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Pathophysiology and Treatment of Canavan Disease

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Keywords

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Canavan Disease

Canavan disease is a recessively inherited vacuolar leukodystrophy caused by ASPA mutations [1–3]. ASPA encodes aspartoacylase, an oligodendroglial enzyme required for cleavage of the abundant brain amino acid *N*-acetyl-L-aspartate (NAA) to acetate and L-aspartate [4]. ASPA mutations are relatively common in Ashkenazi Jews, with carrier frequency estimates ranging between 1:40 and 1:60, but also occur, though substantially less often, in many other human populations [3, 5, 6]. The disease classically presents in infancy with ataxia, hypotonia, and failure to acquire normal developmental milestones, often in association with macrocephaly and seizures [3]. In atypical cases in which some aspartoacylase enzymatic activity remains, disease onset is delayed until several years after birth [2, 7, 8]. Neuroimaging shows brain white matter signal abnormalities, and, at later time-points, ventricular enlargement [9, 10]. In vivo proton nuclear magnetic resonance spectroscopy (^{1}H-MRS) documents a 30% or greater elevation in brain NAA concentration ($[NAA_B]$) [10]. Histological studies reveal brain “spongiform” vacuolation, astrogliosis, and dysmyelination [7, 11–13]. These neuropathological abnormalities are most prominent in superficial white matter and neighboring gray matter of the forebrain, cerebellum, and upper brainstem. In more advanced cases, the cerebral ventricles become enlarged, and numbers of brain neurons diminish [7, 12, 13]. No therapies have yet been proven to be effective in preventing or reversing progression of leukodystrophy in Canavan disease.

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Aspa Mutant Mice

Aspartoacylase-deficient mice are useful for exploring the pathophysiology of Canavan disease and for preliminary evaluation of new treatment options. The histologically best characterized aspartoacylase-deficient mice are homozygous for an ethyl-N-nitrosourea-induced Aspa nonsense mutation ("Nur7", Q193X) [14]. These $Aspa^{Nur7/Nur7}$ mice, which do not express immunochemically detectable aspartoacylase, and maintain an elevated [NAA_B], develop ataxia by postnatal day 21, usually survive into adulthood, but have a diminished median lifespan. Brain astroglial and intramyelinic vacuolation begin between postnatal days 7 and 14 (Fig. 1), followed by cerebral ventricular enlargement and loss of cerebral cortical and cerebellar neurons [14–18].

Linking Aspartoacylase Deficiency with Vacuolar Leukodystrophy

Two alternative, not mutually exclusive, hypotheses have been advanced to explain how aspartoacylase deficiency might cause vacuolar leukodystrophy in Canavan disease and in aspartoacylase-deficient mice. The "oligodendroglial starvation" hypothesis proposes that dysmyelination results from the inability by aspartoacylase-deficient oligodendroglia to derive acetate from NAA for synthesis of the myelin lipid precursor acetyl-CoA, and perhaps also from an inadequate oligodendroglial supply of NAA-derived L-aspartate to support production of high-energy phosphate compounds [19, 20]. The "NAA toxicity" hypothesis proposes, instead, that astroglial and intramyelinic vacuolation in Canavan disease are caused by impaired brain osmolar homeostasis resulting from elevated [NAA_B] [21].

Two sets of data support the oligodendroglial starvation hypothesis. First, in vitro and in vivo isotope studies indicate that carbon atoms derived from NAA are incorporated into CNS myelin lipids [22]. Second, brain acetyl-CoA and ATP concentrations are diminished in aspartoacylase-deficient mice [19, 20]. But the oligodendroglial starvation hypothesis has been somewhat weakened by the demonstration that reducing [NAA_B] to undetectably low levels in aspartoacylase-expressing ($Aspa^{+/+}$) mice by homozygous constitutive deletion of Nat8l, which encodes neuronal N-acetyl transferase 8-like (also referred to as N-acetylaspartate synthetase), an enzyme essential for NAA synthesis [23, 24], does not prevent the mice from achieving full brain myelination [15]. Note, however, that initial central nervous system (CNS) myelination is slowed and CNS myelin composition and structure are altered in $Nat8l^{-/-}$ mice [25, 26].

In support of the "NAA toxicity" hypothesis, homozygous constitutive Nat8l knockout prevents vacuolar leukodystrophy in $Aspa^{Nur7/Nur7}$ mice [15, 16] (Fig. 2a–c). Total ablation of NAA synthesis does not ensure a normal lifespan for A $spa^{Nur7/Nur7}$ mice, and blocks their synthesis of the CNS peptide neuromodulator/neurotransmitter NAAG [16, 27]. However, lowering [NAA_B] toward the normal range in $Aspa^{Nur7/Nur7}$ mice by constitutive knockout of a single Nat8l allele, or by brain delivery of an Nat8l inhibitory short hairpin-RNA via intracerebroventricular administration of an adeno-associated viral vector (AAV), markedly diminishes the severity of vacuolar leukodystrophy (Fig. 2a, b, d), but does not appear to lengthen lifespan [16, 18].

The mechanism responsible for the toxicity of elevated [NAA_B] has not been established. Cultured neural cells are not harmed by direct exposure to purified NAA [28], and oligodendroglial numbers are not diminished in A $spa^{Nur7/Nur7}$ or Canavan brain white matter [7]. Also, elevating [NAA_B] in $Aspa^{+/+}$ mice to an extent comparable to that in aspartoacylase-deficient mice by feeding NAA methyl ester or by engineering transgenic neuronal overexpression of Nat8l does not elicit clinical or neuropathological abnormalities [29, 30]. Thus, elevated overall [NAA_B], is not sufficient to elicit vacuolar leukodystrophy in mice that express aspartoacylase.

Astroglia, but not oligodendroglia, express a sodium-coupled plasma membrane dicarboxylate transporter (NaDC3, encoded by Slc13a3) with sufficient affinity for NAA to maintain normal brain extracellular NAA concentration below 30μM [31, 32]. If astroglia do accumulate NAA from neurons via the action of NaDC3, and then transfer it to oligodendroglia, perhaps via astroglial/oligodendroglial gap junctions, then in the absence of oligodendroglial aspartoacylase, astroglia might over-accumulate NAA and therefore become vacuolated. Providing indirect support for this hypothesis, spontaneous mutations that inactivate ion channel-associated astroglial proteins, or disrupt astroglial/oligodendroglial gap junctions, are sufficient to elicit vacuolar leukodystrophy in A $spa^{+/+}$ brains [33–35]. This hypothesis could be tested by examining the effects on brain morphology in A $spa^{Nur7/Nur7}$ and A $spa^{+/+}$ mice of ablating astroglial NaDC3 by constitutive or conditional Slc13a3 knockout.

Future Directions

Advances in in vivo gene editing may ultimately make it possible to correct ASPA mutations in vivo. Until that approach becomes feasible, AAV-mediated brain ASPA transduction is likely to be the most promising avenue to pursue, based on the good results reported with this approach in aspartoacylase-deficient mice. Interestingly, administration of an AAV-ASPA designed to target either oligodendroglia or astroglia has been successful in preventing vacuolar leukodystrophy in neonatal aspartoacylase-deficient mice [36, 37]. It should be noted that the success of ASPA gene therapy in aspartoacylase-deficient mice is compatible with both the oligodendroglial starvation and NAA toxicity hypotheses, since brain aspartoacylase reconstitution normalizes both brain acetyl-CoA content and [NAA_B].

An initial attempt at direct brain intraparenchymal AAV2-mediated ASPA gene therapy in a Canavan disease cohort was unsuccessful in preventing progression of clinical neurological deficits and cerebral ventricle enlargement, though it did achieve a slight lowering of [NAA_B] [9]. Among the possible explanations for this disappointing result are that vacuolar leukodystrophy was already far advanced in some patients in the cohort; and that direct brain parenchymal vector administration, while successful in preventing vacuolar leukodystrophy in aspartoacylase-deficient mice, did not elicit sufficiently widespread oligodendroglial and astroglial aspartoacylase expression in the much larger human brains.

Aspartoacylase contributes to acetyl-CoA generation from NAA in various non-neural tissues as well as in oligodendroglia, and aspartoacylase deficiency also causes dysfunction of those tissues. For example, the rate of apoptosis by peritoneal macrophages is increased,

and renal distal convoluted tubules are vacuolated, in aspartoacylase-deficient mice. These and other deficits attributable to a lack of extraneural aspartoacylase may contribute to the shortening in median lifespan that has been documented in $Aspa^{Nur7/Nur7}$ mice, and would not be expected to be corrected by ASPA gene therapy that is directed solely to the CNS [16, 38–40].

Lowering [NAA_B] may provide another effective therapy for Canavan disease, as has been shown in aspartoacylase-deficient mice [15, 16]. Oral lithium citrate administration, a therapy currently advocated for Canavan disease, does lower [NAA_B] slightly in children with this leukodystrophy, but this effect, with usually clinically tolerable dosages, was too weak to be more than minimally clinically effective [41]. Instead, it should be possible to lower [NAA_B] toward normal in infants and children with Canavan disease by suppressing brain N-acetyltransferase 8-like activity, either via NAT8L knockdown [18] or by development of a druggable N-acetyltransferase 8-like inhibitor [42].

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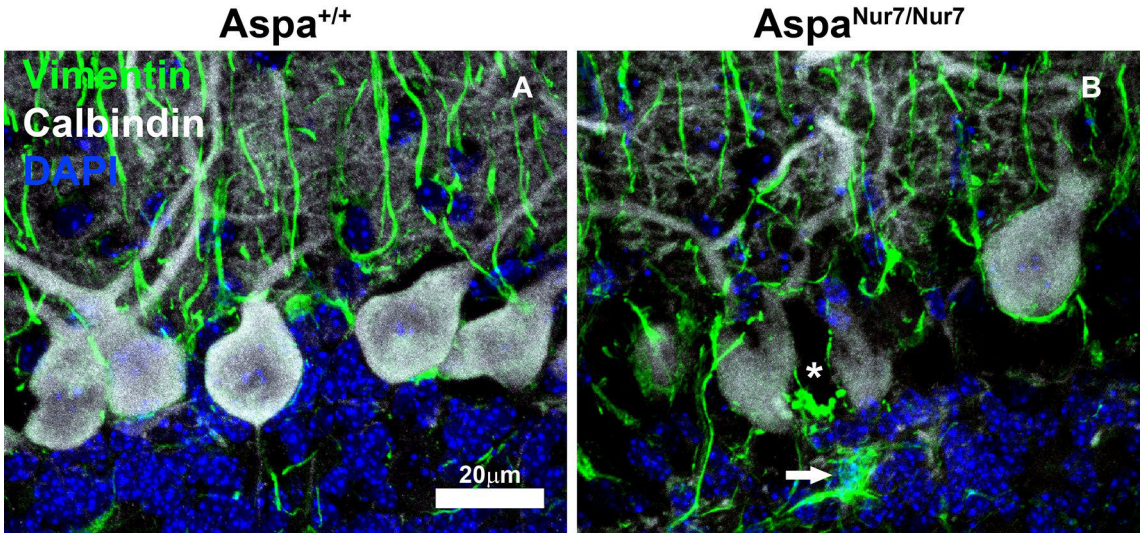

Fig. 1.

$Aspa^{Nur7/Nur7}$ mouse cerebellar vacuolation and astrogliosis. Cryostat sections through cerebellum of a 14 days old wild-type ($Aspa^{+/+}$) mouse (**a**) and of a 14 days old aspartoacylase-deficient ($Aspa^{Nur7/Nur7}$) mouse (**b**) were immunostained for calbindin (white) and vimentin (green), then counterstained with DAPI, then viewed by laser scanning confocal microscopy. In the section from the $Aspa^{Nur7/Nur7}$ mouse, the Purkinje cell layer is disorganized, and there is a vacuole between two Purkinje cells (asterisk) that is surrounded by vimentin^{+} fibrils. Also in the $Aspa^{Nur7/Nur7}$ section, there is a vimentin^{+} hypertrophic astrocyte (arrow) in the internal granule cell layer mouse, and Bergmann glial vimentin^{+} fibrils appear to be fragmented. Scale bar = 20μ m in both panels

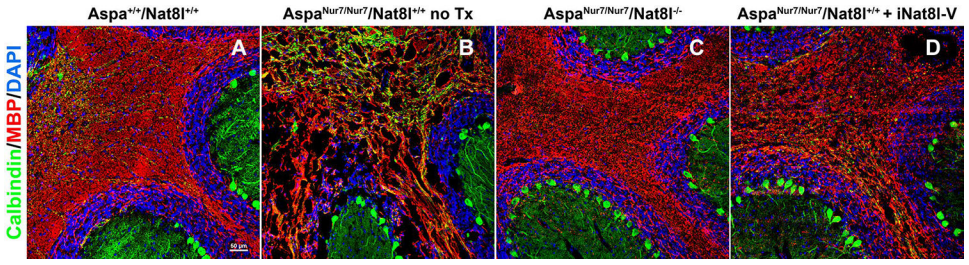

Fig. 2.

Cerebellar vacuolation and dysmyellnation in Aspa$^{Nur7/Nur7}$ mice is prevented by homozygous constitutive Nat8l knockout, and substantially diminished in severity by neonatal brain Nat8l knockdown. Cryostat sections through cerebellum were prepared from brains of 2 months old wild-type (Aspa$^{+/+}$/Nat8l$^{+/+}$) (**a**), a 2 months old untreated A spa$^{Nur7/Nur7}$/Nat8l$^{+/+}$ mouse (**b**), a 2 months old Aspa $^{Nur7/Nur7}$/Nat8l$^{-/-}$ mouse (**c**), and a 2 months old A spa$^{Nur7/Nur7}$/Nat8l$^{+/+}$ mouse that had been given an intracerebroventricular AAV carrying an Nat8l short hairpin inhibitory RNA (iNat8l-V) on postnatal day 1 (**d**). The sections were immunostained for myelin basic protein (MBP) and calbindin, counterstained with DAPI, and viewed by laser scanning confocal microscopy. Cerebellar vacuolation and dysmyelination were prominent in the untreated Aspa$^{Nur7/Nur7}$ mouse, but these abnormalities were prevented by homozygous constitutive Nat8l knockout, and diminished in severity by Nat8l knockdown. Scale bar = 50μ m in all panels