Absent thalami caused by a homozygous 
\textit{EARS2} mutation: expanding disease 
spectrum of LTBL

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ABSTRACT

Leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL) is caused by autosomal recessive EARS2 mutations. Onset is most often in infancy, but in severe cases in the neonatal period. Patients typically have magnetic resonance imaging (MRI) signal abnormalities involving the thalamus, brainstem and deep cerebral white matter. Most signal abnormalities resolve, but in severe cases at the expense of tissue loss. Here we report a patient with an encephalopathy of antenatal onset. His early MRI at 8 months of age showed signal abnormalities in the deep cerebral white matter that improved over time. The thalami were absent with the configuration of a developmental anomaly, without evidence of a lesion. We hypothesized that this was a case of LTBL in which the thalamic damage occurred antenatally and was incorporated in the normal brain development. The diagnosis was confirmed by a novel homozygous EARS2 mutation. Our case adds to the phenotypic and genetic spectrum of LTBL.
INTRODUCTION

In 2012, the novel disease “leukoencephalopathy with thalamus and brainstem involvement and high lactate” (LTBL) was defined based on a distinctive magnetic resonance imaging (MRI) pattern.\(^1\) Patients typically have symmetrical signal abnormalities in the thalamus, brainstem and deep cerebral white matter that improve over time in the majority (70%) of the originally identified cases.\(^2\) The underlying genetic defect of this disorder was identified shortly thereafter. All patients have autosomal recessive mutations in the gene \(EARS2\), encoding mitochondrial glutamyl tRNA synthetase.\(^{1,2}\) Although the core neurological and MRI phenotype was similar, a wide variability in clinical severity was seen in this group, ranging from infantile-onset disease followed by evident clinical improvement or a more severe neonatal-onset encephalopathy with stabilization, but no improvement.\(^2\) The patient discussed here has an earlier, antenatal-onset encephalopathy with in addition a novel feature on brain MRI.

CASE REPORT

An 18-year-old male with a leukoencephalopathy of unknown cause was referred for second opinion. He was born full term after an uneventful pregnancy with a birth weight of 3,600 g. He was the second child to healthy Dutch parents without known consanguinity. From birth on, hypotonia, poor visual contact and total lack of achievement of any developmental milestones were noted. He did not show behavioural problems or signs of distress. He experienced severe feeding difficulties, which led to percutaneous endoscopic gastrostomy tube feeding at 12 years of age. His neurological picture was stable and there were no periods of clinical regression. He developed tonic-clonic seizures at age 18 years. Laboratory investigations, including serum lactate and extensive screening for metabolic diseases, were unrevealing. Lumbar puncture was not performed. Physical examination at 18 years revealed a young man with a severe microcephaly (-4 SD). He had a divergent squint and offered no eye contact. No head control was present. He had hyperreflexia and hypertonia with contractures of arms and legs. He had no intentional movements.

His initial brain MRI, performed at 8 months of age, showed disfiguration and moderate enlargement of the lateral ventricles and confluent, symmetrical signal abnormalities in the deep cerebral white matter with sparing of a periventricular rim and the directly subcortical white matter (Figure 1, B and C). The corpus callosum was complete, but
thin, especially the posterior part and showed T2 signal abnormalities throughout. The basal nuclei, brainstem, and cerebellar white matter had a normal signal (Figure 1, A). Follow-up MRIs, at 3 and 18 years of age showed partial resolution of the signal abnormalities in the cerebral white matter and corpus callosum (Figure 2, B). The basal nuclei, brainstem and cerebellar white matter still had a normal signal. We noticed that the remarkable disfiguration of the lateral and third ventricles was caused by absence of the thalami (Figure 2, A-D). There were no signal abnormalities in this region suggesting a lesion and there was smooth lining of the ventricles (Figure 2, C). In retrospect, the absence of the thalami was also visible on his previous MRI (Figure 1).

We argued that EARS2 mutations could by the cause of the disorder (see discussion) and thus performed DNA analysis of the EARS2 gene, which revealed a homozygous in-frame deletion of three base pairs (c.454_456del), causing a deletion of the highly conserved amino acid lysine at position 152 (p.[Lys152del]). This mutation has not been reported in public databases (1,000 Genomes [release of February 2012], dbSNP137 [available at: http://www.ncbi.nlm.nih.gov/projects/SNP], and NHLBI Exome Sequencing Project, ESP6500 release [available at: http://evs.gs.washington.edu/EVS/]). Parents both carried the mutation. Subsequent detailed analysis of their pedigrees revealed consanguinity with a common ancestor in the 17th century, eight generations back.
The patient described here presented shortly after birth with a severe but stable encephalopathy with lack of all development and spastic tetraparesis. These features are in themselves nonspecific and with the apparent a- or hypoplasia of the thalami they would suggest a genetically determined developmental anomaly of the brain. However, mutations analysis showed that this patient had a novel homozygous one amino acid deletion in the gene EARS2 associated with the disease LTBL.2

Patients with LTBL were initially described in 2012.1,2 These patients could be divided into two clinical groups: (1) patients with neurological regression at 6 to 12 months of age and subsequent stabilization and improvement on follow-up, and (2) patients with a neonatal or early infantile onset severe encephalopathy with seizures and spastic paraparesis with subsequent stabilization but no clinical improvement.1,2 None of these patients had a second period of regression. The MRI early in the disease course showed symmetrical signal abnormalities and swelling of the deep cerebral white matter with
sparing of a periventricular rim and the directly subcortical white matter. Additional signal abnormalities were present in deep gray matter structures, most prominently involving the thalami. The corpus callosum, brainstem and cerebellar white matter were affected as well. Proton MR spectroscopy showed elevated lactate in the affected white matter. Follow-up MRI showed improvement and partial to complete resolution of signal abnormalities and normalization of lactate. In the severe cases, however, part of the affected structures was apparently damaged beyond repair and displayed atrophy, especially the thalami and cerebral white matter, with remaining evidence of scarring. In some patients the posterior corpus callosum was abnormally thin or completely absent.\textsuperscript{2}

The evidence available until now suggests that the phenotype of patients with \textit{EARS2} mutations results from a single episode of deterioration early in life. The degree of damage is thought to be determined by the timing and the severity of this episode.\textsuperscript{2} Superficial inspection of our patient’s MRIs suggested a developmental anomaly with a- or hypoplasia of the thalamus and smooth lining of the ventricles, without evidence at all suggesting a previous lesion and scarring. Closer examination of the MRI, however, showed absence of thalamic nuclei originating from both the thalamus dorsalis and ventralis, which are separated nuclear zones along the diencephalic wall of the neural tube during fetal development,\textsuperscript{3} and no involvement of other derivatives as the habenula or pineal gland. This is more compatible with a very early disruptive event. Damage early during fetal life is incorporated into normal development, making the final configuration look like a developmental anomaly. A comparable phenomenon is seen in schizencephaly, which is characterized by clefts spanning the cerebral hemisphere from the pial surface to the lateral ventricle.\textsuperscript{4,6} Interestingly, the walls of the defect are subsequently lined with ectopic and dysplastic gray matter.\textsuperscript{6} The presence of this gray matter lining indicates that the induced injury, caused by vascular disruption or exogenous environmental factors, occurs prior to termination of neuronal migration with the consequence that the defect is incorporated within further brain development.\textsuperscript{6} A similar scenario could be envisioned for LTBL. Keeping in mind the possibility of an expanding disease spectrum, we hypothesize that the MRI features of our patient could reflect a very early hit resulting in an early antenatal onset of LTBL with disappearance of the thalamus. Some special MRI features supported our hypothesis of LTBL, including abnormalities in the deep cerebral white matter with sparing of a periventricular rim, the improvement of the white matter abnormalities over time and the thin posterior corpus callosum. We considered other early antenatal events disrupting the thalami, such as infection of ischemia, but in view of the special MRI features supporting LTBL, we tested \textit{EARS2} first. Indeed, our hypothesis was confirmed by detection of a homozygous \textit{EARS2}
mutation in our patient. Interestingly, in some of the early onset, severely affected LTBL patients described by Steenweg et al, the posterior part and rostrum of the corpus callosum were absent (see Figure 3, C in Steenweg et al, 2013), and this was ascribed to a developmental anomaly. With the new information, this interpretation should be revised. The corpus callosum abnormality is more likely also the result of an early injury disrupting further development of the corpus callosum rather than a developmental anomaly per se. This revised interpretation indicates two episodes of deterioration in those patients, one early fetal and one early infantile.

Since the discovery of recessive EARS2 mutations in LTBL patients, three more patients with EARS2 mutations were reported. For one patient no detailed clinical features were reported. The patient described by Talim et al., presented with a severe infantile multisystem disease involving the brain and the liver with a very early death at three months of age due to necrotizing bronchopneumia. Also this patient had absence of the posterior part of the corpus callosum. The patient reported by Biancheri et al., had an intermediate clinical phenotype, with an early-onset disease, but subsequent clinical improvement. His MRI, however, showed new lesions in the left caudate nucleus and globus pallidus during follow-up at age 5 years and 10 months, indicating that there could be more than one episode of regression. This is in line with the new view that there can be more than one episode of deterioration. Based on the patients published so far, there is no clear genotype-phenotype correlation.

With the identification of the patient described here we illustrate that an early antenatal “hit” may give rise to a severe clinical phenotype and a specific MRI pattern characterized by absence of the thalami, which expands the disease spectrum associated with EARS2 mutations. Additionally, new evidence indicates that there can be more than one episode of deterioration. It is to be expected that whole-exome sequencing and whole-genome sequencing will lead to a further expansion of the known disease spectrum of LTBL.
REFERENCES
