

Krabbe Disease with Normal Enzyme Assay with a Pathogenic Variant in *GALC* Gene—A Report of Two Indian Cases

Krabbe disease (KD) is an autosomal recessive neurodegenerative disorder characterized by a mutation in *GALC* gene leading to deficiency of beta Galactocerebrosidase (GALC).^[1] The deficiency of GALC leads to abnormal accumulation of galactosylsphingosine.^[2] Four types of KD have been recognized based on the age of onset.^[3] We are describing two cases of Krabbe's disease with normal enzyme levels.

Case one: A one-year-two-month-old developmentally normal girl, born of a consanguineously married couple presented with regression of milestones, seizures, irritability, and spasticity. On examination, the child had microcephaly (size; 40 cm) with spastic quadriparesis and optic atrophy.

Case two: A six-year-old developmentally normal female child born to a consanguineously married couple presented with progressive difficulty in walking, ataxia, and gradual vision deterioration following a febrile illness. On examination, the child had a normal head size, optic atrophy, and spastic quadriparesis.

Clinically we considered possibilities of neurodegenerative conditions like metachromatic leukodystrophy (MLD), adrenoleukodystrophy (ALD), neurometabolic disorders, and mitochondrial disorders. Hence, complete hemogram, renal and liver function, serum ammonia, serum lactate, Tandem mass spectrometry, and urine for abnormal metabolites were done, all were normal in both patients.

Magnetic resonance imaging (MRI) of case one showed T2 hyperintensity involving bilateral centrum semiovale especially in the frontal lobes with extension along the corticospinal tracts to involve the posterior limbs of internal capsules [Figure 1a-c] and thickening of the prechiasmatic optic nerves [Figure 1d]. Case two showed symmetric T2 hyperintensity in the corticospinal tract extending from the white matter of the motor areas to the posterior limbs of the internal capsules, periventricular white matter in the parietooccipital regions, and the splenium of corpus callosum [Figure 1e and f].

Based on MRI, we considered a strong possibility of KD, hence enzyme levels of beta Galactocerebrosidase levels were done in leukocytes and found to be 5.4 and 10 for case one and case two respectively (normal levels 4-40 nmol/h/mg) were normal. Targeted next-generation sequencing (NGS) revealed variants in *GALC* gene in compound heterozygous status in both cases. The variants were, in case one, Ex 11. c.1230delC/p.Phe411LeufsTer46 and Ex 9. c.956A > G/p.Tyr319Cys and in case two Ex 9. c.956A > G/p.Tyr319Cys and Ex 7.c.626T > A/p.Leu209*. In both cases the Y319C variant was common. The variant c.1230delC/p.Phe411LeufsTer46 in ex 11 is pathogenic and others are variants of unknown significance according to ACMG criteria. Sanger sequencing of parents in case one showed mother carrying Ex 11. c.1230delC/p.Phe411LeufsTer46 and father carrying Ex 9. c.956A > G/p.Tyr319Cys in a heterozygous state. Similarly, in case two, the mother was carrying Ex 9. c.956A > G/p.Tyr319Cys and father carrying Ex 7.c.626T > A/p.Leu209* in a heterozygous state.

Here we are describing one infantile-onset and other juvenile-onset KD with normal GALC levels. The imaging findings in case one is consistent with findings described in early-onset KD with the predominant affliction of frontal white matter. Similar imaging findings have also been described in MLD. However, patients with early-onset KD have more pronounced cerebral atrophy and subtle enhancement of gray-white matter junctions which is not observed in MLD patients. The unusual feature of prechiasmatic optic nerve enlargement when present also helps to distinguish KD from MLD. On CT scans hyperdensity of thalami, cerebellum, corona radiata, and splenium have been described in KD but never observed in MLD.^[4] The posterior predominant white matter changes seen in case two along with splenial involvement are well described in late-onset KD. Early-onset MLD also presents with predominant posterior white matter affliction however cerebellar involvement is less commonly seen in MLD than KD. The MRI pattern of parieto-occipital predominance seen in late-onset KD resembles the one seen in

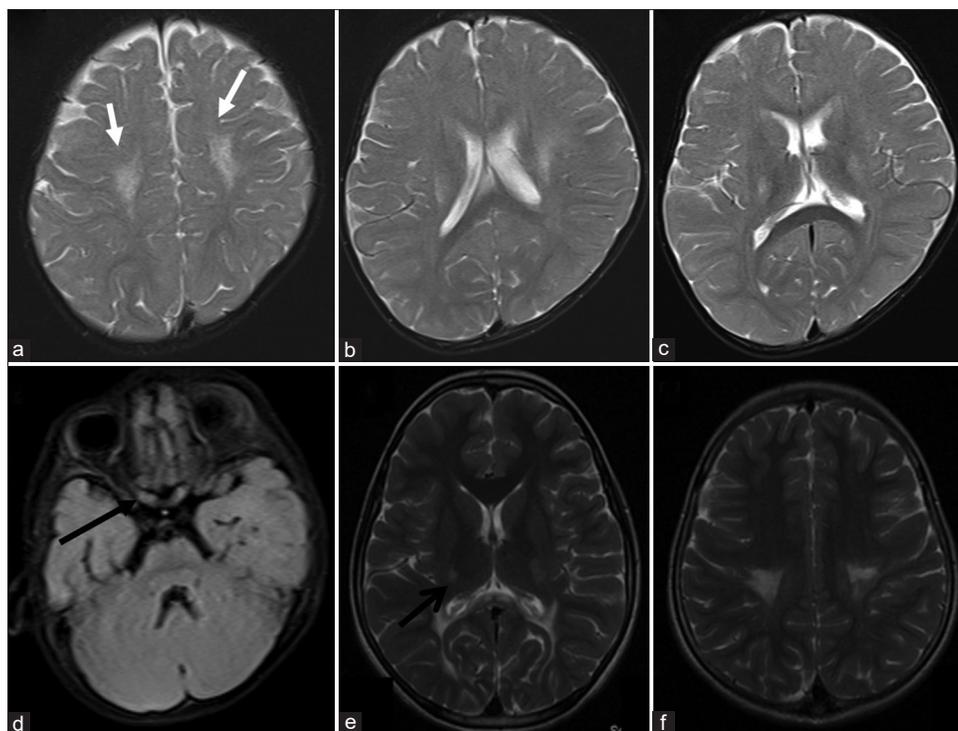


Figure 1: Case one MRI of the brain (a), axial T2WI showing hyperintensities extending along both corticospinal tracts (arrows), (b and c) bilateral symmetrical hyperintensities involving posterior limb of the internal capsule and white matter, (d) axial FLAIR image demonstrating thickening of both prechiasmatic optic nerves (arrow). Case two MRI of the brain, T2 WI axial image (e and f) shows bilateral symmetrical hyperintensity involving corticospinal tracts along the posterior limb of the internal capsule (Black arrow), parieto-occipital lobes, and splenium of the corpus callosum

ALD. However, the presence of two zones of differential signal intensity in the white matter lesions and the peripheral rim of enhancement between the two zones in ALD differentiates it from KD.^[4] Based on the above classical findings on the MRI of the brain and normal metabolic work-up we excluded MLD, neurometabolic, and mitochondrial disorders. Both children are females, and hence ALD is less likely as it is an X-linked disorder.

In case one, a heterozygous deletion (c. 1230delC) is predicted to cause a frameshift and consequent premature termination of protein (p.Phe411LeufsTer46). The second heterozygous variant is a missense substitution (p.Try319Cys) which alters a conservative residue in protein this variant was identified in compound heterozygous state in cases of KD.^[5] In case two, the description of Y319C variant was as in case one. The second variant is a missense variant p.Leu209* leads to a truncated form of protein.

Both these cases had normal enzyme assay of beta Galactocerebrosidase levels in leukocytes. The various possible reasons for normal enzyme levels in our two cases are explained below. Enzyme assays are the gold standard for definitive diagnosis of lysosomal storage disorders (LSDs) by demonstrating deficient enzyme activity in leukocytes/plasma/cultured fibroblast; however, sensitivity is better in fibroblasts compared to leukocytes. We have not done fibroblast enzyme levels as it requires an invasive skin biopsy, expensive, long waiting period for the culture.^[6]

It is known that mutations, changing an amino acid may affect the way the enzyme handles the substrate. With some mutations, the activity is completely knocked out, leaving little or no residual activity, but in some cases, the amino acid change may make the enzyme work less efficiently.^[7] This may be the reason for normal enzyme levels seen in our patients. Duffner PK, *et al.* studied Galactocerebrosidase activity in 19 patients of which 16 patients' results were available, 50% showed 0.07 nmol/hr/mg or less and 50% 0.1 nmol range between 0.0 and 0.26 nmol/hr/mg. The patient with the highest enzyme levels exhibited only one mutation with polymorphism. The patient with p.Try319Cys genotype had slightly higher enzyme levels of 0.2 as compared to other patients in the study.^[8] The normal enzyme is also reported in other LSDs like in MLD due to Saposin B or Arylsulfatase A deficiency,^[7] and the AB variant of gangliosidosis.^[9]

In the published reports, no correlation has been established between residual enzyme activity in either leukocytes or fibroblasts with disease age onset, severity, and genotype.^[10] Our hypothesis for the normal enzyme levels could be, in association with p.Try319Cys variant seen in both cases and reported earlier with higher enzyme level in this variant. Phenotype of one novel variant Ex 11.c.1230delC/p.Phe411LeufsTer46 in case one and rare variant Ex 7.c.626T > A/p.Leu209* in case two may also contribute to normal levels of the enzyme.

To conclude, if clinical and Neuroimaging are suggestive of KB and enzyme level is normal, consider genetic evaluation before investigation for other rare disorders with expensive tests.

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Conflicts of interest

There are no conflicts of interest.

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