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REVIEW ARTICLE

Canavan Disease: A Neurometabolic Disease Caused By Aspartoacylase Deficiency

Ute Lienhard and Jörn Oliver Sass

Abstract:
Canavan disease is a genetic neurodegenerative disorder caused by mutations in the ASPA gene encoding aspartoacylase, also known as aminoacylase 2. Important clinical features comprise progressive psychomotor delay, macrocephaly, muscular hypotonia as well as spasticity and visual impairment. Cerebral imaging usually reveals leukodystrophy. While it is often expected that patients with Canavan disease will die in childhood, there is increasing evidence for heterogeneity of the clinical phenotype. Aspartoacylase catalyzes the hydrolysis of N-acetylaspartate (NAA) to aspartate and acetate. Its deficiency leads to accumulation of NAA in the brain, blood, cerebrospinal fluid and in the urine of the patients. High levels of NAA in urine are detectable via the assessment of organic acids by gas chromatography - mass spectrometry. Confirmation is available by enzyme activity tests and mutation analyses. Up to now, treatment of patients with Canavan disease is only symptomatic. Although it is a panethnic disorder, information on affected individuals in populations of other than Ashkenazi Jewish origin is rather limited. Ongoing research aims at a better understanding of Canavan disease (and of related inborn errors of metabolism such as aminoacylase 1 deficiency). Unraveling underlying mechanisms may provide a basis for future therapeutic approaches.

Keywords: *Canavan disease, aspartoacylase, aminoacylase, leukodystrophy, neurodegeneration, organic acids, N-acetylaspartate, ASPA, ACYI, NAT8L*
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Introduction

Canavan disease is a childhood leukodystrophy which often leads to death in childhood. Its name goes back to Myrtelle M. Canavan who published a case report on a child with a very enlarged head, neurological symptoms such as nystagmus, and psychomotor retardation in 1931 [1]. In retrospect the first clinical description of the disorder was accredited to Globus and Strauss (1928) [2]. Bogaert and Bertrand defined it as a clinical entity [3].

Since the brain of patients with Canavan disease shows a spongy degeneration of the white matter [1,4] the disorder is also known as "spongy degeneration (van Bogaert and Bertrand type)" [5]. Canavan disease follows an autosomal recessive trait of inheritance [6] and is caused by mutations in the *ASPA* gene resulting in deficiency in aspartoacylase (aminoacylase 2) [7], which catalyzes the hydrolysis of *N*-acetylaspartate (NAA) [8]. (Figure 1)

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Figure 1: Aspartoacylase (ASPA; EC 3.5.1.15), also known as aminoacylase 2, catalyzes the hydrolysis of *N*-acetylaspartic acid.

While Canavan disease is particularly frequent among Ashkenazi Jews [3,5,9] it has been reported in many populations [10-14].

Clinical features

Children with Canavan disease are usually considered symptom-free in the first three months of life, although mild retardation, hypotonia and inadequate visual tracking may be detectable in an attentive examination [15]. Traeger and Rapin have reported that about a fifth of the patients present at birth with a poor suck, irritability and poor visual fixation [16].

Usually, the disease manifests not later than at the age of six months [6]. Key clinical features are developmental delay, head lag and macrocephaly [3,15-17]. The head circumference exceeded the 90th percentile in 54 of 59 children with Canavan disease reported by Traeger and Rapin [16].

Observation of the three signs macrocephaly, hypotonia and head lag should lead to suspicion of Canavan disease, especially if there is evidence for white matter involvement [15]. Affected individuals do not meet important developmental milestones. They do not acquire appropriate motor and verbal skills and do also lose abilities. Interaction is often further impaired by ophthalmological symptoms [16]. Optic atrophy, retinal degeneration and horizontal nystagmus are frequent findings in Canavan disease [3,4,15,16].

Early observed irritability usually increases with the progression of the disease [15]. Hypotonia and reduced motor activity are additional early recognizable features.

Later those features can change to spasticity [2,4,15]. The spasticity may resemble cerebral palsy. For this reason some children with Canavan disease have been diagnosed with cerebral palsy [15].

At the age of one or two years most Canavan patients develop sleep disturbances and feeding problems. Some children with Canavan disease suffer from seizures. In 38 of the 60 children included in the survey by Traeger and Rapin, epilepsy was ascertained at various ages, from birth to 15 years [16]. Others describe seizures in about half of the patients, mostly generalized tonic-clonic convulsions. As a reaction to a stimulus, tonic extensor spasms often occur with an excessively ophistotonic posture [4,6].

Common understanding of Canavan disease is that the life expectancy of children with Canavan disease is very short and that children with the classic course of the disease die mostly in the first three years of their life [6]. However, there are more and more patients who achieve an age of more than ten years. Matalon and Michals-Matalon have contributed this to better medical and nursing care [15], although there is still no curative therapy. Increased awareness of the disease and improved diagnostic possibilities deserve also consideration and may have led to more frequent diagnosis of mild manifestations which may previously have escaped investigations and statistics. Notably, early reports on Canavan disease often referred to individuals of Ashkenazi Jewish origin [3,5,17] whereas more recent publications underline the panethnic character of the disease [14,18–21].

Neuropathology

Formerly, pathologic changes in the brain of Canavan

patients could only be detected at autopsy and histopathological examination. In 1931, Myrtelle Canavan's autopsy findings were spongy degeneration of the white matter and an expansion of cerebral ventricles and aqueduct. Cerebrum as well as cerebellum were very soft and gelatinous [1]. In the histopathological examination of brains of affected children Bogaert and Bertrand found a widespread vacuolization change in both white and gray matter [3]. Others reported a marked elevation of the number of protoplasmic astrocytes and a diffuse loss of cortical neurons in Canavan disease [4].

In electron microscopic studies, vacuoles have been found within protoplasmic astrocytes. They are considered the main cause for the vacuoulated appearance of the white matter [5].

Radiology

Nowadays the pathologic changes in the brains of patients with Canavan disease can be visualized by cerebral imaging. Findings include leukodystrophic signs, lack of myelin, brain atrophy and increasing ventriculomegaly [20,22,23]. Affection of the basal ganglia is revealed in some cases [22–24]. On CT scans white matter changes can be demonstrated [23]. Reversal of signal intensities in T1- and T2-weighted MRI scans as well as elevated white matter T1 values are signs for pathologic changes in myelination [20,23,25].

Brismar *et al.* 1990 considered CT and MRI results nonspecific for Canavan disease [26]. They found no correlation of the abnormalities with the severity of the clinical presentation [26]. Proton magnetic resonance spectroscopy (^{1}H-MRS) is used to quantitate the levels of NAA in the brain of Canavan patients *in vivo* [20,24,25,27]. Mostly, elevated NAA concentrations are reported, if compared to the reference compounds choline or creatine [25,27]. The data of Janson *et al.* indicate that in Canavan patients NAA levels increase linearly as a function of age, with a frontal to occipital gradient. This parallels the progression of symptoms and white matter degeneration [20]. In some patients changes are also detectable by cranial ultrasound. Ventricular extension as well as elevated echogenicity of white matter and periventricular gray matter can then be detected [23].

Laboratory Investigations

Metabolite Studies

Following the discovery that patients with Canavan disease present with *N*-acetylaspartic aciduria, the determination of NAA levels in urine has become an important tool for the identification of individuals with this inborn error of metabolism [28–30]. NAA is one of the parameters which are assessed by the analysis of urinary organic acids using gas chromatography-mass spectrometry (Figure 2) [31]. Bal *et al.* have demonstrated that it is the L-enantiomer of NAA which accumulates in Canavan disease [32]. Elevated concentrations of NAA have also been demonstrated in plasma and cerebrospinal fluid of affected individuals [33,34]. However, diagnostic investigations in urine are more easily accomplished.

Aspartoacylase Activity

Since Canavan disease is due to aspartoacylase deficiency, enzyme assays have been developed which allow the confirmation of the diagnosis in cultured skin fibroblasts [28,35,36]. Matalon *et al.* reported that the aspartoacylase activity in cultured fibroblasts from heterozygous mutation carriers was diminished to about half of the activity detected in normal fibroblasts [37].

Mutation Analysis

The human *ASPA* gene encoding aspartoacylase was localized on the short arm of chromosome 17 (17p13-ter) [38]. It comprises six exons coding for 313 amino acids [7,38]. Obviously aspartoacylase was highly conserved during evolution [38].

The highest prevalence of Canavan disease has been reported for Ashkenazi Jews (carrier rate 1:37.7 to 1:57 [39–41]). Two mutations, a missense E285A mutation and a nonsense Y231X mutation, account for about 97% of the affected alleles in patients of Ashkenazi Jewish origin [10]. Genetic screening is possible among high-risk couples in which both partners have Ashkenazi Jewish background [42].

The most prevalent mutation among the non-Jewish population is the missense mutation A305E, in which an alanine residue is replaced by glutamic acid. In different surveys this mutation was found in 39.5% to

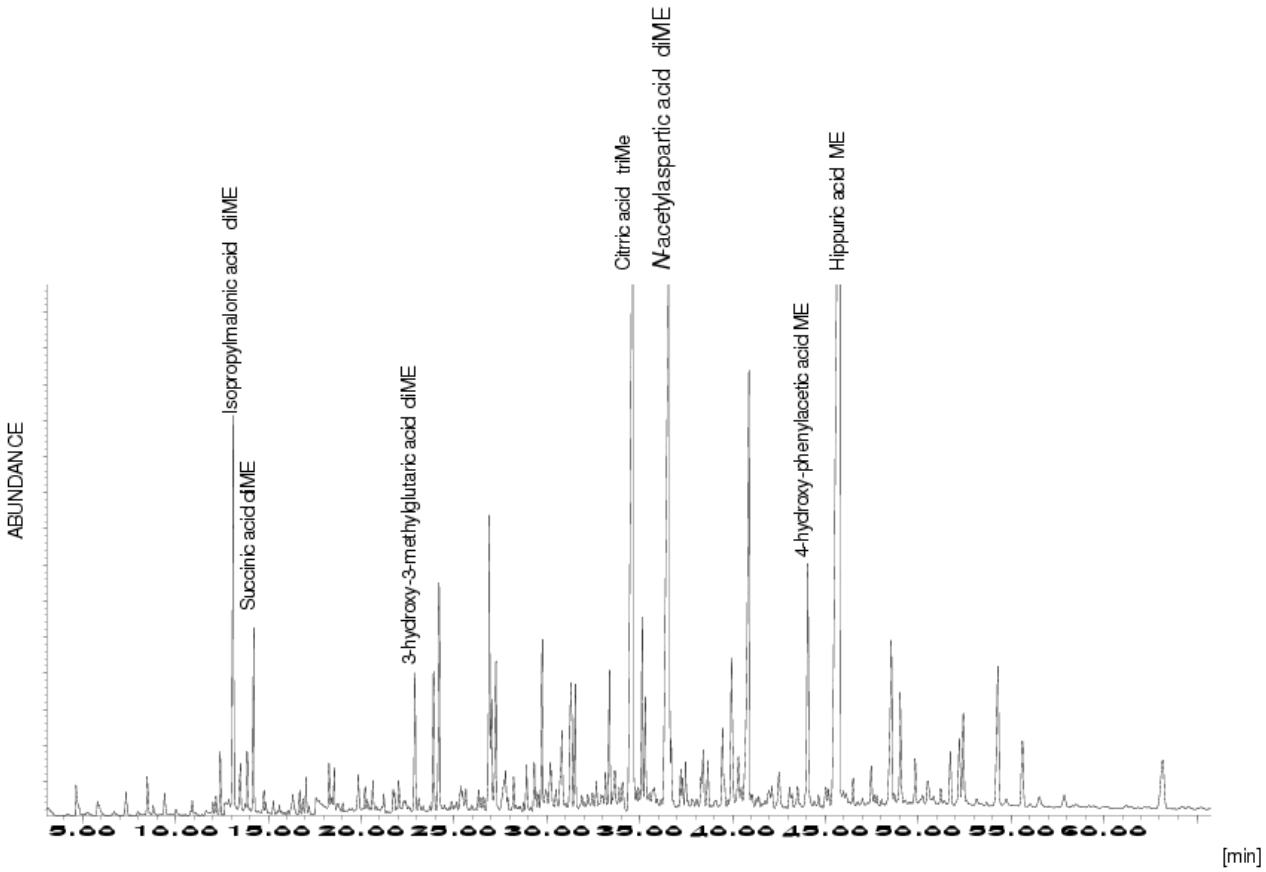

Figure 2: The pattern of urinary organic acids (analyzed as methyl esters [ME] by gas chromatography with mass-selective detection) of a patient with Canavan disease demonstrates accumulation of *N*-acetylaspartic acid which is normally present in trace amounts only. Isopropylmalonic acid served as the internal standard.

60% of the analyzed chromosomes of Canavan patients [10–12]. Besides the three common mutations many others are known and new mutations are still being discovered [11,12,43].

Prenatal diagnosis

DNA analysis is the most reliable approach to prenatal diagnosis of Canavan disease. It is the method of choice when the mutations are known. As among Askenazi Jewish populations two mutations cause almost all cases of Canavan disease, preconception carrier testing and prenatal diagnosis for those two mutations are available. In other groups the candidate mutations are far more numerous. This renders molecular diagnosis more complex and sometimes impossible within the given time frame.

Enzyme activity testing has been considered non-reliable with the samples usually applied for prenatal testing [37,44,45].

A more predictive method for prenatal diagnosis with adequate sensitivity and selectivity is quantification of NAA levels in amniotic fluid by gas chromatography – mass spectroscopy (GC-MS) [33,46,47] or liquid chromatography – tandem mass spectrometry LC-MS/MS [48]. However, slight elevations of NAA levels in the amniotic fluid should be interpreted with caution to avoid false positive results [49].

Recently, preimplantation diagnostics have been used for *in vitro* screening of embryos from parents who

are known carriers of *ASPA* mutations [42]. In countries where such a procedure is legal this represents an additional diagnostic option.

Phenotype-genotype correlation

A strong-genotype phenotype correlation has not been demonstrated in Canavan disease so far. However, there is evidence that patients who are compound heterozygotes for certain (perhaps even protective?) mutations such as K213E, Y288C and G212A may have a rather benign phenotype [18,19,21,50,51]. So far, the small number of patients known to have those and similar genotypes and the lack of mechanistic studies limit our understanding of Canavan disease.

Pathomechanisms as a basis of therapeutic approaches

So far, there is no established treatment of Canvan disease beyond symptomatic measures, e.g., controlling seizures. Some children require feeding via a nasogastric or gastrostomy tube when normal feeding becomes too difficult [15,16].

It is known that lack of aspartoacylase is the underlying defect in Canavan disease. However, the pathogenetic correlation between the enzyme deficiency causing elevated levels of NAA and the neuronal and white matter degeneration leading to the phenotype of Canavan disease is not clear yet. Much work has been done on this topic and theories concerning pathogenesis have been established. Some of them have led to suggestions for new therapeutic strategies.

The elevated NAA levels in tissues and body fluids have been interpreted as a possible indication of toxicity of NAA or its metabolites [52]. Burlina *et al.* focused on *N*-acetylaspartylglutamate (NAAG) [53], a probable product of NAA [54]. They have assumed that high levels of NAAG in the brain caused by high concentrations of NAA could have pathologic effects either by disturbing NMDA receptor-dependent processes or by causing accumulation of glutamate [53,55,56].

Direct injections of 4 μmol of NAA into the brains of rats were found to result in absence-like seizures, injections of 8 μmol of NAA have been shown to cause convulsive seizures. Both types of seizures have been accompanied by epileptiform discharges in the EEG and abnormal behavior [57,58]. The authors concluded NAA to be excitatory [57]. Critics argumented that the high doses they used do not correspond to the far lower NAA concentrations present in the brain of Canavan patients. They considered dysmyelination an alternative explanation for the seizures [59]. Injection of 2 μmol of NAA did not induce seizures in normal rats [57].

Recently, NAA has been detected in a wide range of food [60]. Therefore, Delaney *et al.* have performed toxicity studies following oral administration of NAA. They found that neither one high dose of 2000 mg/kg NAA nor repeated doses of 10, 100, 500 or 1000 mg/kg/day NAA cause any adverse effects or biologically significant changes [61]. On 2009 Karaman *et al.* reported that NAA in food is not mutagenic [62].

Another theory about the pathogenesis of Canavan disease is that NAA may act as an osmoregulator in the brain [63,64]. Investigators found that the efflux of NAA from the neurons to the extracellular fluid (ECF) is associated with water efflux [63] and that the extracellular concentration of NAA in the rat striatum rises during hyposmotic phases [64].

It was also proposed that NAA functions as a molecular water pump in myelinated neurons and that its accumulation leads to osmotic dysregulation in the brain which is responsible for the dysmyelination and subcortical vacuolation observed in Canavan disease [65–67]. Reduced levels of GABA and its precursor glutamate were found in the brains of Canavan patients and knockout mice. Therefore administration of a glutamine analogue and a GABA analogue has been proposed as a possible therapeutical approach particularly to alleviate spasticity [68].

In 2009 the results of Kumar *et al.* have indicated that aspartoacylase may be involved in the epigenetic regulation of myelin genes and genes responsible for the differentiation of oligodendrocytes [69], the cells in which aspartoacylase is localized [70–72]. Another theory attributes the pathology observed in Canavan disease to the inability to liberate free acetate from NAA due to the lack of aspartoacylase. In healthy individuals NAA appears to be the primary source of acetate required for some portion of myelin lipid

synthesis during postnatal axonal myelination [70,72–78]. This hypothesis is supported by data on the incorporation of acetate from NAA into myelin lipid [74,78] and on deficiency of myelin lipid synthesis in the brains of aspartoacylase knockout mice [75]. An argument against this hypothesis is that there are alternative sources for acetate in the brain [6].

Based on the hypothesis of acetate deficiency, dietary acetate supplementation with glyceryl triacetate (GTA) has been proposed as a therapy of Canavan disease. After application of GTA increased acetate levels were detected in mice [77]. There was no elevation of NAA levels in the brain [77] and there was no evidence for adverse effects or clinical deterioration in two infants and in rats [79]. In the tremor rat model of Canavan disease, application of GTA improved motor function and changed the composition of myelin lipids [80].

Recently, the application of lithium has been studied as a new experimental treatment after lithium chloride had been found to reduce NAA levels in some parts of the brain of the tremor rat model for Canavan disease [81]. The application of lithium to patients was shown to be well tolerated and to cause a moderate decrease of brain NAA which was significant only in one examined area [82,83]. In MRI-scans, T1 values indicated a moderate amelioration. However, clinical tests did not show any statistically significant improvement [82,83]. It should be noted that only two open studies with very few patients, seven in total, have been performed to assess this experimental therapeutic approach.

Experimental approaches using gene therapy in humans and animals so far have not opened a therapeutic perspective [37,52,84–86].

Recently, interest in deficiency of aspartoacylase has further grown due to the discovery of deficiency of aminoacylase 1 [87,88]. Aspartoacylase, also called aminoacylase 2, only cleaves *N*-acetylaspartate. Aminoacylase 1 cleaves virtually all *N*-acetylated *L*-amino acids except for *N*-acetylaspartate and *N*-acetylproline [8]. Aminoacylase 1 deficiency was mostly detected in children with neurological abnormalities [87–89] Ongoing research addresses possible interactions between the two aminoacylases. This may contribute to a better understanding of Canavan disease and its etiology.

Following the discovery of the synthesis of NAA by *N*-acetyltransferase 8-like protein (encoded by the *NAT8L* gene) early in 2010, this protein will probably attract attention as a new potential target for affecting NAA levels soon [90].

Conclusions

Canavan disease is a genetic neurodegenerative disease caused by mutations in the *ASPA* gene. Important clinical features are macrocephaly, hypotonia, head lag and developmental delay. Patients show elevated urinary concentrations of NAA. While it is often expected that patients with Canavan disease will die in childhood, there is increasing evidence for a wide variation of the clinical phenotype. Although it is a panethnic disease, information on affected individuals in populations of Non-Ashkenazi Jewish origin is rather limited. Ongoing research aims at a better understanding of Canavan disease and underlying mechanisms as a basis for new therapeutic approaches.

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