A Novel Compound Heterozygous Mutation in the GALC gene in a Tunisian Family

Imen Ketata, MD¹,², Emna Ellouz, MD¹,²

¹Department of Neurology, University Hospital of Gabes, Gabes, Tunisia
²Department of Neurology of Gabes, University of Sfax, Sfax, Tunisia

Krabbe disease (KD) is an autosomal recessive neurodegenerative disorder caused by mutations in the galactocerebrosidase (GALC) gene, which is responsible for the production of the GALC enzyme [1]. Early infantile Krabbe disease (EIKD), which presents before 6 months of age, is the most prevalent, accounting for 85% to 90% of cases [1]. Although numerous mutations in the GALC gene have been identified, new mutations continue to be discovered. In this report, we describe three siblings who exhibited atypical clinical features of EIKD associated with a novel compound heterozygous mutation. Consent for this study was obtained from the family.

The three cases were born at term to non-consanguineous Tunisian parents after an uneventful pregnancy. The first sibling (IV11) was a 7-month-old girl (Fig. 1A). She was brought to our Neurology Department, at this age, for delayed milestones and spasms. She was brought to our Neurology Department at this age due to delayed milestones and spasms. Her medical history included normal developmental milestones until the age of 2 months, when her parents noticed a loss of smiling and eye tracking. At 3 months, she began experiencing spasms in clusters. Subsequently, she was seen in the pediatric department, where an electroencephalogram (EEG) revealed hypsarrhythmia. She was diagnosed with West syndrome (WS), and treatment with vigabatrin was initiated. One month later, a follow-up EEG showed disorganized cerebral electrogenesis and the presence of diffuse spikes, with no differentiation between sleep and wake states. Hydrocortisone therapy was started. At 7 months, a neurological examination showed severe hypotonia and brisk tendon reflexes in all limbs. A fundoscopic examination found optical atrophy. Brain magnetic resonance imaging (MRI) (Fig. 1B) showed periventricular diffuse hyperintensities. A lumbar puncture (LP) found an elevated protein level (1.5 g/L). Electroneuromyography (ENMG) indicated a demyelinating neuropathy. Auditory evoked potentials confirmed sensorineural hearing loss, while visual evoked potentials were normal. Her condition progressed to tonic seizures and spasticity in all four limbs. She passed away at the age of 8 months.

The second and third siblings (IV12 and IV13) (Fig. 1A) exhibited the same clinical features and normal psychomotor development until the age of 5 months. At that point, their parents reported symptoms of irritability, feeding difficulties, and episodes of hyperpnea. The mother observed a regression in visual tracking abilities and poor head control, accompanied by hypertonia. By the age of 8 months, a physical examination revealed failure to thrive, diminished alertness, constant crying, and brisk tendon reflexes with clonus in both feet. The Denver developmental screening...
Fig. 1. (A) Pedigree of the affected family. The arrows indicate the individuals whose cases were studied. The family tree revealed that three siblings (IV11, IV12, and IV13) were heterozygous for the mutations, while the father (III13), the mother (III12), and the youngest child (IV14) each carried one mutation. (B) Brain magnetic resonance imaging (MRI) of the sister (IV11). Axial T2-weighted images (Ba, Bc, Bd) show T2 hyperintensity in the bilateral periventricular area (Ba, arrows) and in the posterior arms of the internal capsule (Bc, arrows), a thin corpus callosum (Bd, rectangle), involvement of the dentate nuclei (Bc, dot arrows), and global cortico-subcortical atrophy (Bc, stars). An axial T1-weighted image (Bb) shows cortico-subcortical atrophy. (C) Brain MRI of the brother (IV12). Axial T2-weighted images (Ca, Cc, Cd) show a tigroid pattern of periventricular hyperintensity (Ca, large arrows), involvement of the dentate nuclei (Cc, dot arrows), and atrophy of the Sylvian fissures (Cd, arrowheads) and periventricular T2 hyperintensity (Cd, arrows). A sagittal T1-weighted image (Bb) shows cortico-subcortical atrophy.
test estimated their developmental age at 2 months. An ophthalmological examination of IV12 revealed bilateral proptosis, and his brain MRI showed periventricular hyperintensity (Fig. 1C). An LP revealed a high level of protein (2.2 g/L), and ENMG identified a demyelinating neuropathy. The auditory evoked potentials showed an absence of brainstem waves. Two months later, both siblings developed axial hypertonia, bilateral nystagmus, and tonic spasms. Repeated EEGs during sleep remained normal. These two children passed away at the ages of 18 and 20 months, respectively.

In the IV12 sibling, GALC enzyme activity in peripheral blood leukocytes was found to be deficient (0 µkat/kg, with normal values ranging from 1.9 to 6.5 µkat/kg). Both parents and sibling IV12 underwent mutation screening of the GALC gene. DNA was extracted from whole blood using the nucleon BACC3 kit (Amer- sham, Piscataway, NJ, USA). Sequencing of the 17 exons and intron-exon junctions of the GALC gene was conducted, along with a search for the large 30 kb deletion that spans exons 11 to 17, using specific polymerase chain reaction techniques. The mother was heterozygous for the p.Ala263_Thr266dup (c.788_799dup) mutation, and the father for the p.Leu149Phe (c.447G>T) mutation. The substitution of leucine (L) by phenylalanine (F) is predicted to be pathogenic by the Alamut software (Sophia Genetics, Lausanne, Switzerland). Sibling IV12 was found to have a novel compound heterozygous mutation. A prenatal diagnosis conducted during the fifth pregnancy enabled the couple to have a healthy daughter (IV14), who inherited only the maternal heterozygous

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**Fig. 2.** (A) Evaluation by MutationTaster, showing the substitution of guanine (G) by thymine (T) and the conserved guanine amino acid at position 149 in other investigated species. (B) G is completely conserved across all investigated species in the galactocerebrosidase (GALC) protein amino acid sequences. (C) Evaluation of the pathogenicity using the PolyPhen database shows a score of 1,000, indicating a probably damaging variant.
mutation (Fig. 1A).

Tunisia has been classified as having a low risk of KD [2,3]. To the best of our knowledge, there have been only two reported cases of EIKD in the Tunisian population. The first case was observed by Kraoua, and the second by Fiumara et al. [3]. EIKD presents a wide clinical spectrum with non-specific symptoms [1]. In our cases, proptosis and WS were the most notable clinical findings. Although febrile seizures, myoclonic seizures, and generalized tonic-clonic seizures are the most common seizure types in EIKD, WS has been infrequently reported [4,5]. It has been documented in one French and one Chinese study [4,5]. Common characteristics of KD patients with WS include female sex, an onset age of 3 months, and resistance to medication [4,5]. Kliemann et al. [6] noted that the EEG features of WS in KD differ from those typically seen in infantile spasms. The amplitudes were often between 50 and 200 µV, without any spikes or sharp waves [6]. The EEG of our patient displayed a classic hypsarrhythmia pattern.

Visual abnormalities were primarily characterized by discordant eye movements (48%), aberrant pupillary responses (56%), strabismus (14%), and nystagmus (11%) [1]. Sibling IV12 presented with bilateral proptosis, which may be attributable to the accumulation of globoid cells in the optic nerve. However, proptosis has not been previously reported in this context.

According to the ClinVar database, 992 variations of the GALC gene in Homo sapiens have been identified to date (https://www.ncbi.nlm.nih.gov/clinvar). Of these, only 176 variants are considered pathogenic. In our study, the mother carried a frameshift mutation characterized by a duplication of 12 nucleotides from position 788 to position 799 (c.788_799dup), which may alter the structure and function of the enzyme. In the father, the guanine (G) nucleotide was replaced by thymine (T) in the 447th position of the GACL gene (c.447G>T), and phenylalanine (F) replaced the leucine (L) (Fig. 2A). An alignment of GALC protein amino acid sequences indicated that guanine (G) is completely conserved across all investigated species (Fig. 2B). We utilized the PolyPhen database (http://genetics.bwh.harvard.edu/pph2), SIFT software (https://sift.bi.a-star.edu.sg), and MutationTaster (https://www.genecascade.org/MutationTaster2021) to evaluate the pathogenicity of the father’s variation. The PolyPhen database indicated that the missense mutation (p.Leu149Phe) is expected to be deleterious (Fig. 2C). The SIFT score was equivalent to 0.00 (<0.05), which indicates that the substitution is expected to be damaging.

Our case reports are constrained by the lack of a detailed genetic assay for the patients since the testing was performed abroad, and we only possess the results related to the novel mutation.

In conclusion, we have reported these cases featuring a novel compound heterozygous mutation with significant clinical findings to underscore the importance of considering EIKD in the presence of non-specific clinical features and to contribute to the expansion of the GALC pathogenic mutation database.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

ORCID

Imen Ketata, https://orcid.org/0000-0002-3057-8028
Emna Ellouz, https://orcid.org/0000-0002-2180-9445

Author contribution

Conceptualization: IK and EE. Data curation: IK. Project administration: IK and EE. Visualization: EE. Writing-original draft: IK. Writing-review & editing: EE.

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