Protracted Course of Krabbe Disease in an Adult Patient Bearing a Novel Mutation

Laura B. Jardim, MD; Roberto Giugliani, MD, PhD; Ricardo F. Pires, MD; Sergio Haussen, MD; Maira G. Burin, MSc; Mohammad A. Rafi, PhD; David A. Wenger, PhD

**Background:** Krabbe disease, or globoid cell leukodystrophy, is an autosomal recessive disorder caused by the deficiency of galactocerebrosidase (GALC) activity. Although most cases are diagnosed in infancy and show a fatal outcome in childhood, adult patients have been identified, showing progressive spastic hemiparesis to tetraparesis, followed by optic atrophy, dementia, and neuropathy. The disease can be diagnosed by detecting the deficiency of GALC activity (less than 5% of normal) in any available tissue sample. The cloning of the human GALC gene allowed the molecular characterization of newly diagnosed patients. More than 75 disease-causing mutations and polymorphisms in this gene have been identified.

**Objective:** To describe a 28-year-old woman with Krabbe disease, correlating clinical and biochemical abnormalities to a novel mutation on the GALC gene.

**Methods:** Clinical investigation was enriched by neurophysiological and neuroimaging data. The activity of GALC was assayed in white blood cells using radiolabeled natural substrate. Genomic DNA was isolated from peripheral blood, and the GALC gene was sequenced. The mutated gene was expressed and GALC activity was measured in transfected COS-1 cells.

**Results:** The patient had progressive and bilateral amaurosis starting at 8 years of age. Although she was experiencing weakness in all her extremities, her intellect remained intact. She was found to be homozygous for a previously unreported missense mutation (T1886G), which leads to low, but not totally deficient, GALC activity.

**Conclusions:** Expression of this mutation in COS-1 cells using the pcDNA3 expression vector (Invitrogen, Carlsbad, Calif) resulted in low, although not null, GALC activity, which can explain the protracted clinical course in this patient. Patients carrying the mutation described herein might be potential candidates for therapeutic trials, such as bone marrow transplantation or gene therapy.

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Krabbe disease, or globoid cell leukodystrophy (GLD), is an autosomal recessive disorder caused by the deficiency of galactocerebrosidase (GALC) activity. While most patients present with symptoms of spasticity, developmental delay, and irritability before 6 months of age, the disorder has also been diagnosed in older patients, including adults. The clinical features of patients with adult GLD described in the literature are very heterogeneous, including age at onset and severity of symptoms; however, many patients with adult GLD show progressive spastic hemiparesis to tetraparesis, followed by optic atrophy, dementia, and neuropathy. The disease can be diagnosed by detecting the severe deficiency of GALC activity (less than 5% of normal) in any available tissue sample. However, some individuals who do not have GLD, as we now understand it, have GALC activity that is less than 10% of normal activity. This so-called pseudodeficiency state may complicate the correct diagnosis of GLD, especially in atypical cases.

With the cloning of the human GALC complementary DNA (cDNA) and gene, molecular analysis of the GALC gene became possible in all cases of GLD. At this time, one common mutation has been found in 40% to 50% of the mutant alleles in infantile patients of European or Mexican ancestry. Nearly 65 disease-causing mutations and polymorphic changes have been identified. We describe a 28-year-old woman whose symptoms began when she was 8 years old. She was found to be homozygous for a T to G transversion at the cDNA position 1886.
which results in an arginine substitution for a leucine at polypeptide location 629.

REPORT OF A CASE

A 28-year-old woman of German ancestry was the sixth of 7 children born healthy to consanguineous parents who are first cousins. Her birth and delivery were uncomplicated, and her developmental milestones were normal. She was asymptomatic until the age of 8 years, when a progressive and bilateral amaurosis started to develop. Her school performance was normal, with the use of the Braille system. She was healthy and working as a manufacturing laborer until the age of 23 years, when weakness was noted in her right leg. Five of her siblings were normal, but her 35-year-old brother had the same clinical presentation starting at 7 years of age.

At 25 years of age, the patient’s neurological examination revealed an alert and oriented young woman with a normal mental status. The ophthalmological abnormalities included a bilateral optic atrophy, poor peripheral vision with central amaurosis, and a divergent strabismus in the left eye. Her gait was spastic on the right side, with a normal station. She demonstrated decomposition of movements, dysdiadochokinesia, and Holmes rebound phenomenon in the right arm. Her muscle tone was increased in all extremities, although more in the right leg. On testing of muscular strength, weakness was observed in her right leg. All stretch reflexes were brisk bilaterally with sustained clonus at both ankles and wrists. The plantar responses were extensor. Her sensory examination revealed a reduction in pallesthesia and in the sense of motion and position in the left leg. Her cerebrospinal fluid sample was acellular, with a protein level of 0.39 g/L and a glucose level of 2.7 mmol/L (49 mg/dL) and no oligoclonal bands.

Magnetic resonance imaging with T2-weighted techniques showed abnormalities in the periventricular white matter of both hemispheres, more intense near the occipital horns, basal ganglia, and left cerebellar pedunculus (Figure 1). A computed tomographic scan revealed no abnormalities.

The GALC activity in different leukocyte samples varied from 0 to 0.07 nmol/h per milligram of protein (control mean, 4.2 nmol/h per milligram of protein). Cultured skin fibroblasts showed a GALC activity of 0.03 nmol/h per milligram of protein (control mean, 3.2 nmol/h per milligram of protein). These values are consistent with a diagnosis of GLD and do not differ from those found in samples from other patients, including infants, confirmed to have GLD. The affected brother was not examined; however, his GALC activity in leukocytes was 0.02 nmol/h per milligram of protein, confirming the diagnosis of GLD.

RESULTS

Genomic DNA was isolated from the cultured skin fibroblasts of our patient, and all exons and exon-intron boundaries were amplified by polymerase chain reaction and sequenced using methods previously described. A transversion of T to G at nucleotide position 1886 (counting from the A of the initiation codon) was the only change found. The patient was found to be homozygous for this change, consistent with the parental consanguinity. This nucleotide substitution changes codon 629 for leucine to one for arginine. The more common nucleotides, C and T, respectively, were present at the 2 polymorphic positions (502 and 1637). Changes from C to T at position 502 and T to C at position 1637 have effects on the measured GALC activity and seem to have higher association with other mutations in patients with GLD than in normal controls. To delineate the effects of T1886G change on GALC activity, we introduced this nucleotide change into the normal human GALC cDNA in the pcDNA3 expression vector (Invitrogen, Carlsbad, Calif), and measured the GALC activity produced in transfected COS-1 cells. As shown in Figure 2, expression of the GALC cDNA with the normal sequence (GALC/Nl) resulted in a 31-fold increase in GALC activity (20.2 nmol/h per milligram of protein compared with 0.65 nmol/h per milligram of protein in mock-transfected cells). Expression of a mutation found in an infant with GLD (T to C at position 860, S287F) resulted in no net GALC activity (0.45 nmol/h per milligram of protein). However, expression of the mutation found in this patient resulted in a small, but significant, increase in GALC activity (1.45 nmol/h per milligram of protein). The transfection was repeated 3 separate times, with the same result. This rise in GALC activity is about 7% of the increase in activity produced by the normal sequence, and it could explain the relatively mild phenotype in our patient and her brother.
As late-onset GLD,25 other cases demonstrate a distinctive phenotypic pattern, which may include a slow downhill course, including periods of stability, sometimes lasting for years.3-17 Although the adult form of GLD is relatively rare, an increasing number of cases with survival to adulthood have been reported recently.11-15 One of us (D.A.W.) has diagnosed more than 20 cases of confirmed GLD in which the patient has survived past 10 years of age. A brief review of some cases was presented by Verdru et al.8 Some of these adult patients with GLD became symptomatic in late childhood, and these cases are usually classified as late-onset GLD.25 Other cases demonstrate a distinctive phenotypic pattern, which may include a slow downhill course, including periods of stability, sometimes lasting for years.3-17

To correlate the prolonged survival rate and common phenotypic pattern of adult GLD to the nature and location of the disease-causing mutations, we examined all the mutations delineated to late-onset or adult cases of GLD. All mutations responsible for the late-onset or adult form of GLD are simple mismatched mutations mainly located at the 5-prime end of the gene, whereas most mismatched mutations responsible for the infantile form of the disease are concentrated at the 3-prime end of the gene.13,26 Twelve of 14 mutations (G147C, A198G, G188A, G283A, A286G, A301T, A512T, G643A, T701C, G802A, A809G, A893G, T1853C, and T1886G) reported to be responsible for late-onset or adult GLD are located in the 50-kd subunit.12-15,17,26 One previously reported mutation (T1853C)15 and the novel mutation described herein (T1886G) are located on 30-kd subunit (exons 16 and 17, respectively). The T1853C mutation was found in association with 2 polymorphic changes (T1637C and A865G), while no polymorphic changes were found associated with the T1886G mutation. Expression of the T1886G mutation demonstrated a small amount of residual activity, which could explain the protracted clinical course in the proband and her brother. There is a similarity between the distribution of the mutations responsible for late-onset GLD and the distribution of the mutations known as polymorphic changes. Three of 4 reported polymorphic changes (C502T, G694A, and A865G)17,26 are located in the 50-kd subunit, and T1637C is the only polymorphic change found in the 30-kd subunit.26 All 4 polymorphic changes have been demonstrated to decrease the in vitro expression of GALK alone or in combination, and are thought to be partially responsible for the low GALK activity seen in pseudodeficient people.12,13 Pseudodeficiency is defined as the in vitro measurement of low enzymatic activity (sometimes under 10% of the normal mean for controls) in a healthy or non-GLD individual.18 It probably results from inheriting multiple copies of the polymorphic changes mentioned above.

The T1886G mutation is the most 3-prime located missense mutation reported to date. The siblings described herein are homozygous for this mutation. It changes the leucine located at 629 for the arginine. This substitution is just 39 amino acids before the end of the 699-amino acid–long polypeptide. The small, but significant, residual activity seen in the in vitro expression of the GALK cDNA with this mutation may result from the nature of the mutation and its extreme 3-prime location. Fundamental explanation regarding the age at onset for patients with GLD and the pattern in which the symptoms develop must be related to the level of residual GALK activity in situ.

As the patients with adult GLD present a slow-progressing clinical condition, staying free of dementia for many years, they could be seen as potential candidates for any kind of therapeutic trials. The symptoms, including spastic hemiparesis, bilateral pyramidal syndrome, optic atrophy, dystonia, and dementia, and the neuroradiological findings suggestive of white matter lesions must lead us to the measurement of GALK activity in leukocytes or cultured skin fibroblasts. The young adult or adult patient who receives an early diagnosis may have the opportunity for therapeutic trials, such as bone marrow transplantation or gene therapy.

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