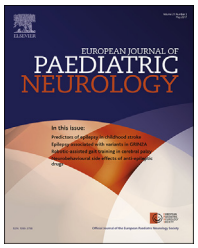
Official Journal of the European Paediatric Neurology Society



Original article

Late onset Krabbe disease due to the new *GALC* p.Ala543Pro mutation, with intriguingly high residual GALC activity *in vitro*

Inge Krägeloh-Mann [a], *Klaus Harzer* [a,*], *Kevin Rostásy* [b], *Stefanie Beck-Wödl* [c], *Antje Bornemann* [d], *Judith Böhringer* [a], *Andrea Bevot* [a], *Verena Beck* [a], *Gisela Merkel* [a], *Maria Pechan* [a]

[a] *Department of Neuropediatrics and Neurometabolic Laboratory, Children's Hospital of the University of Tübingen, Hoppe-Seyler-Str. 1, 72076 Tübingen, Germany*
[b] *Children's Hospital Datteln, University Witten/Herdecke, Dr.-Friedrich-Steiner-Str. 5, 45711 Datteln, Germany*
[c] *Department of Medical Genetics and Applied Genomics, University of Tübingen, Calwerstr. 7, 72076 Tübingen, Germany*
[d] *Department of Pathology, Division of Neuropathology, University of Tübingen, Calwer Str. 3, 72076 Tübingen, Germany*

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form
18 December 2016
Accepted 23 December 2016

Keywords:
Galactosylceramide degradation
Late onset Krabbe disease
Mutation introducing proline
Residual GALC activity
Skin ultrastructure
Specific inclusions

ABSTRACT

Background: Krabbe disease (KD) is an inherited leukodystrophy due to a defect in the GALC gene which encodes the lysosomal galactosylceramide β-galactosidase (GALC). About two thirds of patients show the early onset form of KD dominated by cerebral demyelination leading to death in early infancy. Late onset forms include a spectrum of late infantile, juvenile and adult clinical courses. The deficiency of GALC leads to a galactosylceramide lipidosis in which lysosomal storage phenomena are seen almost only at the ultrastructural level.

Results: In a 4-year-old boy, the clinical suspicion of KD was high according to neurologic and neuroimaging findings. However, laboratory results were inconclusive; white blood cell GALC activity being at 23 to 25% of the normal level, and GALC genotyping revealing the new homozygous p.Ala543Pro variant which, ex silico, was of unclear significance. Studying a skin biopsy, cultured fibroblasts showed the GALC activity at 21 to 30% of the normal level; ultrastructurally, clearly KD-specific inclusions were seen in the eccrine sweat gland cells, confirming a KD diagnosis.

Conclusion: The high clinical suspicion combined with the morphologic evidence for KD predict that the p.Ala543Pro variant is pathogenic for (late onset) KD. A hypothesis linked to the proline in the mutant GALC may explain the in vitro effect with high residual GALC activity. This patient would not have been correctly diagnosed, despite the strong clinical criteria of KD, if the electron microscopic results had not been available. The detailed

Abbreviations: GAC, galactosylceramide; GALC, galactosylceramidase; KD, Krabbe disease; LOKD, late-onset Krabbe disease; LSD, lysosomal storage disease; MR, magnetic resonance; T2w, T1w, T2-, T1-weighted image; TLC, thin-layer chromatography.

* *Corresponding author*. Klinik für Kinder-und Jugendmedizin, Abt. Neuropädiatrie und Neurometabolisches Labor, Universitätsklinikum Tübingen, Hoppe-Seyler-Str. 1, 72076 Tübingen, Germany.
E-mail address: harzer-rottenburg@t-online.de (K. Harzer).

http://dx.doi.org/10.1016/j.ejpn.2016.12.012

knowledge of neurologic and neuroimaging signs is important in diagnostically problematic KD patients in which also an electron microscopic approach can be crucial.

1. Introduction

Krabbe disease (KD) is a classic enzyme-deficient leukodystrophy (OMIM:245200) with a clinical onset at <6 months of age in 62–90% of patients.[3,4] The first signs in early infantile KD are hyperirritability and developmental stagnation. Then severe motor and mental deterioration, marked muscle hypertonicity with scissoring of the legs and opisthotonic posturing appear; the decerebrate and blind children usually decease before the second year of age.[15] The progressive demyelination does not only include the central but also the peripheral nervous system.[19] MR imaging especially on T2w scans is characteristic with signal changes of the central cerebral white matter and dentate nucleus[13]; spinal nerve roots use to enhance with contrast.[20,6,10] Ten to 38% of KD patients present as late onset KD (LOKD) patients with a wide spectrum not only of clinical onset (late infancy up to late adulthood[9]) but also of predominant clinical symptoms.[3,4,2,17] MR scans typically show demyelination of the pyramidal tracts and parieto-occipital white matter.[2] The enzymatic laboratory diagnosis of KD usually is problem-free (provided a radio-labelled natural enzyme substrate is used) with the assay of the activity of galactosylceramide β-galactosidase (GALC), the lysosomal enzyme deficient in KD. Similarly effective is the analysis of the *GALC* gene in which according to a 2015 update more than 140 pathogenic mutations have been described (http://ommbid.mhmedical.com/content.aspx?bookid=971&sectionid=62644214). The GALC deficiency leads to the cellular accumulation of galactosylceramide (GAC, the main but not only lipid storage substrate in KD). However, in some patients a KD diagnosis requires additional tests because the enzymatic and molecular findings are not conclusive.[19] Exceptionally, a LOKD-like disease is not caused by a *GALC* defect, but by the deficiency in an indispensable, non-enzymic cofactor protein of GALC called saposin A.[18,12] Moreover, if saposin A is intact, but despite inconclusive laboratory results, the neurologic and neuroimaging signs are suggestive or reminiscent of KD.[1] a peripheral biopsy, e. g., of skin and nerve, may be performed[16,8] which, by contrast to most other sphingolipidoses, is promising to specifically detect KD.[5] KD is a GAC lipidosis with formal, though no strict morphologic analogy to, for example, Gaucher disease (a classic lysosomal storage disease, LSD); its discrete storage pathomorphology usually is studied at the electron microscopic level. Here we describe an up to now unreported constellation in a patient in whom the residual GALC activity was intriguingly high and the molecular findings initially were ambiguous, whereas the clinical and neuro-imaging signs were highly suspicious of KD. A LOKD diagnosis was eventually made with the crucial support by the positive ultrastructural result in dermal eccrine gland cells.

2. Material and methods

Biochemical materials Thin layer chromatography (TLC) plastic sheets (20 × 20 cm) came from Macherey–Nagel (no. 805,013), Düren, Germany; galactosyl-[stearoyl-1-^{14}C]ceramide from Biotrend (no. ARC-1453-50), Köln, Germany; sodium taurocholate from Sigma–Aldrich (no. T4009), Taufkirchen, Germany; unlabelled C_{16}-ceramide from Enzo Life Sciences (no. SL-115), Lörrach, Germany.

Assay of GALC activity In a volume of 130 μl the following component concentrations were used: 1.02 μM galactosyl-[stearoyl-1-^{14}C]ceramide (assay amount, 132.5 pmol, with 2.035 dps/pmol); 46.2 mM sodium acetate pH 5.0; 0.23% (w/v) bovine serum albumin (inactivated 8 h at 50 °C); 0.8% (w/v) sodium taurocholate (assay amount, 1 mg); about 12,000 white Blood cells/μl (total, about 1.5 × 10^6 cells) or, in the case of cultured fibroblasts, 0.23–0.62% (w/v) cell protein (total, 30–80 μg) of about 0.8–2.2 × 10^6 cells. The radioactive substrate was added to the assay vial in a calibrated ethanol solution, and the taurocholate detergent in chloroform/methanol (2/1 by vol.) solution, and dried before the other components were added. White blood cells or fibroblasts were added as 100 μl homogenised cell suspensions in which the cell number had been counted prior to homogenisation. Incubation was for 4 h at 37 °C; 50 μl of the assay was then applied to a TLC silicagel plastic sheet. When dried, 7 μg unlabelled ceramide (in chloroform/methanol, 1/1 by vol.) was applied to the same start line. As a blank, 50 μl of the prepared assay were taken and immediately stopped with 50 μl methanol, then separately applied to TLC together with unlabelled ceramide. The sheet was chromatographed in chloroform/methanol/conc. acetic acid (47/1/1 by vol.) up to 15 cm. The dried sheet was stained with iodine vapours, and the start line containing the uncleaved radioactive substrate as well as the band containing unlabelled and radioactive, enzymatically released ceramide were marked in pencil. The marked bands were cut out (the ceramide band was distended by 5 mm upwards, to reach all of the [^{14}C]ceramide with the C_{18} fatty acid), and their radioactivity determined by β-counting. The GALC activity was calculated for Table 1 in proportion to the ratio of released [^{14}C]ceramide to the sum of released [^{14}C]ceramide and uncleaved [^{14}C]substrate, and, alternatively for Fig. 2, in direct proportion to the [^{14}C]ceramide released from the substrate with its constant initial concentration in the assays; both evaluations yielding very similar results.

Sanger sequencing of the GALC gene Blood leukocytes were isolated using standard procedures. Genomic DNA was isolated from the leukocyte pellets using the DNA isolation Kit from Roche®. Molecular genetic analysis of the 17 coding exons of *GALC* (OMIM:606890, ENST261304) and the flanking