metabolic analyte levels. Metabolomic analysis was performed at Baylor Genetics (www.BaylorGenetics.com) (Miller et al., 2015; Kennedy et al 2017).

Figure legends

Figure 1. a: Diffuse cerebellar volume loss and hypertrophic olivary degeneration in Case 1. b: Distal toe hypoplasia in Case 1. c: Abnormal T2 prolongation in basal ganglia and thalami with volume loss of bilateral caudal heads and thalamus in Case 2.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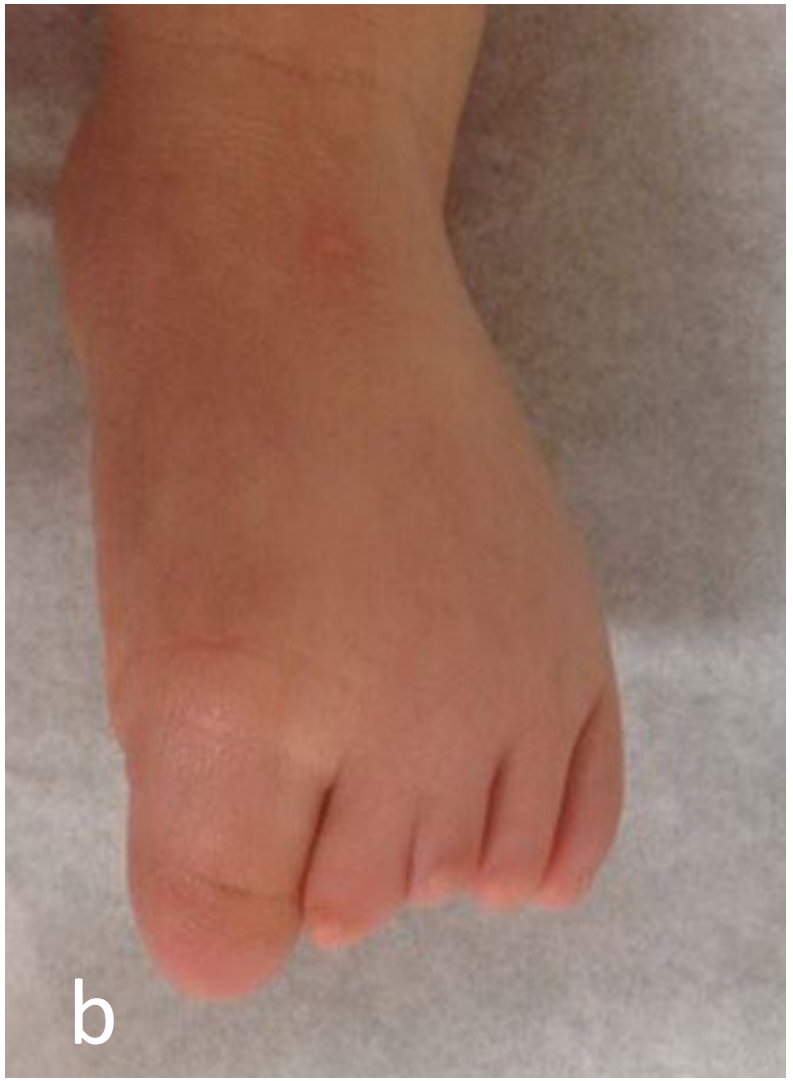
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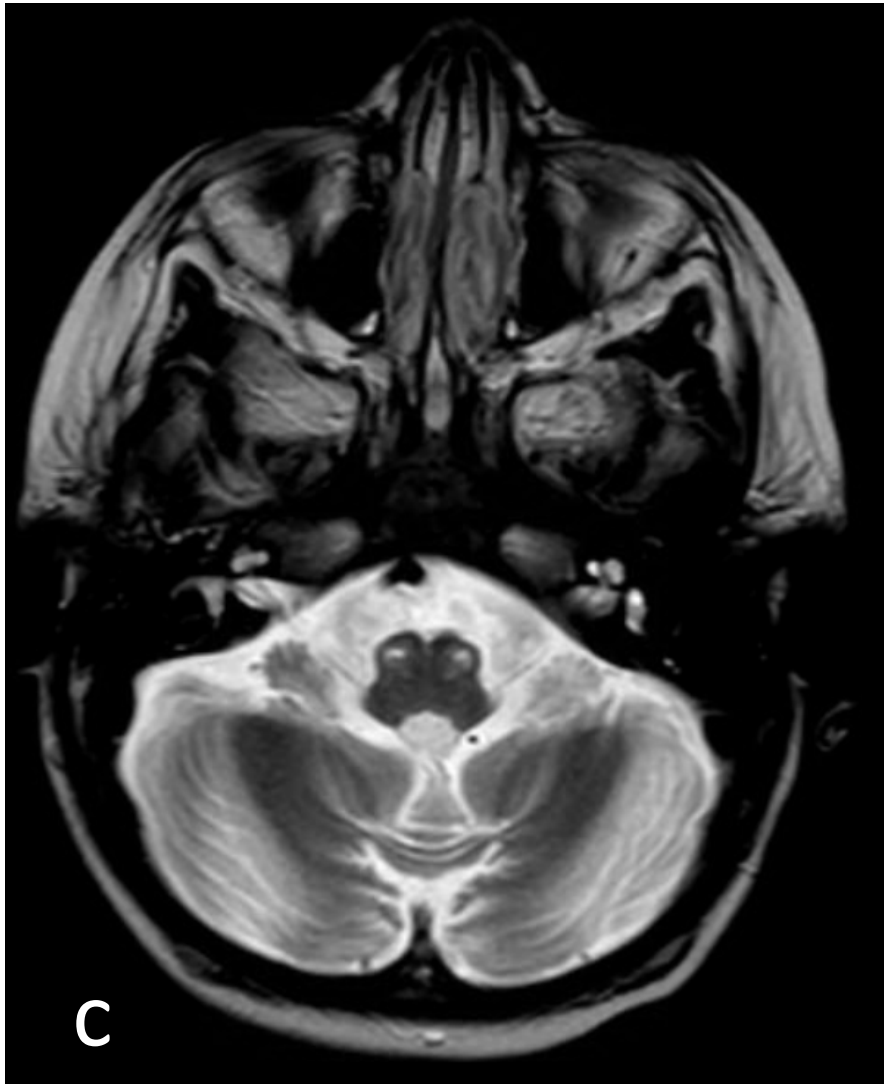
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