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Published in:
Neuropathology and Applied Neurobiology

DOI:
10.1111/nan.12557

Publication date:
2019

Document version
Accepted manuscript

Citation for published version (APA):

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Download date: 17. Apr. 2021
First report of the neuropathological findings in a patient with leukodystrophy and compound heterozygous variants in PIGT gene

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Keywords: Brain, white matter, hypomyelination, childhood epilepsy, sudanophilic leukodystrophy

Short running title: PIGT leukodystrophy

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nan.12557
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Here, we report the first case of a leukodystrophy diagnosed by post mortem neuropathological examination, in which whole exome sequencing (WES) revealed compound heterozygous variants in PIGT.

Leukodystrophies are rare heritable myelin disorders affecting the white matter of the central nervous system with myelin sheath abnormalities. Furthermore, leukodystrophies involve glial cells or other non-neuronal cells and should be distinguished from the more general term leukoencephalopathy, used to describe any disease of white matter, also including acquired or toxic diseases of white matter.[1] In several leukodystrophies, the biochemical defects, the genetic backgrounds, the neuropathological patterns, the appearance on MRI scans and the clinical presentations are well known, and their aetiologies are clear. Yet, there is still a large group of leukodystrophies without a known underlying disease mechanism.

Congenital disorders of glycosylation (CDG) are genetic defects in the synthesis and attachment of glycoprotein and glycolipid glycans. There are many types of CDG with a very broad clinical spectrum, covering multiple phenotypes including neurological disease in some cases.[2,3] In one subtype, a case with CDG-Ih, a leukoencephalopathy was reported based on imaging.[4] Inherited congenital deficiencies in GPI anchor biosynthesis and attachment comprise a subset of CDG. PIGT [MIM, 610272] encodes phosphatidylinositol-glycan biosynthesis class T, which is a subunit of the glycosylphosphatidylinositol (GPI) transamidase complex that facilitates the attachment of GPI anchors to proteins and subsequently attaching them to the outer layer of the cell membrane.[5] GPI synthesis and GPI-anchored protein modification are mediated by at least 29 genes and loss-of-function pathogenic variants in 19 of these genes have been described to cause neurological impairments including intellectual disability (ID), developmental delay (DD), epileptic seizures and multiple congenital anomalies.[6] So far, 26 patients with a GPI anchor deficiency due to recessive PIGT variants have been described.[7,8,9,10,11,12,13,14] The predominant clinical presentation is that of an epileptic encephalopathy including common but subtle craniofacial dysmorphisms and
hypotrichosis, profound ID/DD, hypotonia, cortical visual impairment, nystagmus and cortical/cerebellar atrophy.[13,14]

We report the case of a male who was born at 40 weeks of gestation after a normal pregnancy as the third child of non-consanguineous healthy Caucasian parents. His older sister suffered from severe ID/DD and neonatal onset seizures. After delivery, he was admitted to the neonatal intensive care unit due to severe hypotonia, and within a few hours, he developed refractory epileptic seizures.

Ophthalmological examination revealed cortical visual impairment and abnormal motility of the eyes including strabismus and nystagmus. A brain MRI performed at the age of twelve days revealed delayed myelination and a hypoplastic corpus callosum with no signs of cerebellar atrophy. He died of pneumonia at the age of eleven months (for clinical details, see [14]).

Standard paediatric and neuropathological post mortem examinations were performed. There is full consent from the parents to publish all findings.

The findings of the paediatric autopsy included severe bronchopneumonia, retention of the testes, and a small hepatocellular adenoma. There were no other abnormalities outside the brain, in particular, no abnormalities of the heart, adrenals or kidneys.

The neuropathological examination revealed a normally formed brain with a weight within the normal range. Externally, the brain had a normal pattern of sulci and gyri and there were no abnormal features. On the cut surface, the white matter of the cerebrum was gelatinous whereas the grey matter had a more normal appearance. Histologically, the most striking feature was a substantial reduction of myelination and a pronounced astro- and microgliosis in the white matter (Figure 1a-d). Furthermore, there were macrophages in the white matter containing lipid deposits that became visible when stained with Oil Red O and Sudan black (Figure 1e-f). There was no perivascular preservation of myelin, no perivascular lymphocytic cuffing, no spongiosis, and no dysmorphic neurons. We found no Rosenthal fibres or globoid cells, there was a negative staining for PAS, and there was no metachromasia. The most severe reduction of myelination was found in the white matter of the cerebrum and the corpus callosum. Hypomyelination to a lesser extent was also observed in the white
matter of the cerebellum and the brainstem with no side differences. Overall, there was only a slight astro- and microgliosis in the grey matter.

In conclusion, the pathological findings in the brain were consistent with an orthochromatic (sudanophilic) leukodystrophy not otherwise specified.

WES was performed on genomic DNA from the patient, as previously described.[11] Compound heterozygous variants in PIGT (NM_015937) were found: c.T1472A:p.L491H and c.1484+2T>A. Segregation analysis by Sanger sequencing demonstrated that both variants were identified in the affected sister, and that p.L491H was paternally inherited and c.1484+2T>A maternally inherited, respectively. The c.T1472A:p.L491H variant was predicted damaging by PolyPhen-1 and Mutation Taster, and only seen in heterozygous state in 1/251388 controls in the gnomAD database. The c.1484+2T>A variant was predicted pathogenic by SpliceSiteFinderLike, MaxEntScan, NNSPLICE, GeneSplicer and not reported in gnomAD. Flow cytometry of GPI-anchoring proteins on granulocytes from the siblings supported the diagnosis of a PIGT-CDG

Similar to the other PIGT-CDG families, the clinical presentation in the present case was with progressive neurological features: hypotonia and intractable seizures, with cortical visual impairment, nystagmus and strabismus, and skeletal abnormalities.

Integrating the data from the medical history, metabolic screening, imaging, and the paediatric and neuropathological autopsies, the final diagnosis was orthochromatic (sudanophilic) leukodystrophy with hypomyelination.

A study from 1997 [15] demonstrated that the GPI anchor is responsible for the selective association of GPI-anchored proteins with glycosphingolipid-rich microdomains during maturation of oligodendrocytes and targets these molecules to the myelin sheath, thus acting as a myelin sorting signal. Thus, the study suggests a crucial role of GPI anchored proteins in onset and maintenance of myelination. The pathogenic variants in a subunit of the GPI transamidase causing inherited congenital deficiencies in GPI anchor biosynthesis and attachment is therefore a plausible explanation for the pathological mechanism resulting in hypomyelination. In conclusion, the PIGT-CDG leukodystrophy that we report must belong to the first category (myelin disorders) in a new classification system of genetic white matter disorders proposed by a recent paper.[16]
This is to the best of our knowledge the first time that neuropathological findings revealed in a post mortem examination are linked to a PIGT-CDG. This case illustrates that compound heterozygous variants in PIGT can explain the disease mechanism in some orthochromatic (sudanophilic) leukodystrophies.

The combination of basic, genetic and clinical sciences has led to substantial progress in our current understanding of leukodystrophies. Using clinical, genetic, pathological, imaging, and molecular biology approaches together, we will continue to learn more about the disease mechanisms that are involved in these disorders.

There are no conflicts of interest

References


Figure 1.

Photomicrographs from the neuropathological post mortem examination. H&E stain of the white matter (a). Klüver-Barrera stain revealing a reduction in myelination in the white matter (b). A GFAP immunostain in the white matter demonstrating a gliosis (c). The CD68 immunostain supports the morphological interpretation of macrophages and activated microglia (d). Oil Red O and Sudan black staining respectively, demonstrating lipid deposits in the white matter, see arrows (e-f). This article is protected by copyright. All rights reserved.
Author contribution:

Karen Bonde Larsen drafted the initial manuscript and arranged the photodocumentation and approved the final manuscript as submitted. Lisa Leth Maroun performed the pediatric autopsy, reviewed the manuscript and approved the final manuscript as submitted. Eva Løbner Lund performed the neuropathological autopsy, reviewed the manuscript and approved the final manuscript as submitted. Allan Bayat followed the probands clinical course, provided his clinical neurological expertise, reviewed the manuscript and approved the final manuscript as submitted. Rikke Stensbjerre Møller supervised the genetic investigation, reviewed the manuscript and approved the final manuscript as submitted.