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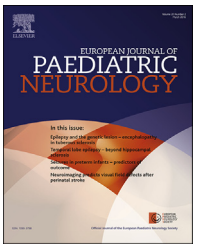

Case study

Absence of biochemical evidence at an early age delays diagnosis in a patient with a clinically severe peroxisomal biogenesis disorder

Natalia Lüsebrink [a,*], *Luciana Porto* [b], *Hans R. Waterham* [c], *Sacha Ferdinandusse* [c], *Hendrik Rosewich* [d], *Gerd Kurlemann* [e], *Matthias Kieslich* [a]

[a] *Department of Pediatric Neurology, Goethe University Hospital, Frankfurt, Germany*
[b] *Institute for Neuroradiology, Goethe University Hospital, Frankfurt, Germany*
[c] *Laboratory Genetic Metabolic Diseases, Department of Pediatrics/Emma Children's Hospital, Academic Medical Center, University of Amsterdam, The Netherlands*
[d] *Department of Pediatrics and Pediatric Neurology, Georg August University, Göttingen, Germany*
[e] *Department of Pediatric Neurology, University Hospital Muenster, Germany*

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ABSTRACT

Analysis of the plasma levels of very long chain fatty acids (VLCFA) is a primary screening method for peroxisomal disorders and usually identifies severe peroxisomal biogenesis defects reliably. We report a patient presenting with typical facial stigmata, a treatment resistant seizure disorder and polymicrogyria, whose plasma VLCFA levels were within normal limits until the age of 18 months. Only thereafter an elevation was found. Subsequent enzymatic and molecular genetic analysis revealed compound heterozygous mutations in the *PEX6* gene. In conclusion, normal VLCFA levels do not necessarily exclude global peroxisomal biogenesis defects and the analysis should be repeated subsequently. Persisting clinical suspicion justifies further enzymatic and molecular evaluation.

1. Introduction

Peroxisome biogenesis disorders (PBD) are autosomal recessive multi system disorders marked by craniofacial stigmata, muscular hypotonia, seizures, psychomotor retardation and sensorineural deficits. The clinical phenotype is constituted of the severe classic Zellweger syndrome and atypical milder variants,[1] formerly classified as neonatal adrenoleukodystrophy and infantile refsum disease. Laboratory testing,

Abbreviations: PBD, peroxisomal biogenesis disorder; VLCFA, very long chain fatty acids; CGH Array, comparative genomic hybridization array; DHAPAT, dihydroxyacetonephosphate acyltransferase; NALD, neonatal adrenoleukodystrophy.

* *Corresponding author*. Schwerpunkt Neurologie, Neurometabolik, und Prävention Klinik für Kinder- und Jugendmedizin Universitätsklinikum Frankfurt Theodor-Stern-Kai 7, 60590 Frankfurt am Main. Tel.: +49 69 6301 5560; fax: +49 69 6301 5765.
E-mail address: Natalia.Luesebrink@kgu.de (N. Lüsebrink).

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correlation of biochemical and clinical phenotype and prognosis vary considerably.[2] PBDs are caused by mutations in *PEX* genes which encode PEX proteins involved in recruiting and translocating peroxisomal proteins synthesized in the cytosol into the organelles.[3] Defective PEX proteins result in a lack of functional peroxisomes and consequently defects in the various metabolic processes the organelles are involved in, including β oxidation of VLCFA, α oxidation of phytanic acid, plasmalogen biosynthesis, and glyoxylate detoxification. Mutations in the *PEX1* and *PEX6* genes are the two major causes of PBD.[4]

2. Patient presentation

Our index patient was the firstborn child to healthy, non-consanguineous parents. Pregnancy was unremarkable. The girl was delivered vaginally at 41 weeks gestation. APGAR was 6/7/7, umbilical cord pH 7.15. Length and weight were between the 75. and 90. percentile, head circumference was at the 25. percentile. Postnatally, she presented with marked muscular hypotonia requiring gavage feeding and multiple dysmorphic features, including a high forehead, hypertelorism, epicanthus and an arched palate. Audiometry revealed sensorineural hearing loss requiring hearing aids bilaterally. On the 5th day of life, she suffered her first generalized seizure and she developed treatment resistant epilepsy presenting as West syndrome with hypsarrhythmia within the first year.

Further development was characterized by severe psychomotor delay. No major milestones were reached, motor skills culminated at stretching and very discreet head control, abilities that were subsequently lost. At 26 months of age neurologic examination was remarkable for a lack of fixation, severe truncal- and extremity hypotonia, lack of spontaneous movements, head control or reflexes. Non-verbal communication was not possible, there was no vocalization. Electrophysiologic examination was inconclusive.

Cerebral MRI at 7 weeks of age showed corpus callosum hypoplasia and perisylvian polymicrogyria, at 20 months delayed myelination and frontal pachygyria became apparent. There was no peritrigonal leukodystrophy Fig. 1.

The initial neurometabolic and genetic workup revealed no pathologies. Newborn screening, serum amino acids, urine organic acids and VLCFA were all within normal limits (Tables 1 and 3). Karyotype, CGH array and sequencing of TUBB2B and SRPX2 in regard to the polymicrogyria showed no abnormalities.

Despite normal VLCFA levels, the clinical phenotype, particularly the treatment refractory epilepsy, sensorineural deficits and MRI findings were highly suggestive of a peroxisomal disorder. A repeated evaluation at the age of 18 months revealed elevated VLCFA and reduced plasmalogens for the first time. By this time a ketogenic diet had been initiated as treatment trial for the persisting epilepsy, however the VLCFA pattern with normal C24:0 and elevated C26:0 as well as elevated ratios was compatible with a peroxisomal defect (Table 1).[5] After discontinuation due to treatment failure,

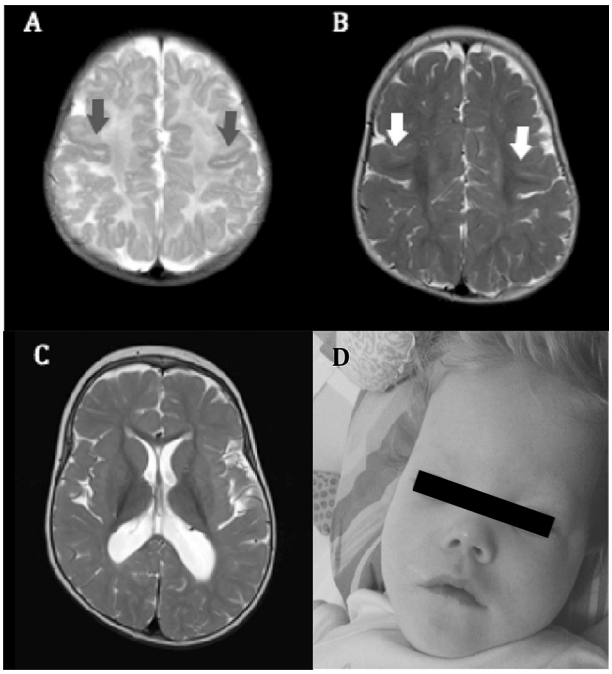

Fig. 1 – MR imaging (A–C) and clinical appearance of the index patient (D). A. T2-image at 7 weeks showing polymicrogyria of the central regions (arrows). B. Follow up T2 image at 19 months reveals a progression at the same area to mild pachygyria (arrows). C. For 19 months hyperintensity within the white matter related to the delayed myelination. D. Facial stigmatas at the age of 20 months showing the high forehead, hypertelorism, and epicanthus. Also notable, hypotonia of the facial musculature. (Photo published with parents' consent).

VLCFA levels consequently remained elevated. Further diagnostic steps including fibroblast culture and complementary testing were initiated, revealing partial cytoplasmatic catalase, pathological profiles for DHAPAT activity, acyl-CoA oxidase and peroxisomal thiolase (Table 2, Fig. 2). Immunofluorescent microscopy analysis showed aberrant peroxisomal membrane structures (Fig. 3), indicative of a severe global peroxisomal biogenesis defect. Genetic analysis identified two heterozygous mutations in *PEX6* that had previously been described and are predicted to be deleterious (c.1801C > T (p.Arg601Trp) Exon 8 and c.2362G > A (p.Val788-Met) Exon 12).

3. Discussion

Our case exemplifies the pitfalls sometimes encountered in the diagnosis of peroxisomal disorders. The clinical picture, including severe psychomotor retardation, muscular hypotonia, deafness and facial stigmata reflected the typical signs and symptoms present in up to 75% of patients suffering from this group of disorders. Moreover the cerebral MRI showed