

Review:

Canavan disease: Genomic interaction and metabolic levels

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ABSTRACT

Canavan disease (CD) is an autosomal recessive disorder, characterized by spongiform degeneration of the white matter of the brain. Aspartoacylase (ASP) hydrolyses N-acetylaspartic acid to aspartate and acetate. Mutation of the gene results in enzyme deficiency to result CD. The clinical features seen in the disease are head lag, macrocephaly, hypotonia and mental retardation. More than forty five mutations have been identified in the ASPA gene. Pathophysiological abnormalities seen in CD is likely due to abnormal metabolic levels of NAA, aspartate, acetate, aspartate aminotransferase, glutamate, glutamate dehydrogenase, γ -aminobutyric acid, and ketoglutarate dehydrogenase complex. These pathways are useful to understand possible therapeutical targets and pharmacological manipulations in CD.

Keywords: Canavan disease, aspartoacylase, ASPA knockout mouse, tremor rat, spongiform degeneration, neurodegeneration

INTRODUCTION

Canavan disease (CD) is a neurodegenerative disorder. Aspartoacylase/aminoacylase II (ASP; EC 3.5.1.15) hydrolyses N-acetylaspartic acid (NAA) to aspartate and acetate (Birnbaum, 1955; Birnbaum et al., 1952). Aspartoacylase gene has been mapped to the 17p13-ter region (Kaul et al., 1993; 1994a). ASPA activity is mainly present in brain and kidney (Kaul et al., 1993). ASPA has a vital role in the compartmentalization of NAA in brain. While neurons are rich in NAA (Jacobson, 1957; Birken et al., 1989; Moffett et al., 1991; Baslow et al., 1997), ASPA is predominantly found in

oligodendrocytes (Baslow et al., 1999). ASPA mutation (Kaul et al., 1993) resulting enzyme deficiency (Matalon et al., 1988) leads to CD (Globus and Strauss, 1928; Canavan, 1931; van Bogaert and Bertrand, 1949; Adachi et al., 1972; Adornato et al., 1972). The clinical features of the disease include psychomotor retardation, megalencephaly and hypotonia (van Bogaert and Bertrand, 1949). The brain of the patient showed swollen astrocytes and elongated mitochondria (Globus and Strauss, 1928; Canavan, 1931; van Bogaert and Bertrand, 1949; Adachi et al., 1972; Adornato et al., 1972). Although CD is pan ethnic, the

disease is more prevalent in Ashkenazi Jewish population.

An ASPA knockout mouse (Matalon et al., 2000) and a tremor rat (Kitada et al., 2000; Seki et al., 2004) are available to study molecular mechanism involved in CD. These animal models showed ASPA deficiency, NAA accumulation and spongiform degeneration in the brain (Kitada et al., 2000; Seki et al., 2004). Aspartoacylase deficiency in the mouse induced vacuolation not only in the white matter of the brain but also at all levels of the spinal cord (Surendran et al., 2005). These animal models are useful to understand molecular events involved in CD.

Therapeutical attempts are being made to recover the lost enzyme as well as the lost function in CD. Aspartoacylase gene transfer to the brain improved neurodegeneration close to the injection site (Matalon et al., 2003; Leone et al., 2004; McPhee et al., 2005). Implantation of neural progenitor cells to the mouse brain differentiated into oligodendrocytes (Surendran et al., 2004a). Whether therapy affects all the events seen in CD is yet to be studied.

Clinical symptoms of CD

Infants with CD show mild delays, hypotonia, and inadequate visual tracking. These infants become progressively irritable, and remain hypotonic with poor head control (Matalon et al., 1989). Developmental delay and larger head are visible after 6 months of age. Patients can not sit, stand, walk or talk. Children with CD develop optic atrophy and have difficulty focusing, but are able to recognize their surroundings. Feeding

difficulties increase with age, and feeding by a nasogastric tube or permanent gastrostomy will be needed.

Aspartoacylase gene

Aspartoacylase gene was located in the short arm of chromosome 17 (17p13-ter) (Kaul et al., 1993; 1994a). The human ASPA gene spans about 29 kb of DNA, with 6 exons and 5 introns. Human ASPA complementary DNA (cDNA) contains 1,435 base pairs that encode 313 aminoacids (Kaul et al., 1993; 1994b), an enzyme with a molecular weight of approximately 37 kDa. The enzyme converts NAA into aspartate and acetate (Fig 1). There have been over 45 mutations identified in the human ASPA gene. The genomic organization of the gene with various mutations is shown in Table 1. The deduced amino acid sequence of mouse ASPA is 86% identical and 94% similar to that of human ASPA and the catalytic domains are 100% identical (Namboodiri et al., 2000). The fifth aminoacid seen in human ASPA protein "H" (histidine) is not present in the mouse protein. Hence, mouse ASPA cDNA encodes 312 aminoacids.

The two common mutations, E285A and Y231X that account for over 76% of the mutations among Ashkenazi Jewish population (Kaul et al., 1993). The most common non-Jewish mutation is A305E (Kaul et al., 1994b). Mutation D114Y was found in a small geographical region in Norway and D249V mutations was specific to Norwegian and Swedish population (Olsen et al., 2002) suggesting some mutations in ASPA gene are founder mutations.

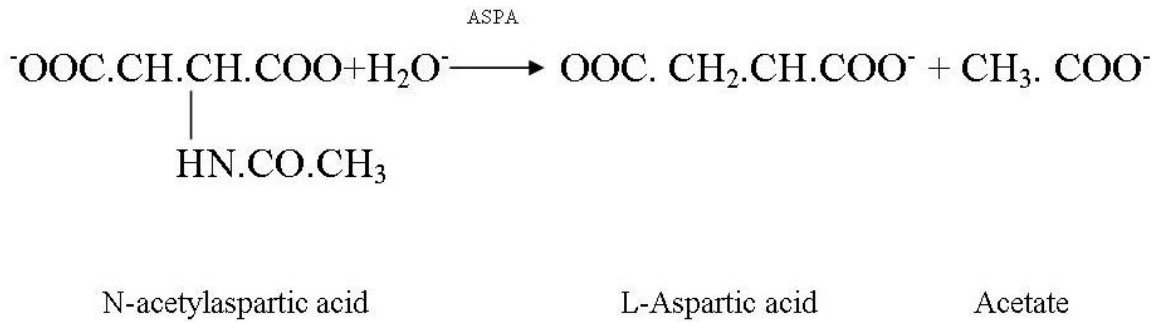


Figure 1: N-acetylaspartic acid is hydrolyzed into L-aspartic acid and acetate by aspartoacylase (ASPase), the enzyme deficient in CD.

Exon 1	Exon2	Exon 3	Exon4	Exon5	Exon6
I16T, E24G, G27R, D68A, 10T>G, 11insG, 32delT, 33del13bp	Y109X, D114E, D114Y, G123E, I143T, 244delA, 245insA, IVS1 - 2A>T	C152R, C152Y, C152W, R168C, IVS2 del- 2A, -3C	P181T, P183H, Q184X, V186F, M195R, 527 del6bp, 566del7bp	K213E E214X, C218X, Y231X, H244R, D249V 698insC, 635del10bp	G274R, P280S, P280L, E285A, A287T, Y288C, F295S, X314W, 827delGT, 870del4bp, 876del4bp, 923delT

Table 1: Sketch of Aspartoacylase with five introns and six exons. The human ASPase gene spans 29Kb. Mutations E285A and Y231X are common in Jewish population. The A305E mutation is common in European population, the C218X mutation is found in Gypsy population, the D249V mutation is identified in Norwegian and Swedish populations and the D114Y mutation is identified in region specific Norwegian CD patients.

The Y288C variant (Surendran et al., 2003a; Tacke et al., 2005) does not result in all the events seen in classical CD and therefore this mutation may be classified as mild CD. Preimplantation genetic diagnosis (PGD) using a single-cell nested PCR approach (Yaron et al., 2005) helps

to identify embryos with CD and therefore CD birth may be prevented.

NAA metabolism in the brain of CD

N-acetylaspartic acid, the substrate for ASPase is synthesized from acetyl-coenzyme A and aspartate by L-aspartate N-acetyltransferase (Goldstein, 1976),

located in the mitochondria of the brain (Patel and Clark, 1979; Bates et al., 1996). NAA may be involved in osmotic regulation, neuromodulation and lipogenesis during myelination (Birken and Oldendorf, 1989). NAA level is three times higher in the brain of CD (Grodd et al., 1990; Wittsack et al., 1996). The accumulation of NAA in the brain leads to approximately 50 times higher urinary NAA. NAA-derived acetate is a major source for the synthesis of fatty acids/lipids used for myelination (Kirmani et al., 2003; Madhavarao et al., 2004) and therefore reduced level of acetate seen in CD is likely to contribute to neurodegeneration.

Neuropathology in the brain of CD

Aspartoacylase deficiency resulting NAA metabolism affects the brain. The concentration of NAA in the human fetus brain in utero is approximately 2.5 μ mol/g (Kato et al., 1997), while in the normal adult human brain the level varies 8-10 μ mol/g (Frahm et al., 1989; Kreis et al., 1993). Nuclear magnetic resonance spectroscopy (MRS) of CD brain showed approximately 3 fold higher NAA in CD (Grodd et al., 1990; Wittsack et al., 1996). The accumulation of NAA in the brain results in an increased urinary NAA in CD. Patients with CD have more than 50 fold higher urinary NAA (1440 \pm 873.3 μ mol/mmol) compared to the normal subjects (23.5 \pm 16.1 μ mol/mmol). ASPA deficiency resulting NAA accumulation in the brain suggests that abnormal NAA level contributes to spongiform degeneration.

The brain of CD showed swollen astrocytes and distorted and elongated mitochondria (Adachi et al., 1972; Adornato et al., 1972; Luo and Huang, 1984). Computed tomography (CT) of the head or magnetic resonance imaging

(MRI) of the brain showed white matter degeneration in CD (Rushton et al., 1981; Brismar et al., 1990). These studies suggest that mitochondrial defect is likely one of the contributing factors of neurodegeneration seen in CD.

ASPA deficiency in the mouse affects the central nervous system

ASPA knockout in the mouse leads to vacuolation throughout white matter of the brain (Matalon et al., 2000). In contrast, spinal cord of the mouse showed severe vacuolation in the gray matter than white matter (Surendran et al., 2005). These studies in the mouse suggest that ASPA knockout affects normal development of the brain and spinal cord to contribute in abnormal function. Lesion in any one of these parts of the spinal cord would impede functions including walking (McKenna et al., 1996). The changes observed in the ASPA knockout mouse spinal cord suggests that spinal cord pathology is one of the important contributing factors of behavioral deficit seen in CD.

ASPA knockout affects glutamate-ketoglutarate dehydrogenase complex pathway

ASPA knockout in the mouse reduced the levels of potential neurotransmitters, glutamate and GABA in the brain (Surendran et al., 2003a). During low levels of external glutamate, the glutamine synthetase pathway is turned on and when external glutamate levels are high, the oxidative pathway (Tri carboxylic acid cycle; via 2-oxoglutarate) is turned on with considerable glutamate being consumed via the astrocytic malate dehydrogenase (McKenna et al., 1996).

The enzyme which reversibly converts aspartate to glutamate, aspartate

aminotransferase (AAT; EC 2.6.1.1) was found to be lower in the brain of ASPA knockout mouse (Surendran et al., 2003b) (Fig 2). Both neurons and astrocytes oxidize glutamate via glutamate dehydrogenase (GDH; EC 1.4.1.3), a major route for the entry of glutamate carbon into the TCA cycle. GDH converts

glutamate into 2-oxoglutarate or the reverse (Rajeswari et al., 1984) and this enzyme was also found to be lower in the mouse (Surendran et al., 2004b). Downregulation of GDH leads to abnormal glutamate metabolism to induce neurodegeneration via neuroexcitotoxic mechanisms (Chokroverty et al., 1984).

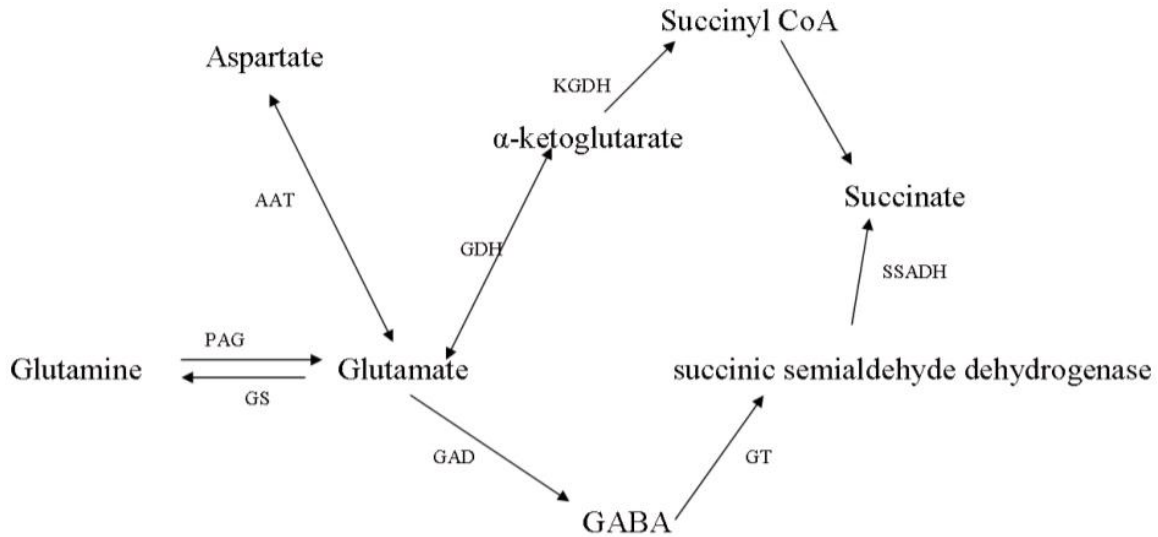


Figure 2: Enzymes involved in the aspartate-glutamate-GABA metabolic pathway. AAT=Aspartate aminotransferase, PAG=phosphate activated glutaminase, GS=glutamine synthase, GAD= glutamic acid decarboxylase, GT= GABA transaminase, SSADH= succinic semialdehyde dehydrogenase, GDH= glutamate dehydrogenase, KGDH= α -ketoglutarate dehydrogenase.

α -Ketoglutarate dehydrogenase complex (KGDHC; EC 1.2.4.2, EC 2.3.1.61, and EC 1.6.4.3) converts α -ketoglutarate into succinyl CoA. Lower levels of KGDHC induce caspase 3 to resulting in neurodegeneration (Huang et al., 2003). The metabolic enzyme activity was found to be lower in the brain of ASPA knockout mouse (Surendran et al., 2004b). These studies suggest that lower levels of neurotransmitters and metabolic enzymes seen in the ASPA knockout mouse brain are likely to contribute to neurodegeneration.

Transport of glutamine from astrocytes to neurons is important for the synthesis of γ -aminobutyric acid (GABA). The converted glutamate from glutamine, serves as a precursor for GABA (Petroff et al., 1999) (Fig 2). GABA is lower in the ASPA knockout mouse brain (Surendran et al., 2003c) suggests that ASPA deficiency hampers inhibitory function of GABA.

While N-acetylaspartylglutamate (NAAG) is synthesized in neurons, the enzyme that cleaves NAAG to NAA and glutamate (Fig

3), carboxypeptidase II is limited to astrocytes (Robinson et al., 1987; Stauch et al., 1989; Slusher et al., 1990; Williamson et al., 1991). In neurons, NAA and glutamate are converted into NAAG in the presence of N-acetylaspartate-L-glutamate ligase (Tyson et al., 1998). NAAG level is approximately 20 fold higher in CD

(Burlina et al., 1994, 1999; Krawczyk et al., 2003). Meantime, NAAG was not affected in the brain of ASPA knockout mouse. Spinal cord neurodegeneration resulting NAAG disposal may be one of the contributing factors of elevated NAAG seen in CD.

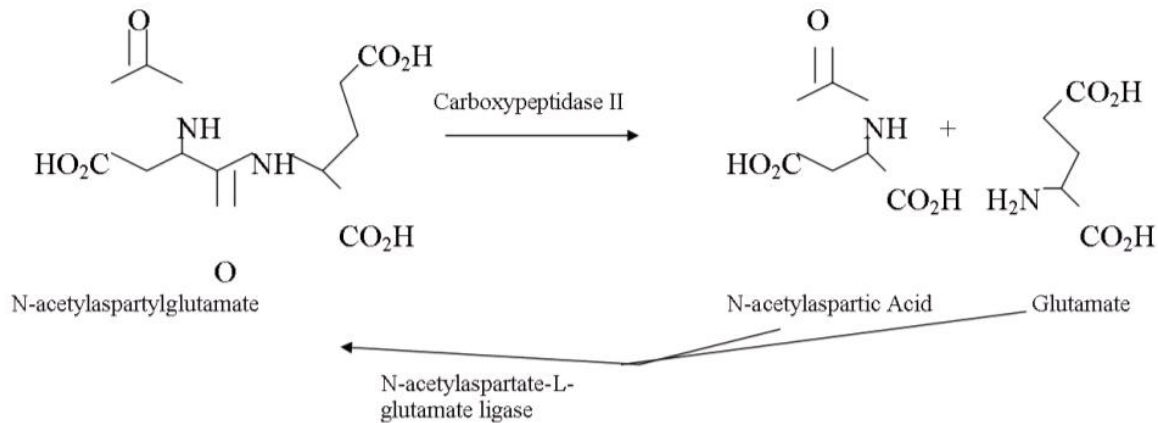


Figure 3: NAAG is hydrolyzed into N-acetylaspartic acid and glutamate by NAALADase/glutamate carboxypeptidase II. NAA and glutamate are converted into NAAG by N-acetylaspartate-L-glutamate ligase.

Therapeutical attempts to recover neurodegeneration in CD

Gene transfer and cell implantation are the attempts made to treat neurodegeneration seen in CD. ASPA gene transfer to the brain of children with CD improved brain NAA levels (Leone et al., 2000). MRI images in one of the ASPA injected child's subcortical white matter showed improvement of signal intensity (Leone et al., 2000) suggests that ASPA gene transfer improves myelination. Adenoassociated virus mediated ASPA gene transfer to the brain of ASPA knockout mouse, improved spongiform degeneration and brain NAA levels (Matalon et al., 2003). However the effect was close to the injected site. Although gene transfer improves neurodegeneration, whether the gene transfer affects the entire

CNS and improves all the events seen in CD is to be studied.

Cell therapy is one of the approaches to recover the lost enzyme and to replace the lost cells. Implantation of neural progenitor cells to the knockout mouse brain differentiated into oligodendrocytes, the ASPA synthesizing cells and fibrous astrocytes (Surendran et al., 2004a) suggests that neural progenitor cells may be used to recover the lost enzyme as well as the lost cells in CD.

Conclusion

ASPA knockout in the mouse affects metabolic levels. ASPA knockout not only affects the brain but also the spinal cord. Whether gene transfer affects all the events seen in CD is to be studied.

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