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Krabbe disease: Clinical, biochemical and molecular information on six new patients and successful retrospective diagnosis using stored newborn screening cards

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ABSTRACT

Purpose: To present clinical, biochemical and molecular information on six new clinically diagnosed Krabbe disease patients and assess the sensitivity of retrospective galactocerebrosidase measurement in their newborn screening samples.
Methods: Medical records were reviewed. Galactocerebrosidase activity was measured in leukocytes and, retrospectively, in the patients' newborn screening cards (stored for 1.4 to 13.5 years). *GALC* gene mutation analysis was performed.
Results: Five patients with Krabbe disease, one of whom also had hydrocephalus, became symptomatic during infancy. A sixth patient presented with seizures and developmental regression at age two and had a protracted disease course. Galactocerebrosidase activity in leukocytes ranged from 0.00 to 0.20 nmol/h/mg protein. Low galactocerebrosidase activity (range: 3.2% to 11.1% of the daily mean), consistent with Krabbe disease, was detected in each of the newborn screening samples. *GALC* molecular analysis identified six previously unreported mutations and two novel sequence variants.
Conclusion: Our cases highlight the clinical variability of Krabbe disease. Galactocerebrosidase activity in newborn dried blood spots is a highly sensitive test, even when samples have been stored for many years. The high frequency of private mutations in the *GALC* gene may limit the use of genetic information for making treatment decisions in the newborn period.

1. Introduction

Krabbe disease (OMIM #245200) or globoid-cell leukodystrophy is a severe neurodegenerative disorder caused by deficiency of galactocerebrosidase (GALC), a lysosomal enzyme responsible for the degradation of certain galactolipids found in myelin [1–4]. Early infantile Krabbe disease presents with sudden onset irritability, startle response to stimuli and developmental arrest between three and six months of age in a previously healthy infant [5,6]. Over the course of several months, affected infants develop hypertonicity, seizures and blindness and most do not survive past their second birthday [7]. Feeding difficulties and aspiration pneumonia are frequent complications. Krabbe disease may present later in infancy, in childhood, or even adulthood. Children with late infantile Krabbe disease present after 6 months of age with symptoms similar to the early infantile form, followed by rapid disease progression and death within two years [8]. Juvenile Krabbe disease usually presents acutely with ataxia or spastic paresis between three and eight years of age, followed by a slowly progressive disease course [8–13]. Intellect, often intact at the onset of symptoms, may deteriorate or remain stable for years to decades after symptoms begin. A high incidence of late-onset Krabbe disease has been reported in Southern Italy [13].

The diagnosis of Krabbe disease may be aided by neuroimaging, nerve conduction studies and/or spinal tap. Cerebral spinal fluid protein is increased [5]. Diffuse, symmetric cerebral atrophy and demyelination is evident by brain CT and/or MRI [7]. Hydrocephalus has been reported in only three cases [14–16]. Peripheral neuropathy, demonstrated by reduced nerve conduction velocities, is evident in all cases of early infantile Krabbe disease and some patients with later onset [17].

In symptomatic individuals, the diagnosis of Krabbe disease can be made by enzyme analysis of GALC activity in leukocytes or cultured skin fibroblasts. Affected patients usually have severely decreased activity levels ranging from 0 to 5% of the normal mean; there is no correlation, however, between residual enzyme activity and disease

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severity [4,18]. Leukocyte GALC enzyme activities of 8–20% in individuals without any of the classical presentations of Krabbe are inconclusive and require molecular confirmation.

The cDNA encoding *GALC* was cloned by Chen et al. in 1993, permitting molecular analysis and investigation of possible genotype–phenotype correlations [19]. Among early infantile patients, a common 30-kb deletion beginning in intron 10 and continuing beyond the end of the gene (c.502T/del) occurs with an allele frequency of 40–45% in patients of Northern European descent and 35% in patients from Mexico and South America with Spanish ancestry [4,18]. The frequency of this deletion is highest (75% of mutant alleles) among patients from Sweden, where it likely originated [7]. Homozygosity for the 30-kb deletion or compound heterozygosity for this deletion and another severe mutation is always associated with early infantile Krabbe disease [18]. Two point mutations, c.1538C>T (p.T513M) and c.1652A>C (p.Y551S), make up another 10–15% of mutant alleles among infantile patients of Northern European descent [7,18]. A substitution of aspartic acid for glycine at position 270 (c.809G>A) has only been found in cases of late-onset, although clinically variable, disease, regardless of the second mutation [4].

Treatment for Krabbe disease is currently limited to pre-symptomatic hematopoetic stem cell transplantation (HSCT) [20,21]. With the promise of pre-symptomatic HSCT and the advent of assays to detect lysosomal enzyme deficiencies in newborn dried blood spots, newborn screening programs began to consider the addition of Krabbe disease [22,23]. In 2006, New York became the first state to screen for the disorder, using measurement of GALC enzyme activity in dried newborn blood spots, followed by enzyme activity in leukocytes/molecular analysis, when indicated. Although diagnosis of the disease in the newborn period can be achieved, significant challenges remain to select patients who will benefit from HSCT and to predict post-transplant neurodevelopmental outcome [24–28]. While further investigation into the long term outcome of pre-symptomatic HSCT for Krabbe disease is needed, other benefits of newborn screening for the disorder are apparent. These include avoidance of diagnostic delay and the recognition of couples at risk who may benefit from available reproductive options. Recently, the state of Illinois also began screening for Krabbe disease. It is likely that other programs will do so in the future.

The sensitivity of GALC enzyme analysis in newborn screening samples obtained from known affected individuals, however, has not been validated. If this is a reliable screening test for Krabbe disease, one would expect to identify many pre-symptomatic infants and, therefore, correlations between genotype and clinical outcome are needed.

The purpose of this report is to present clinical, biochemical and genetic information on six patients with Krabbe disease not previously described in the literature, as well as to assess the sensitivity of GALC enzyme analysis in newborn dried blood spots obtained from affected individuals.

2. Materials and methods

2.1. Patients

Between 2003 and 2008, five patients were diagnosed with Krabbe disease by the Metabolic Division at CHOC Children's (Orange, California). An additional patient with late onset disease and prolonged survival was referred to the Division during this time.

2.2. Enzyme analysis

Analysis of GALC activity in leukocytes was performed on all patients and on the parents of patients 1, 2 and 5 at the laboratory of David A. Wenger, PhD, (Lysosomal Diseases Testing Laboratory, Jefferson Medical College, Philadelphia, PA) using the radiolabeled natural substrate galactosylceramide. Results were compared with that laboratory's reference ranges.

2.3. Enzyme analysis of newborn dried blood spots

Dried blood spots were obtained on all patients for routine newborn screening. The remainder of each of the specimens was stored at −20° with desiccant, and, after parental consent was obtained, shipped to the Krabbe Laboratory at the New York State Department of Health (Albany, New York) for retrospective measurement of GALC activity. Thirty control samples (5 for each patient) obtained at the same time and stored in the same manner were also included. The laboratory was blind regarding the sample identity (patient or control). A result is considered abnormal by the New York State Newborn Screening Program when the GALC activity is <20% of the daily mean. Normally, the New York State protocol calls for re-testing of all abnormal results in duplicate, followed by molecular analysis of samples with activity ≤12% of the daily mean. As this study was performed retrospectively on a limited amount of sample from each patient, enzymatic analysis was performed only once, followed by molecular testing.

2.4. Mutation analysis

Whole blood or isolated DNA from patients 2 to 6 and from the parents of patient 1 was sent to the Neurogenetics Laboratory at New York University (New York City, New York) for *GALC* mutation analysis. Results were confirmed in the Krabbe Laboratory at the New York State Department of Health using DNA isolated from the newborn dried blood spots of all six patients. Polymerase chain reaction and agarose gel electrophoresis are used by both laboratories to detect the common 30-kb deletion and, by New York State, to detect a recurrent 7.4-kb deletion. Prior to sequencing, the New York State lab employed a LightCycler real-time polymerase chain reaction assay (Roche Applied Science, Indianapolis, IN), to test for three common *GALC* polymorphisms known to attenuate enzyme activity (p.I546T, p.R168C, and p.D232N) and three common point mutations (p.T513M, p.Y551S, and c.1424delA). Both laboratories performed semiautomated bidirectional DNA sequencing on all 17 exons and two promoter regions of the *GALC* gene.

3. Results

3.1. Clinical presentation

3.1.1. Patient 1

Patient 1, the second child born to his parents, was delivered at 37 weeks gestation by emergency cesarean section due to decreased fetal movements. A nuchal cord was noted at birth; however, the patient had good Apgar scores and no perinatal complications. From birth, he was noted to have difficulty with breast feeding and taking formula. Weight and length were below the 5th centile, but followed a parallel curve. Early developmental milestones were normal. At 14 months, a clumsy, unsteady gait was noted. Neurological examination demonstrated lower extremity hypertonia and scissoring. EEG and MRI of the brain were reportedly normal. Occupational and physical therapy were initiated, however, over the following weeks, he stopped walking and lost the ability to sit up or hold up his head. He remained alert and interactive. At 21 months, the patient was admitted for decreased PO intake, swallowing difficulties and concerns for aspiration. Review of systems was significant for two episodes of pneumonia, treated as an outpatient. Physical examination revealed relative macrocephaly (weight and length <3rd centile and head circumference at the 25th centile), irritability, hypertonia and increased deep tendon reflexes. MRI of the brain demonstrated markedly abnormal T2 prolongation in the periventricular and subcortical