

## The impact of structural biology on neurobiology

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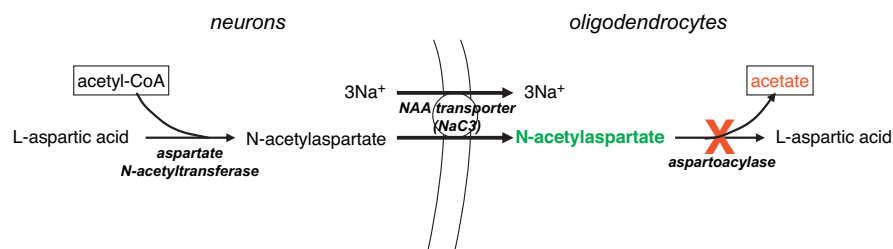
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Canavan disease is a fatal neurodegenerative disorder whose symptoms, including loss of motor skills and muscle control, appear in early infancy and typically progress very rapidly, with death usually occurring within the first decade of life. Unlike the case with many neurological disorders where the underlying genetic defects remain to be elucidated, Canavan disease is caused by defects in a single gene, the *acy2* gene that encodes for the enzyme aspartoacylase. Recent biochemical studies have begun to characterize the mechanistic properties (1) and structural properties (2) of aspartoacylase, but progress in our understanding of this disease has been slowed by the absence of high-resolution structures of this critical metabolic enzyme. This gap has now been filled by the determination of the structures of both the rat and human forms of aspartoacylase reported by Bitto *et al.* (3) in this issue of PNAS.

The substrate for aspartoacylase, N-acetyl-L-aspartate (NAA) is one of the most abundant amino acids in our brain (4), and the pathway for its production and utilization is quite straightforward (Fig. 1). NAA is produced by an as-yet-uncharacterized acetyltransferase using a CoA-activated acetate group to couple to L-aspartic acid. The NAA synthesized in neuronal cells is transported by a membrane-bound sodium/dicarboxylate symporter (NaC3) that moves three sodium ions across the cell membrane for each NAA transported (5) and is coexpressed in cell types that also express aspartoacylase (6). In the brain, these aspartoacylase-containing cells called oligodendrocytes (7) are responsible for the synthesis of myelin (8), and the increase in aspartoacylase activity parallels the occurrence of myelination in the central nervous system (9).

DNA taken from infants with Canavan disease has identified numerous mutations that result in a loss of aspartoacylase activity (10); however, there have been no systematic studies of how and why these alterations affect catalytic activity and little detailed characterization of aspartoacylase itself. The absence of a properly functioning enzyme in these patients leads to abnormally high levels of the substrate NAA (11), but there have been no definitive studies that show whether the symptoms of the disease are caused by the accumulation and subsequent misprocessing of NAA or whether the failure to form



**Fig. 1.** Metabolism of NAA in brain. NAA is produced in neurons by the condensation of activated acetate (in the form of acetyl-CoA) with L-aspartate, catalyzed by an as-yet-uncharacterized acetyltransferase. The product is transported to oligodendrocytes by a sodium/dicarboxylate cotransporter (NaC3) and then cleaved to release L-aspartate and acetate by aspartoacylase. This enzyme is defective in Canavan disease patients, leading to an accumulation of NAA and the failure to release acetate in these myelin-synthesizing cells.

the products L-aspartate and acetate leads to these symptoms. Because acetate is the precursor for fatty acid biosynthesis, it is likely that the loss of aspartoacylase activity is the cause for the decreased myelin lipid production. Analysis of lipid levels in a mouse knockout model confirms a correlation between diminished aspartoacylase activity and a decline in lipid synthesis (12). These studies provide a link between the decline in acetate levels in oligodendrocytes (Fig. 1) and the demyelination that is observed in the brains of Canavan disease patients. However, there have been a number of other reasonable hypotheses advanced to explain the symptoms of Canavan disease, each of which must still be critically evaluated.

The value of this new structure of human brain aspartoacylase is the framework that it provides for researchers in this field to test hypotheses regarding the catalytic mechanism and the possible modes of regulation of this enzyme. These authors have, for the first time, been able to map the known clinical mutants of aspartoacylase onto a three-dimensional structure and correlate these point mutations with proposed functional roles for many of these amino acids (3). This new structure shows the proximity of the zinc binding site to the likely substrate binding site, thereby confirming the previously proposed carboxypeptidase-type mechanism of aspartoacylase (2). This study also demonstrates a critical difference in active site accessibility that allows this enzyme to hydrolyze its physiological substrate NAA with high specificity but is altered compared with other members of the carboxypeptidase family through the addition of a carboxy-terminal domain,

thereby preventing access by peptide-like substrates.

This initial structure of aspartoacylase will facilitate additional structural studies aimed at elucidating the detailed catalytic mechanism of this key enzyme and the possible role of posttranslational modifications in regulating enzyme activity and will lead to a better understanding of how the genetic defects lead to the symptoms of this fatal disease. Gene therapy trials are underway in which a functional copy of the *acy2* gene is delivered by means of an adenoviral vector (13). The initial results from these trials showed that the relentless progress of this disease can be slowed but not reversed in these patients. Genetic screening of potential carriers for the defective gene has led to prenatal identification and the possibility for earlier gene therapy intervention. The hope is that treatment before the development of symptoms will lead to a cure for Canavan disease.

Further structural studies of aspartoacylase will provide mechanistic insights that can lead to the development of more active and more stable forms of this enzyme to be incorporated into gene therapy trials. Coupled with a characterization of the acetyltransferase and the NAA transporter, and an examination of the regulatory relationships between these proteins, future structural biological studies have the potential to significantly advance our fundamental understanding of the neurobiology of Canavan disease.

Author contributions: R.E.V. wrote the paper.

The author declares no conflict of interest.

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