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Case report

Early-onset leukoencephalopathy due to a homozygous missense mutation in the *DARS2* gene☆

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ABSTRACT

Mutations in the *DARS2* gene are known to cause leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), a rare autosomal recessive neurological disorder. It was originally described as juvenile-onset slowly progressive ataxia and spasticity, but recent reports suggest a broader clinical spectrum. Most patients were found to carry compound heterozygous *DARS2* mutations, and only very few patients with homozygous mutations have been described so far. We present here an 8-month-old boy carrying a homozygous missense mutation in *DARS2* who clinically showed severe neurological deterioration after a respiratory tract infection, followed by an almost complete remission of symptoms. This report further extends the knowledge about the clinical and molecular genetic spectrum of LBSL.

1. Introduction

Mutations in the gene encoding mitochondrial aspartyl-tRNA synthetase (*DARS2*, located on chromosome 1q25.1) have been shown to cause leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL; MIM #611105), a rare autosomal recessive neurological disorder [1]. It was first described as a juvenile-onset disease mainly characterized by slowly progressive ataxia and spasticity, and most affected individuals were reported to become wheelchair-dependent in their teens or twenties [2]. However, more recent reports showed that the clinical spectrum may vary from early-onset severe disease, sometimes even fatal within the first few years [3], to an adult-onset mild phenotype. The majority of patients was found to carry compound heterozygous mutations in *DARS2*, mostly a combination of an intron 2 splice site mutation and a second variable mutation [1]. Only very few patients with homozygous mutations have been described so far [4,5], and these showed varying phenotypic expression. We report here on a little boy of German origin carrying a homozygous missense mutation in *DARS2* who clinically presented with severe neurological deterioration following a respiratory tract infection at 9 months of age, followed by a remarkable almost complete remission of symptoms.

2. Material and methods

2.1. Case report

The index patient is the second child of healthy consanguineous German parents (2nd degree cousins, see Fig. 1) with Ukrainian roots. After uneventful pregnancy and delivery, psychomotor development was normal (except for sensorineural deafness of the right ear) until the age of ~8 months, when the parents noticed

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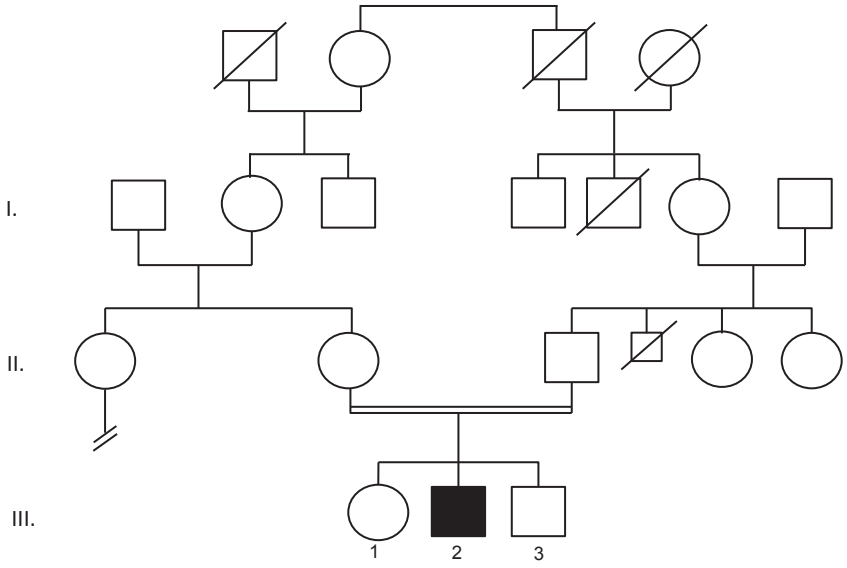

Fig. 1. Pedigree of the consanguineous German family.

reduced attentiveness, irritability and psychomotor regression. The neurological problems were further intensified after a febrile respiratory tract infection at the age of 9 months. The patient was first presented to our Children's Hospital at the age of 10.5 months; he showed an overall stable condition and had measurements of height, weight and head circumference within the normal percentiles for his age. Dysmorphic stigmata were not evident. Neuropediatric examination revealed marked psychomotor abnormalities with irritability, reduced eye-contact, truncal hypotonia, lack of head control upon traction and loss of targeted motor functions such as sitting up, picking up objects or rolling over. Muscular reflexes were regular; there were no extrapyramidal signs, eye movements and swallowing also appeared normal. Blood as well as cerebrospinal fluid (CSF) parameters, including CFS analysis for lactate, neurotransmitters, MOG antibodies and oligoclonal bands, did not reveal abnormal results. Amino acid analysis in plasma and urine, analysis of very long chain fatty acids in plasma and organic acid analysis in urine were inconspicuous, as were enzymatic analyses for arylsulfatase A (Krabbe disease) and galactocerebrosidase (metachromatic leukodystrophy). The first MRI scan at the age of 11 months showed diffuse symmetrical signal intensities of the white matter (see 2.2).

We started metabolic supplements with vitamin C, co-enzyme Q10 and creatine. Treated with regular physiotherapy over the following months, the neurological functions of our patient showed gradual improvement. At the age of 12.5 months, he started grabbing objects again, could hold his head up in the prone position and sit unsupported for a few minutes. Further, he showed more interest in his surroundings and started smiling socially. With 14 months, he could roll over and sit up by himself again, he made first attempts at crawling and speaking first words. From that point on, psychomotor development was approximately appropriate for his age, except for a slight ataxia. The boy is now 3 years old and is talking in complete sentences with slight problems of pronunciation. He does not show any cognitive delay, his social behaviour is sensible towards children and adults, he can run and climb stairs without any help; however slight atactic signs can still be observed.

2.2. MRI data

A first MRI scan was performed at the age of 11 months (Fig. 2a–d) and revealed bilateral symmetric signal abnormalities of the frontal and parietal white matter mainly involving periventricular and central components. The U fibres were spared. In addition, abnormal signal was present in both posterior limbs of internal capsules and splenium of corpus callosum. Diffusion weighted imaging, including the apparent diffusion coefficient (ADC), revealed very low ADC values of the above mentioned lesions, corresponding to restricted water diffusion. No signal abnormalities of basal ganglia, thalamus, brainstem or cerebellum were present. No contrast enhancement was detected. The second MRI, acquired at the age of 15 months (Fig. 2e–h), showed progression of the above mentioned signal changes from central to peripheral and posterior to anterior. This is best visualized by diffusion-weighted imaging and ADC. Compared to the initial MRI, restricted diffusion is now present at the edges of the lesion (dark on the ADC maps), but the ADC of more central lesion components has increased, suggesting demyelination. Basal ganglia, thalamus and infratentorial structures remained normal. Again, no contrast enhancement was detected.

2.3. Genetic analyses

Based on clinical and radiological findings, the *NDUFV1*, *EARS2* and *SDHAF1* genes were analyzed by direct Sanger sequencing without detection of causative mutations. In addition, a SNP array (Affymetrix 6.0) was performed which did not show pathogenically relevant microdeletions or duplications but – in accordance with consanguinity of the parents - revealed several regions with loss of heterozygosity, including a region on chromosome 1q23.3q25.3 where the *DARS2* gene is located.

As the MRI findings strongly pointed towards a mitochondrial encephalopathy, we subsequently carried out a diagnostic gene panel “mitochondrial leukodystrophy” based on next generation sequencing (NGS) technology using the Illumina^{R} MiSeq system. This panel included five genes (*AUH*, *DARS2*, *EIF2AK3*, *GFAP*, and *SDHAF1*). DNA capture of all coding exons as well as exon/intron boundaries was performed with the SureSelect^{XT} Target Enrichment Custom Kit (ID:5190/4816–4824) followed by parallel sequencing. Data analysis was conducted with the following bioinformatics methods: BWA version 0.7.5a-R405, SAMtools version 0.1.19–44428cd, snpEff version 3.3e and Alamut HT version 1.1.8.

The GRCh37/hg19 human genome assembly was used as the reference sequence. Assessment and interpretation of the pathogenicity of sequence variants was done with the Alamut-HT software and PolyPhen-2 prediction tools. With this approach we identified a homozygous missense mutation in exon 2 of the *DARS2* gene (NM_018122.4: c.172C > G, p.Arg58Gly, see Fig. 3). Both parents were confirmed to be heterozygous carriers of the mutation by direct Sanger sequencing. The parents gave informed consent to all investigations as well as data recollection and presentation.

3. Results and discussion

So far, the vast majority of LSBL patients were found to be compound heterozygous for two different *DARS2* mutations, mostly for a combination of an intron 2 splice site mutation and a second variable mutation [1]. It was suggested that such splice mutations, leading to skipping of exon 3 and absence of the functional protein, may be “leaky”, that means that part of the mutated mRNAs may still contain exon 3 and secure some amount of full-length protein [1]. Therefore, the original hypothesis was that homozygous mutations might not be compatible with life [6]. However, very few patients with homozygous *DARS2* mutations have been identified since then. Three siblings of a consanguineous family from Singapore were found to carry a homozygous splice mutation in intron 2 [4], and a homozygous missense mutation (Arg609Trp, exon 17) was identified in a German adult patient with episodic ataxia [5]. We describe here a German patient with early-onset leukoencephalopathy due to another homozygous missense mutation in the *DARS2* gene (Arg58Gly). This mutation affects a highly conserved amino acid and has already been described as a pathogenic mutation in the literature [1,7]. It was found in compound heterozygous state together with another missense mutation (Thr136Ser) and functional analyses indicated that it may influence dimerization [7].

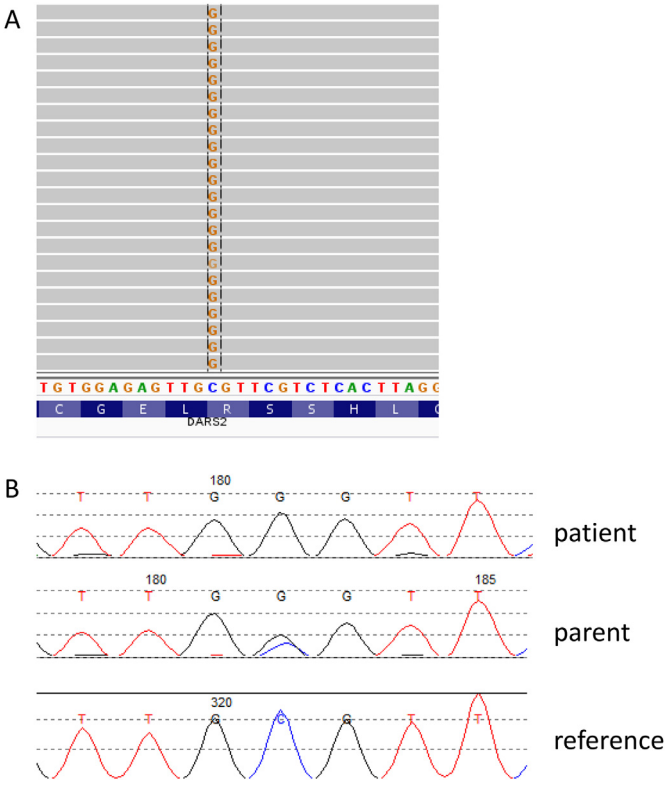

Fig. 3. Sequence data of the *DARS2* mutation c.172C > G, p.Arg58Gly on (A) next generation sequencing of the patient, (B) Sanger sequencing of the patient and his parents.

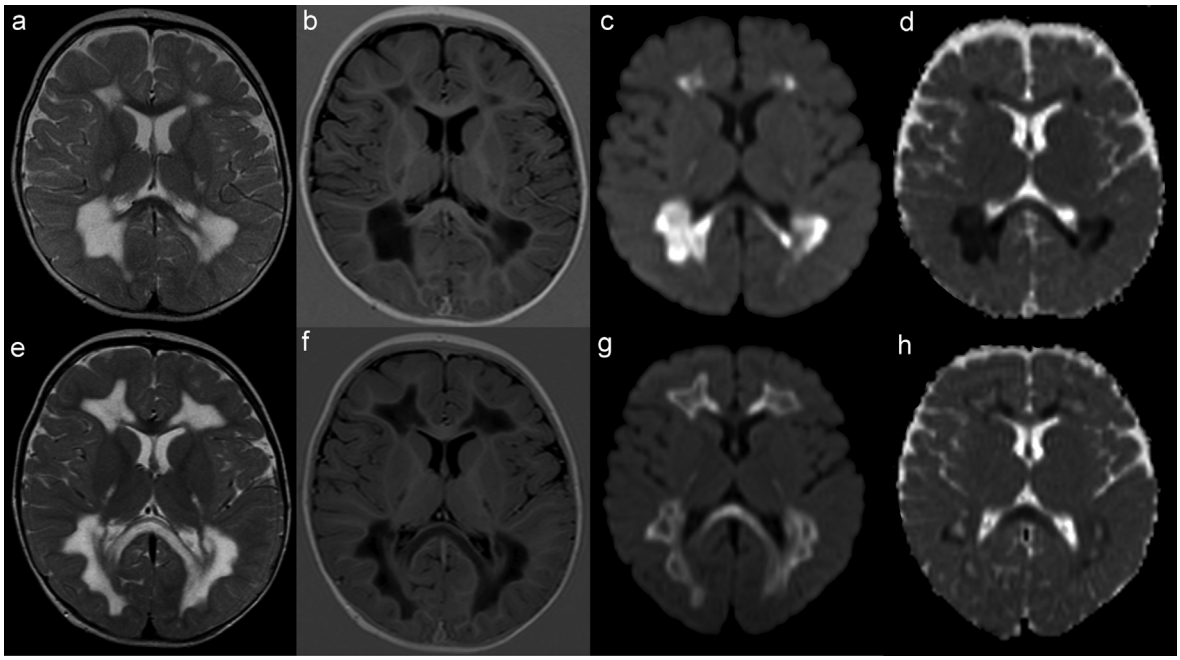

Fig. 2. Comparison of MR images of our patient at 11 (a–d) and 15 months of age (e–h). T2-weighted (a, e) and T1-weighted inversion recovery (b, f) images show bilateral symmetric signal changes mainly affecting periventricular and central white matter. These changes show a centripetal pattern of progression on follow-up. In addition, posterior limbs of internal capsules and corpus callosum are affected; U fibres are spared. Diffusion weighted imaging (c, g) and apparent diffusion coefficient [ADC] (d, h) show areas of restricted diffusion which also progress towards the periphery.

Table 1
Revised MRI criteria for LBSL as given in Steenweg et al. [3].

Signal abnormalities of:	In our patient
Major criteria	
Cerebral white matter (relative sparing of the subcortical white matter)	Yes
Dorsal columns and lateral corticospinal tracts of the spinal cord	n.a.
Pyramids at the level of the medulla oblongata or decussatio of the medial lemniscus or both	No
Minor criteria	
Splenium of the corpus callosum	Yes
Posterior limb of the internal capsule	Yes
Superior and inferior cerebellar peduncles	No
Intraparenchymal part of the trigeminal nerve	No
Mesencephalic trigeminal tracts	No
Anterior spinocerebellar tracts in the medulla oblongata	No
Cerebellar white matter	No

n.a.: not applicable since MRI scans of the spinal cord have not been performed yet.

Since only very few patients with homozygous mutations have been described so far, genotype–phenotype correlations are difficult to establish. However, there does not seem to be convincing evidence that homozygous mutations may be related to a more severe clinical course. While the three siblings from Singapore showed a severe phenotype with two of three affected individuals deceased during childhood/adolescence, the German patient with episodic ataxia exhibited a mild, adult-onset type of disease. Our patient showed rapid deterioration after an infection in early infancy with an almost complete recovery; intensive follow-up will therefore have to reveal the further disease course in this patient. One LBSL patient described by Steenweg et al. also showed acute deterioration after surgery with partial recovery afterwards [3]. Further, for some patients with *EARS2* mutations, leading to another early-onset leukoencephalopathy, milder disease courses with partial recovery have been documented [8]. The combination of acute impairment after an infection followed by substantial recovery without lactate elevation in our patient seems to be compatible with metabolic weakness and vulnerability in stress conditions. Initial disease manifestation following a viral infection has also been described, for example, in vanishing white matter disease and mitochondrial tRNA-related disorders (e.g., MELAS syndrome) [9,10]. A few patients with LBSL were described in the literature that did not show elevated lactate levels so that this feature does not necessarily be present for the diagnosis of LBSL [11].

In contrast to most LBSL patients described in the literature, our patient does not fulfil the MRI criteria for LBSL as given in Ref. [3] (see Table 1). Out of three major criteria, only one can be unequivocally called positive, namely involvement of the cerebral white matter with sparing of the subcortical white matter. The pyramids at the level of the medulla oblongata or the decussation of the medial lemniscus are (so far) not involved, and spinal cord involvement cannot be evaluated at the moment, since the parents did not consent to another MRI scan of their son. Of the minor criteria, only involvement of the splenium of the corpus callosum and the posterior limb of the internal capsule were proven in our patient. Thus, applying strict MRI criteria, the diagnosis LBSL cannot be made in our patient. However, in most other studies patients were recruited through MRI signs suggestive of LBSL, therefore there is a bias towards patients with typical MRI criteria in these studies. The homozygous *DARS2* mutation in our patient, on the other hand, was identified though a panel analysis simultaneously evaluating several genes associated with mitochondrial leukodystrophy. Since next-generation sequencing technologies will increasingly be used to detect mutations in complex and phenotypically overlapping disorders such as leukodystrophies [12], it can be hypothesized that more patients with *DARS2* mutations may be identified that do not fulfil the classical MRI criteria. Therefore, both, the clinical as well as the genetic spectrum of LBSL may further broaden in the future and it has already been proposed by some authors to group the varying phenotypes under '*DARS2*-related spectrum disorders' [13].

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