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KRABBE DISEASE

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CONTENTS

ABSTRACT

Krabbe disease is an inherited demyelinating disease caused by a deficiency of the lysosomal enzyme galactocerebrosidase (EC 3.2.1.46). The gene encoding the enzyme is GALC mapped on chromosome 14q31. Clinical phenotypes were recently divided into two main types: infantile form and late-onset form. Patients with the infantile form typically manifest visual impairment, spasticity and regression of development within the first 6 months of life. The disease should be considered during differential diagnosis of patients who show leukodystrophy on brain magnetic resonance imaging. Treatment is limited but hematopoietic transplantation has proven effective if performed in the early stages of the disease. Enzyme replacement treatment and some pharmaceutical agents are also under consideration. The clinical phenotype, molecular diagnosis and possible future treatment of Krabbe disease are reviewed.

INTRODUCTION

Krabbe disease, an inherited, progressive, demyelinating disease, was first described by the Danish neurologist Knud Haraldsen Krabbe in 1916 in a paper entitled 'A new familial, infantile form of diffuse brain-sclerosis' (1). In 1970 Suzuki and Suzuki established a biochemical diagnosis, having detected a galactocerebrosidase (GALC) deficiency in a patient's lymphocytes and fibroblasts (2). The causative gene *GALC* was identified in 1993 by Chen et al. (3) and our group was the first to report a gene mutation in a patient in 1994 (4). A mutation in the activator protein of GALC, saposin A (SAP-A), reportedly leads to infantile-type Krabbe disease (5). Hematopoietic stem cell transplantation (HSCT) for Krabbe disease was reported by Krivit et al. in 1998 (6).

EPIDEMIOLOGY AND DIAGNOSIS

Krabbe disease occurs in approximately 1 in 100,000 births in the USA and Europe and in 1 in 50,000 births in Scandinavian countries. A higher prevalence of about 1 in 6,000 has been reported in some Arab communities (7). Clinical diagnosis is suggested by disease history and neurological examination including tendon reflex and pyramidal signs. Magnetic resonance imaging, especially the FLAIR method, diffusion and magnetic resonance spectrometry, is essential to detect leukodystrophy in the central nervous system (CNS). Motor nerve conduction velocities, auditory brainstem responses and visual evoked potentials are useful for detecting peripheral nervous system demyelination. Cerebrospinal fluid (CSF) protein concentration is increased in most patients. Enzymatic diagnosis can be performed using peripheral lymphocytes or skin fibroblasts, but only by specialized institutes or companies. Residual enzyme activity may be slightly higher in patients with late-onset Krabbe disease than in patients with typical infantile Krabbe disease. Several polymorphisms affect enzyme activity and make diagnosis difficult. People who have very low GALC enzyme activity but are asymptomatic are called pseudodeficient, and those who have homozygous pseudodeficiency do not show the Krabbe phenotype, which makes the disease difficult to diagnosis correctly. Mutation analysis of the *GALC* gene with the DNA sequence for all exons detects more than 90% of mutated alleles. For some patients with a special mutation set, the phenotype might be speculated. There are a few reports of patients who are not deficient in GALC but in SAP-A. These patients have low activity in lymphocytes but higher activity in skin fibroblasts (5).

A report on a new method of screening newborns for lysosomal diseases has recently been published (8) and a pilot study was performed in New York State in 2006 (9). Several infants were identified as candidates for Krabbe disease and those children positive for the infantile form underwent HSCT (10).

GENOMICS AND PATHOGENESIS

Krabbe disease is caused by mutations in the *GALC* gene encoding the GALC enzyme. The *GALC* gene is 57-kb long with 17 exons coding 669 amino acids (11) and is mapped on chromosome 14q31. Several mutations have been reported; these mutations differ according to ethnicity. In individuals of European ancestry, a 30-kb deletion is the most frequent mutation with 40 - 50% allele frequency (12). Patients with this homozygous mutation present with infantile-type disease, while other mutations such as G270D and L629R

are considered to contribute to the late-onset phenotype. There are also some genotype-phenotype correlations in Japanese patients (13). Recently, an activator protein for GALC, SAP-A, was reported as the second causative gene of Krabbe disease (5,14). From the genetic counseling point of view, it is important to diagnose the proband and the carrier state of the parents. If both parents are carriers, each child is affected in 25% of cases, is an asymptomatic carrier in 50% of cases and is unaffected and not a carrier in 25% of cases. Patient and prenatal diagnoses are possible by enzyme assay using lymphocytes, skin fibroblasts or amniotic cells. However, carrier detection using an enzyme assay can be difficult because the normal range of enzyme activity overlaps with that of carrier patients. When a family member is pseudodeficient, genetic diagnosis is only possible for the patient or carrier.

Krabbe disease involves demyelination, the destruction of established myelin, in the central and peripheral nervous systems. However, in the infantile form of Krabbe disease, the phenotype begins within 6 months after birth, during development of myelination: the pathogenesis therefore involves dysmyelination, ie, impairment of normal development of myelin. Myelin-forming cells, oligodendrocytes in the CNS and Schwann cells in the peripheral nervous system, are affected by the accumulation of psychosine (galactosyl sphingosine), which is cytotoxic (15). The mechanism of psychosine toxicity involves energy depletion, loss of oligodendrocytes, induction of gliosis and inflammation by astrocytes in the CNS. The lysosomal enzyme GALC hydrolyzes galactocerebroside, a major substrate in myelin, lactosylceramide and psychosine. Oligodendrocytes are damaged by accumulated psychosine and undergo apoptosis (16). Monocyte/macrophage lineage cells in the CNS are microglial cells that are damaged by psychosine and become multinuclear cells (globoid cells) via the psychosine receptor (17). Pathologically, the existence of globoid cells in the CNS is characteristic of Krabbe disease. In the peripheral nervous system, Schwann cells proliferate and are damaged by psychosine, leading to peripheral nerve demyelination, which is difficult to ameliorate even with HSCT (18). Recently, it was reported that psychosine is localized at lipid rafts, destroys their architecture and deregulates signal activity, leading to demyelination (19).

CLINICAL FEATURES (PHENOTYPE)

Krabbe disease has two main clinical phenotypes: the infantile form (disease onset within the first 6 months of life) and the late-onset form (disease onset after 6 months). Late-onset Krabbe disease is further subdivided into three groups: the late-infantile form (disease onset at 6 months to 3 years); the juvenile form (disease onset at 3 to 8 years); and the adult form (disease onset after 8 years of age).

Approximately 85 - 90% of Krabbe disease patients have the infantile form, presenting with extreme irritability, spasticity and delayed developmental milestones before the age of 6 months. The patients suffer from frequent respiratory infections and apneic seizures leading to respiratory failure. Their average life span is about 1 - 3 years. This phenotype develops in three stages as follows (20):

Stage I: This stage is characterized by irritability, stiffness, arrest of motor and mental development and failure of temperature control. The child appears normal for the first several months after birth and then becomes hypersensitive to auditory or visual stimuli. Feeding problems and arrested weight gain occur frequently and seizures are possible. CSF protein concentration is already increased at this stage.

Stage II: This stage is characterized by rapid deterioration of motor and mental development. The child shows marked hypertonicity and hyperactive tendon reflexes while their reaction to light is sluggish. Peripheral neuropathy is not clear compared with CNS deterioration.

Stage III: The child does not respond to light or sound stimuli. There is no voluntary movement and no response to his or her environment.

The late-onset form is heterogeneous and the first symptom differs between patients. This phenotype has a slower progression than the infantile form. Late infantile patients show irritability, stiffness, ataxia, psychomotor regression and loss of vision as first symptoms. In the juvenile type, patients develop loss of vision, ataxia and psychomotor regression; a rapid deterioration period is followed by a gradual progression phase. Patients who manifest their first symptoms later than 8 years of age develop spastic paralysis, intention tremor or vision loss slowly, at the latest in their 60s.

TREATMENT

Hematopoietic stem cell transplantation (HSCT)

HSCT was first used to treat Hurler syndrome, a metabolic disease, in 1981. After this successful trial, it has been used in many other metabolic diseases, including metachromatic leukodystrophy, adrenoleukodystrophy, mannosidosis and Krabbe disease. Krivit et al. (6) reported the effect of HSCT in patients with late-onset Krabbe disease in 1998 and Escolar et al. (21) described the use of HSCT to treat infantile Krabbe disease in 2005. When HSCT was performed in presymptomatic infants before 2 months of age, central myelination progressed and the patients' motor and mental development continued as normal. The clinical effect of HSCT is clear even for adrenoleukodystrophy; however, it should be used only in presymptomatic infants (22). This means that disease diagnosis before onset is essential for HSCT, which is possible only for the younger siblings of affected patients or for infants tested using newborn screening.

Enzyme replacement therapy

The effects of enzyme replacement therapy were investigated in the twitcher mouse model of Krabbe disease; an early improvement in motor performance and increase in lifespan were noted (23). The effect was more pronounced if the enzyme was administered intracerebroventricularly (24). An enzyme replacement therapy for Krabbe disease, HGT-2610, is being developed by Shire Pharmaceuticals and is currently in preclinical studies (25).

Cellular transplantation

Transferring mouse neural progenitor cells that had been genetically modified to produce increased GALC activity into twitcher mice resulted in remyelination and a reduction in pathology. There was also a limited increase in lifespan and body weight (26).

Gene therapy

CNS-directed gene transfer might be effective if the vector is safe and expression is stable. There have been several trials to transfer the *GALC* gene into the CNS in murine models (27,28).

Pharmacological treatment

Krabbe disease is often fatal; however, several pharmacological treatments based on the molecular pathology of the disease have been investigated.

L-cycloserine, an inhibitor of 3-ketodihydrosphingosine synthase, reduced levels of the cytotoxic substrate psychosine and increased lifespan by 45% in twitcher mice that had been genetically modified to represent late-onset Krabbe disease (29).

AICAR, 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide, a pharmacological activator of 5'-adenosine monophosphate-activated protein kinase (AMPK), attenuated psychosine-mediated down-regulation of AMPK and restored altered lipid biosynthesis in oligodendrocytes and astrocytes. AICAR targets psychosine toxicity by changing the cellular energy switch in these cell types. This might modify the course of the disease when combined with another treatment (30).

Prostaglandin D2 is known to be a mediator of inflammation and has been proven to mediate the microglia/astrocyte interaction, leading to demyelination in twitcher mice. HQL-79, 4-(benzhydryloxy)-1-[3-(1*H*-tetrazol-5-yl)propyl]piperidine, is an inhibitor of hematopoietic prostaglandin D2 synthase that reduced oligodendroglial apoptosis and suppressed astrogliosis and demyelination in twitcher mice (31).

CONCLUSIONS

Krabbe disease is a rare leukodystrophy that is diagnosed by enzyme assay. The disease progresses very rapidly in patients presenting with the typical infantile-type phenotype. HSCT is the only established effective treatment for asymptomatic patients or those at a very early stage of the disease. Children with the infantile form of the disease who receive HSCT before onset of symptoms develop more normally than untreated patients. Rapid diagnosis after birth is essential and so family history of the disease or a newborn screening system is consequently important. Enzyme replacement therapy and other pharmacological agents are being developed as supportive therapy for the disease. Future research on the details of pathogenesis and the development of enzyme replacement and gene therapies are expected.

DISCLOSURE

The author states no conflict of interest.

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