[Frontiers in Bioscience 5, d307-311, March 1, 2000]

SPONGY DEGENERATION OF THE BRAIN, CANAVAN DISEASE: BIOCHEMICAL AND MOLECULAR FINDINGS

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1. ABSTRACT

Canavan disease is a severe progressive leukodystrophy characterized by swelling and spongy degeneration of the white matter of the brain. It is an autosomal recessive disease found more frequently among Ashkenazi Jews. The clinical features are those of severe mental retardation with inability to gain developmental milestones. Hypotonia, head lag and macrocephaly are characteristic of Canavan disease and become apparent after 5-6 months of age. Massive excretion in the urine of N-acetylaspartic acid is the biochemical marker for Canavan disease, which is caused by deficiency of the enzyme aspartoacylase. This discovery allowed for accurate diagnosis of Canavan disease, while prior to that, a brain biopsy was needed. The gene for aspartoacylase has been cloned and two mutations predominate among Ashkenazi Jewish individuals with Canavan disease and account for more than 98% of the Ashkenazi Jewish patients. The mutations among other ethnic groups are more diverse. The carrier frequency for the two common mutations among Ashkenazi Jews was found to be surprisingly high, 1:37. Screening for carriers is now common practice for this population. A knock-out mouse for Canavan disease is being genetically engineered in our laboratory. The mouse model will allow for development of strategies for gene therapy.

2. INTRODUCTION

Spongy degeneration of white matter of the brain was described in 1931 by Canavan (1). The description of spongy degeneration of the brain by Canavan was thought to represent Schilder's disease in a child. An earlier report in 1928, by Globus and Strauss, described similar brain pathology and was also thought to be a case of Schilder's disease (2). Spongy degeneration of the brain came to be recognized as a specific entity in 1949 by van Bogaert and Bertrand, who described three Jewish children with spongy degeneration of the brain (3). Since then, numerous cases have been reported, with prevalence among Jewish individuals (4). Although the recognition of spongy degeneration of the brain as a specific genetic disease was by van Bogaert and Bertrand, the description by Canavan dominated the medical literature and the disease is commonly referred to as Canavan disease. The enzyme defect of Canavan disease was identified by Matalon *et al.* in 1988 (5). Aspartoacylase deficiency leads to increased urinary excretion of N-acetylaspartic acid (NAA), making the diagnosis of Canavan disease easy to ascertain. The gene for aspartoacylase was cloned in 1993, and mutations causing Canavan disease were identified (6). Interestingly, only two mutations are the basis for Canavan disease among 98% of individuals of Ashkenazi Jewish ancestry, while the molecular basis of Canavan disease among non-Jewish individuals is caused by a wide range of mutations. Therefore, because of the limited number of mutations in Jews, screening programs to identify carriers can be performed in the Ashkenazi Jewish population (7).

3. CLINICAL PRESENTATION

Infants with Canavan disease appear normal in the first few months of life, although careful examination should reveal mild delays, hypotonia and inadequate visual tracking. They become increasingly irritable, remain hypotonic, and have poor head control. Head lag is a constant finding in Canavan disease and is often detected between 3-6 months of age. Developmental milestones are not attained and the head gets progressively larger, especially after the sixth month of life. The triad of hypotonia, head lag and megalencephaly should suggest

Figure 1. A 23 month old girl with Canavan disease with macrocephaly. The head needs to be supported. She is homozygous for the mutation 285 Glu>Ala.

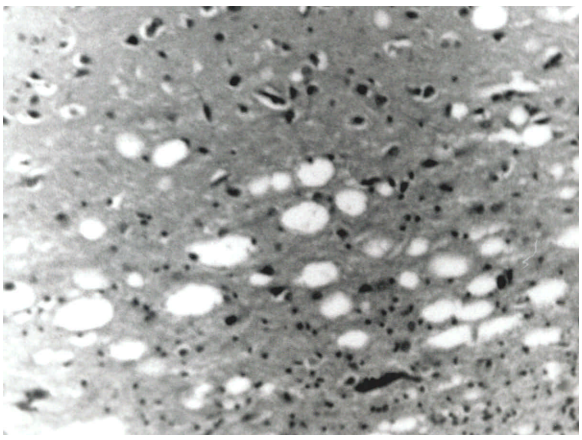

Figure 2. Subcortical spongy changes of the white matter. The cortex above is spared. The dark stained neurons are not affected.

Canavan disease, when white matter involvement is suspected. A 23 month old girl showing the characteristic large head, and inability to support her head is shown in figure 1.

As children with Canavan disease become older, the delay in development becomes obvious, especially in motor and verbal skills. These children are able to interact, laugh, smile, reach for objects and lift their head when in the prone position. However, they are unable to attain the skills for sitting, standing, walking or talking. As they get older the hypotonia gives way to spasticity, similar to cerebral palsy and in fact, some children with Canavan disease have carried the diagnosis of cerebral palsy. After the first or second year of life children with Canavan disease often are irritable and develop feeding difficulties, and sleep disturbances. As feeding difficulties increase assisted feeding by a nasogastric tube or permanent gastrostomy may be needed. Children with Canavan disease develop optic atrophy, however, they are not blind and can follow objects with their eyes. Some children with Canavan disease develop seizures, although seizures are not a common feature of Canavan disease. Life span for patients with Canavan disease has gotten longer due to improved medical and nursing care. Many such children live beyond the first decade of life.

4. PATHOPHYSIOLOGY

Canavan disease is caused by a deficiency of the enzyme aspartoacylase, which hydrolyzes NAA to aspartate and acetate. Aspartoacylase is abundant in the white matter of the brain and deficiency of this enzyme leads to accumulation of NAA. The increased levels of NAA lead to “swelling” or sponginess of the brain and disruption of the white matter. Severe neurological problems found in Canavan disease are the result of the derangement of the normal metabolism of NAA (1-4).

N-Acetylaspartic acid discovered in mammalian brain in 1956 is second to glutamic acid in its abundance in mammalian brain (8). The level of NAA in human brain is 8 mmol/gm tissue. In spite of this high concentration, the role of NAA in normal brain remains an enigma (9). The discovery of the enzyme defect in Canavan disease suggests that the normal metabolism of NAA is important in the maintenance of healthy white matter (5). Although NAA synthesis occurs in the gray matter, aspartoacylase is found mainly in the white matter (10,11). The different localization of the enzyme and substrate suggests chemical compartmentation of the enzyme and substrate. It has been suggested that NAA may act as an Osmolite, and its lack of hydrolysis leads to water accumulation in brain cells (12). As Canavan disease progresses, the brain becomes atrophic, and the gray matter becomes involved as well. Microscopy shows spongy degeneration throughout the white matter and in the subcortical regions as well. Figure 2 shows swollen astrocytes from the brain of a child with Canavan disease, while the neurons are spared. Electron microscopy shows distorted and elongated mitochondria (13-16).

5. DIAGNOSIS

Computed Tomography (CT) scan of the head or Magnetic Resonance Imaging (MRI) of the brain reveal diffuse white matter degeneration in Canavan disease (17-19). The involvement is primarily in the cerebral hemispheres with less involvement in the cerebellum and brain stem. The MRI or CT scan may be

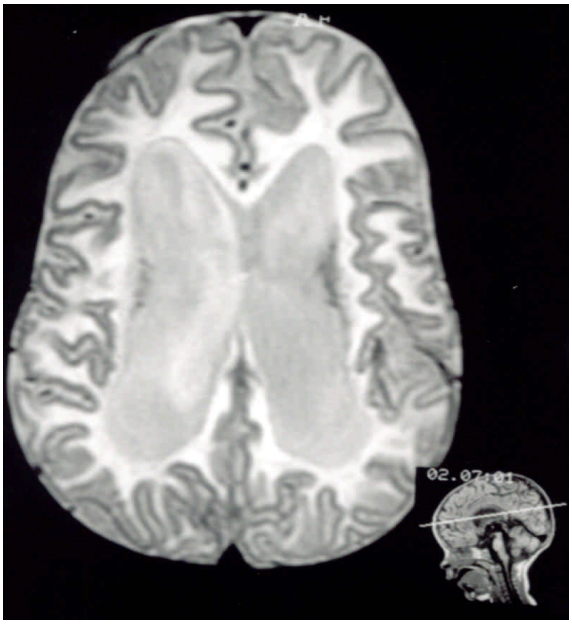

Figure 3. A MRI of the brain of a two-year-old girl with Canavan disease showing advanced demyelination with diffuse white matter changes. Demyelination also involves the entire subcortical white matter.

interpreted as normal early in life, therefore, follow-up evaluations are indicated when Canavan disease is suspected (19). Figure 3 shows the MRI of a 2-year-old girl with Canavan disease, which reveals severe white matter degeneration. Nuclear magnetic resonance (NMR) spectroscopy of the Canavan brain has revealed an increased level of NAA in comparison to normal brain (19-21). This technique can be helpful in the initial work up of Canavan disease.

The specific tests that lead to the diagnosis of Canavan disease are biochemical. Levels of urine NAA are elevated greatly, often more than 100 times the normal urinary levels. There are cases with leukodystrophy with slight increase (4- to 6-fold) urine NAA and these patients may be confused as having Canavan disease. Cultured skin fibroblasts manifest the enzyme deficiency. Enzyme determination may be difficult, and it is not needed since NAA is diagnostic of Canavan disease. Blood is not helpful for enzyme determination. N-Acetylaspartic acid is also elevated in blood and in CSF, but these tests are not required for diagnosis. Brain biopsy is no longer needed to confirm the diagnosis of Canavan disease.

6. DIFFERENTIAL DIAGNOSIS

The macrocephaly, characteristic of Canavan disease, can be found in Alexander disease, Tay-Sachs and other neurodegenerative diseases. 3-OH Glutaric acidemia also leads to macrocephaly and white matters involvement (22). An autosomal recessive form of megalencephaly with vacuolating leukoencephalopathy and a rather mild clinical course has been reported. This is a distinct entity and seems to be common in India (23-25). Autosomal dominant megalencephaly can also be confused with Canavan disease. These individuals are only slightly mentally impaired. Attenuation of the white matter on MRI or CT scans should exclude Tay-Sachs disease, particularly when it is universal. The U fibers are typically involved with Canavan disease. Spongy degeneration of the brain can also occur with viral infections, mitochondrial diseases and other metabolic diseases (26-31). The unique feature of Canavan disease is the increased urinary excretion of NAA (31).

7. THE MOLECULAR LESION

The molecular basis for Canavan disease is caused by mutations in the gene coding for aspartoacylase resulting in a deficient enzyme. This gene has been cloned and localized on the short arm of chromosome 17 (17p13-ter) (6,32). The cDNA for aspartoacylase spans approximately 1,500 bases and is comprised of 6 exons with 5 intervening introns. The cDNA predicts a protein with 313 amino acids for aspartoacylase in man. The mouse cDNA for aspartoacylase codes for a protein with 312 amino acids and the gene is localized on the long arm of chromosome 11 which is syntenic to the short arm of chromosome 17 in man (32). The gene for aspartoacylase has been conserved throughout evolution (32).

Canavan disease is pan-ethnic. It is more prevalent among Ashkenazi Jews of Eastern European extraction. Mutations leading to Canavan disease have been located on all 6 exons. There are two specific mutations common among Jews. The predominant mutation among Jewish individuals is a missense mutation (Glu285Ala) with substitution of glutamic acid to alanine. A nonsense mutation where the codon for tyrosine is substituted by a termination codon (Tyr231X), is also prevalent among Jews (Table 1) (33). Screening of healthy Jews reveals that 1/37-1/40 is a carrier of one of these two mutations which is a high incidence (34-35). Now screening for carriers of Canavan disease among healthy Jewish individuals is done routinely for prevention purposes.

In non-Jewish patients the mutations are different and more diverse. The most common Canavan mutation (about 35%) in non-Jewish patients is Ala305Glu which is a missense mutation substituting alanine to glutamic acid (Table 1) (33). Many of the other mutations in non-Jewish patients often occur only in one family or in few patients (Table 2) (36-42).

8. TREATMENT

Therapy for Canavan disease at this time is symptomatic. Seizures need to be controlled. Children with Canavan disease may need nasogastric feedings or feeding gastrotomy. Gene therapy on two children with Canavan disease has been done (43). The trial seemed to have been ineffective, although, a detailed report is lacking. A trial with acetazolamide to reduce white matter water concentration and NAA was tried for a period of 5 months. Acetazolamide was helpful in reducing the intracranial

Table 1. Common Canavan Disease Mutations

Mutation	Type	Ethnic group
Glu285Ala	missense	Jewish
Tyr231X	nonsense	Jewish
Ala305Glu	missense	non-Jewish

Table 2. Less Common Canavan Disease. Mutations Among Non-Jewish Individuals

Mutations			
Cys152Arg	Gly123Glu	566del7	Met195Arg
876del4bp	Arg168Glu	527del6	Pro280Leu
32delT	Cys218X	527del108	Pro280Ser
Ile16Thr	Phe295Ser	Ile143Thr	Ala287Thr
Gly27Arg	Gly274Arg	Tyr109X	245insA
Asp114Glu	827delGT	Pro183His	Tyr231Cys
870del4	Val186Phe	IVS4GT>TT	Test

pressure, but did not reduce water concentration or NAA levels (44).

9. PREVENTION

If both parents are carriers, the risk of an affected baby is 1:4. Carrier determination and preventive counseling can be attained using DNA analysis (45,46). The high carrier rate observed in the Ashkenazi Jewish population warrants screening, similar to the carrier screening programs for Tay-Sachs disease. Carrier testing for Canavan disease however, requires DNA analysis since the enzyme is not detectable in the blood. In a couple with informative DNA, prenatal diagnosis could be offered using DNA analysis. Other methods of prenatal diagnosis include determination of NAA in amniotic fluid, which should be increased in an affected pregnancy (47,48).

10. ANIMAL MODEL

A knock-out mouse for Canavan disease is being created. These mice should allow for the studies on the pathophysiology of Canavan disease and gene therapy.

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Key Words: N-acetylaspartic acid, aspartoacylase, Canavan disease, Macrocephaly, Spongy Degeneration Of The Brain, Review

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