Review Article

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Unraveling the Mystery of Canavan Disease: A 100 Year Chronicle of Key Events

M. H. Baslow

Nathan S. Kline Institute for Psychiatric Research, Orangeburg, New York, USA

*Corresponding author
M. H.Baslow, Nathan S. Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY 10962, USA; Tel: 845 398 5573, Fax: 845 398 5472; E-Mail: baslow@nki.rfmh.org

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Abstract

In the early 1900's, in a small geographical region in northeastern Europe, a mysterious early-onset human malady was brought to the attention of physicians. In this disease, children appeared normal at birth and met usual developmental milestones up to the age of about 4-5 months. At that time, infants began to lose muscle tone and could no longer control their head movements. The disease was progressive, and by age 2, characterized by enlargement of the head, spastic responses to stimulation, and a general listlessness. By age 3, blindness was apparent and there was extension and posturing of limbs due to uncontrolled muscle contractions. Death usually occurred between ages 3-4. In large family's common at that time, there could be 2-3 afflicted children out of a total of 8-9 siblings. There is no metric to measure pain and suffering of CD children, but we can measure progress made over the past century in solving the mystery. At the present time, there are great expectations that a human cure is possible since a mouse model of CD was recently shown to be completely cured by an unusual genetic engineering outcome. In this short review are chronicled key findings made over the past 100 + years that have led to identification of the genetic and cellular etiology of CD, and the reasons for such encouragement.

Keywords: Brain, Canavan disease, Inborn errors, Genetic engineering

Abbreviations: Asp, aspartate; ASPA, aspartoacylase; CD, Canavan disease; ECF, extracellular fluid; Glc, glucose; Glu, glutamate; GM, gray matter; mGluR3, metabotropic Glu receptor 3; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; NVC, neurovascular coupling; WM, white matter

Introduction

Canavan disease (CD) is a rare human brain white matter (WM) disease, whose clinical name is taken from a case report in 1931 [1]. This disease is present in all human populations on all continents, as well as in several animal species. While human CD is extremely rare globally, it is very common in present day Ashkenazi Jews descended from highly inbred Jewish populations that lived in towns and villages in Lithuania, Poland, and western Ukraine for hundreds of years [2]. In large families, the presence of the affliction in several siblings, but not in all of the children, suggested a Mendelian inheritance of a recessive trait. The mystery of the cause of CD and its potential cure has taken more than a 100 years to resolve since its first published report and important stages in scientific inquiry, not necessarily related to the disease itself, have resulted in a solution to the mystery [3]. This short review highlights the critical findings made by researchers through the years that have led to an understanding of the WM etiology of the disease and to its possible cure.

Canavan Disease

The most common form of CD is the infantile form in which the child initially appears to be normal, alert and happy, but at about 4 months of age, appears to become sluggish and to have difficulty holding the head in an upright position when being held. At about this time, the head begins to swell and the head circumference increases well over the 90th percentile. By the end of the first year there are attacks of increased muscle tone and uncontrolled spastic episodes brought on by various forms of external stimulation. During the second year, spasticity and periods of rigidity increase leading to abnormal posturing. At this time, hearing loss and blindness also become apparent. In the terminal stages of the disease there may be episodes of sweating, hyperthermia, vomiting and convulsions. Death usually occurs shortly thereafter at about age 3-4 years. Upon autopsy, CD is observed to be a spongiform leukodystrophy, the most striking feature of which is the presence of fluid-filled vacuoles in the deeper cortical layers of from 5-100 μm in diameter, and in subcortical WM, even larger vacuoles of up to 200 μm in diameter, whereas in gray matter (GM), the cortex appears to be spared. In addition, while myelin sheaths in WM are no longer histologically demonstrable, axonal fibers and oligodendroglial cells appear to be preserved [4]. Although the mystery of the etiology and pathogenesis of CD was obscure, these authors observed features that were consistent with the accumulation of excessive fluid within astrocyte cytoplasm and between myelin lamellae in WM. In the following chronicle of historical events we are brought up to the present time where a specific WM etiology of CD has been identified, and a genetic engineered human cure appears to be possible.

Chronicle of Key Events

1912 *First published case report of the disease*

The first published report of the mystery WM disease in which it was named "perixialis diffusa", was a disease where the medullary sheaths surrounding neuron axons were attacked, but leaving the axons intact [3].

1931 *The foundation case report: "Canavan disease"*

A clinical case report was published which was thereafter considered to be the index case for the disease and it was named after the author, Dr. M. Canavan [1].

1952 *Discovery of two acylases in hog kidney*

Two acylases were discovered in hog kidney [5]. Acylase I was a broad spectrum acylase that hydrolyzed N-acetyl-L-histidine and other N-acetylated amino acids. Acylase II had a high specificity for N-acetyl-L-aspartic acid and was named N-acetyl-L-aspartate amidohydrolase, now called aspartoacylase (ASPA). Both are also present in brain.

1953 *Discovery of the structure of DNA*

A probable structure of DNA and its genetic implications was published [6]. Over time, this monumental discovery led to the ability to identify mutations in specific genes and also to the ability to intervene in certain diseases, using genetic engineering techniques.

1956 *Discovery of N-acetylaspartic acid (NAA) in cat brain*

Four years after the discovery of ASPA, a large amount of bound aspartic acid was found in cat brain. Upon isolation and acid hydrolysis, it was reported to be N-acetyl-L-aspartic acid, the natural substrate of ASPA [7].

1964 *Discovery of N-acetylaspartylglutamate (NAAG)*

A new dipeptide acetylconjugate, N-acetyl-aspartyl-glutamic acid was discovered in nerve tissue [8]. NAAG was subsequently found to be an adduct of NAA and glutamate (Glu).

1988 *Discoveries of ASPA deficiency and large amounts of NAA in CD urine, connecting for the first time: (1) abnormal NAA metabolism, (2) reduced ASPA activity, and (3) the recessive genetic defect in CD*.

NAA aciduria and ASPA deficiency in skin fibroblasts of young patients with progressive cerebral atrophy were observed in three studies between 1986 and 1988. In a fourth study based on three clinically diagnosed cases of CD, these findings were duplicated [9]. The presence of NAA in urine is now used in the initial diagnosis of CD.

1990 *Proton spectroscopy used to measure brain levels of NAA non-invasively*

Proton magnetic resonance spectroscopy (MRS) was used to measure the buildup of NAA in brain of a CD patient, and it was found to be elevated by 20-25% [10]. Elevation of brain NAA using MRS is now also used in diagnosis of CD.

1993 *Discovery that NAAG peptidase activity is present in cultured astrocytes*

It was reported that pure mouse brain astrocytes in culture expressed high levels of NAAG peptidase activity [11].

1995 *Mutations in the ASPA gene in CD discovered*

Several specific mutations in the genetic code for ASPA were found to be present in high frequency in descendants of European Ashkenazi Jews. These mutations resulted in the expression of ASPA with greatly reduced enzymatic activity [12]. About 100 mutations in this gene are presently known that affect the activity of ASPA to varying degrees. Genetic testing is currently used to identify CD carriers, and to determine specific ASPA mutations in CD patients.

1996 *First genetic engineering trial for CD*

Two US CD patients were administered recombinant DNA in a phase I safety trial conducted in New Zealand, the details of which were subsequently published [13].

1998 *First observation of the synthesis of NAAG from NAA and Glu*

Isotope labeling of NAA and NAAG from 1- ^{13}C glucose (Glc), and of NAAG from NAA and labeled Glu in rat brain reported [14].

1999 *Discovery that ASPA expression is limited to oligodendrocytes*

In cultured astrocytes and oligodendrocytes, expression of ASPA activity was only observed in oligodendrocytes [15]. This finding was the final piece of the puzzle that led to the description of the tri-cellular metabolism of NAA

2000 *Elucidation of the tri-cellular metabolism of NAA*

Results of studies indicated that the synthesis and hydrolysis of NAA and NAAG in brain are metabolically unique and highly compartmentalized in that they require three brain cell types; neurons, astrocytes and oligodendrocytes, and four enzymes differentially expressed by these cell types for completion; NAA and NAAG synthases in neurons; NAAG peptidase in astrocytes; and ASPA in oligodendrocytes [16]. Neurons synthesize NAA from acetyl-coenzyme A, derived from Glc, and aspartate (Asp), and then synthesize NAAG from NAA and Glu. Remarkably, neither NAA nor NAAG can be hydrolyzed by neurons. However, the neurotransmitter NAAG, containing an NAA moiety, is released to extracellular fluid (ECF) and targeted to the metabotropic Glu receptor 3 (mGluR3) on the astrocyte surface. After docking, NAAG is hydrolyzed by NAAG peptidase liberating NAA and Glu. Astrocytes cannot hydrolyze NAA and it is released to ECF, taken up by oligodendrocytes and hydrolyzed by ASPA producing acetate and Asp which are then completely metabolized. All of the components of this tri-cellular system are found in both GM and WM. In WM, the site of interaction is at each node of Ranvier where astrocyte endfeet, myelinating oligodendrocyte paranodal loops, and naked neuron axons exist together in an extremely confined 2-3 μm wide space.

2009 *Development of the astrocyte WM hypothesis of the etiology of CD*

CD is primarily a WM disease, although there is a buildup of the osmolyte NAA in whole brain. It was therefore proposed that CD was an osmotic brain disease in which astrocytes in WM at nodes of Ranvier played a pivotal role [17]. In GM there may be two sources of NAA in ECF. One from direct release of NAA by neurons, and the second a result of the release of NAAG by neurons to astrocytes and then its hydrolysis by NAAG peptidase. In GM in CD, NAA in ECF is elevated by 20-25%, and as it is continuously removed, it is the major source of the NAA aciduria in CD. NAA is not a neurotransmitter and is not excitatory, which may explain why GM is spared in CD even though NAA is elevated. As a hypothesis, in order to explain the WM pathology in CD, it was proposed that

NAA is not released directly in WM, and that the only source of NAA in WM is as a product of the hydrolysis of NAAG released to astrocytes from axons at nodes of Ranvier. Further, that in CD, in the confined lipid-rich dehydrated space of each node of Ranvier, unhydrolyzed NAA builds up and penetrates between the oligodendrocyte paranodal loops, exerting osmotic-induced hydrostatic pressure and splitting the myelin sheaths, resulting in the fluid-filled vacuoles and leukodystrophy component observed in CD. The proposed tri-cellular metabolism of NAA, the neurotransmitter role of NAAG and the proposed site of the WM etiology of CD at a node of Ranvier are illustrated in figure 1. In this figure, note how intracellular axon NAA is transported into extracellular nodal space during this process.

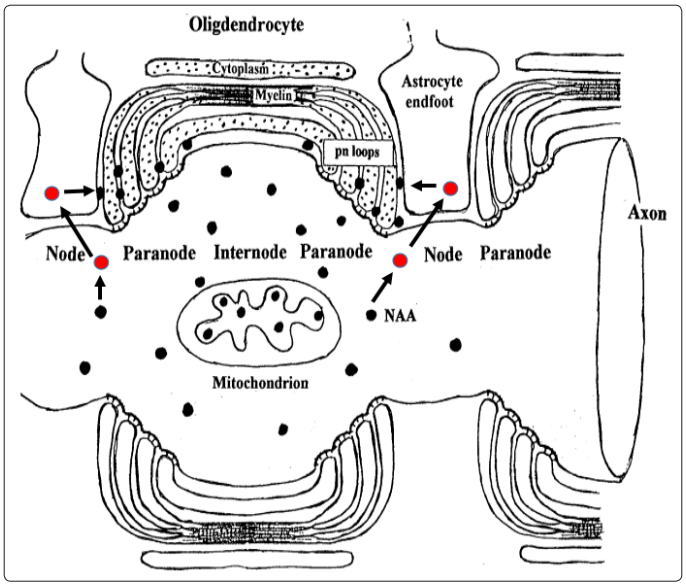

Figure 1. Cartoon of proposed site of liberation of NAAG at neuron axonal nodes of Ranvier in response to neurostimulation. Neuron NAA (black dots) is the precursor of NAAG (red filled circles), but is not itself released in WM upon stimulation. However, NAAG containing an NAA moiety is released and docks with the astrocyte mGluR3 signaling the astrocyte to increase availability of energy. It is then deactivated by astrocyte NAAG peptidase producing NAA and Glu. Astrocytes metabolize the Glu, but cannot hydrolyze the NAA, which is released to nodal ECF, from where it is normally taken up by oligodendrocytes and hydrolyzed by ASPA. In CD, it is proposed that in the absence of ASPA activity there is a buildup of nodal NAA, initiating the WM demyelinating pathology. By redirecting ASPA activity to astrocytes, a new sink for NAA is created in WM, eliminating the NAA-induced myelin pathology in CD. From (Baslow and Guilfoyle, 2017)

2016 *Function of the tri-cellular metabolism of NAA in GM and WM*

Neuron cell bodies and axons can transmit impulses at high rates, up to 900 Hz, and require large amounts of energy on an irregular time schedule. To do this, neurons interact with astrocytes that in turn control the rate of focal blood flow. Two neurovascular coupling (NVC) mechanisms of blood flow control have been reported, one is fast (phasic) that increases blood flow in 2-3 seconds, and the other slow (tonic) that increases flow in 10's of seconds [18]. The neurotransmitter for fast phasic changes was identified as Glu released during synaptic transmission, however the neurotransmitter for slow tonic changes was unknown. It has been proposed that the neurotransmitter for slow tonic NVC responses in both GM and WM is NAAG released by neurons and targeted to the astrocyte mGluR3 [19]. In GM, astrocytes are the conduit for about 90% of traffic between cells and the vascular system. However, in WM, astrocytes are perhaps the sole conduit to supply energy from the vascular system to myelinating oligodendrocytes and neuron axons, as well as for removal of waste products of their metabolism. Thus, in WM, where there are few synapses, the tri- cellular metabolism of NAA and liberation of the neurotransmitter NAAG appears to be a critical factor in maintaining WM integrity.

2017 *Rescuing Canavan disease by redirecting metabolic processing*

In a recent genetic engineering attempt to cure CD using a human adeno-associated virus ASPA construct in a murine CD model, a complete cure was accomplished [20]. However, it was found that the wrong cell was engineered [21]. Instead of transfecting oligodendrocytes to restore normal ASPA activity, the construct was serendipitously inserted into astrocytes. The method of administration appears to be important in that it was introduced globally via the vascular system by intravenous injection, which is the best way to reach capillary-surrounding astrocytes in both WM and GM. In GM in CD, the NAA product of astrocyte NAAG hydrolysis in ECF is readily removed even in the absence of ASPA activity. However, in WM in CD, in the restricted environment of the nodes of Ranvier, it was proposed that the NAA product of astrocyte NAAG hydrolysis builds up, penetrates the paranodal loops of myelinating oligodendrocytes and in this way was responsible for demyelination and the spongiform leukodystrophy in this disease [17]. If the astrocytes in WM were redirected to express ASPA activity, this "rescue" may have been due in large part to eliminating the buildup of NAA at nodes of Ranvier [22]. If true, the possibility of redirecting additional metabolic activities to astrocytes may prove useful in treatment of a variety of WM diseases.

Discussion

CD has long been recognized to be a WM disease. Based on evidence provided in this review, the likely site of the lesion in CD is the node of Ranvier where the neurotransmitter NAAG is released. In the confines of each node of Ranvier, bordered by the naked axon, oligodendrocyte paranodal loops, and astrocyte endfeet, NAA content may rise to pathogenic levels due to the lack of ASPA activity. A logical cure would be to transfect myelinating oligodendrocytes with a normal ASPA gene. However, because of their high degree of specialization in WM and lack of direct connection to vascular system, this is difficult. Another plausible treatment would be to transfect astrocytes in WM to express ASPA at nodes of Ranvier. If so, the normally expressed astrocyte NAAG peptidase would produce NAA from axon liberated NAAG, and then the induced ASPA could hydrolyze the NAA, the products of which could then be taken up and metabolized by oligodendrocytes. This may be how the CD mouse model was completely cured by intravenous injection of the adenovirus-gene construct, even though in this case the wrong cells, "astrocytes", were transfected. Could this treatment work in in CD in the more complex brain of humans? This is unknown, but several commercial firms have been started including "ASPA Therapeutics" and "Voyager Therapeutics" in order to try to develop appropriate human vector-gene constructs and then carry out clinical trials of ASPA gene therapy for human CD.

Conclusions

In this historical review, the etiology of CD is traced to a failure

of myelinating oligodendrocytes to maintain neuron axon sheaths in WM, leading to progressive failure of CNS connectivity and to the CD syndrome. It has been proposed that the likely site of failure is at the node of Ranvier. In this scenario, at each node the neuron axon liberates NAAG to the mGluR3 to communicate with astrocytes, and astrocytes then hydrolyze the NAAG forming NAA and Glu. The Glu is recycled by astrocytes, and the NAA is normally taken up and hydrolyzed by oligodendrocyte ASPA. In CD, inborn errors in the gene for ASPA result in reduced ability of myelinating oligodendrocytes to hydrolyze nodal NAA derived from NAAG, leading to its buildup and to the eventual destruction of the node and to spongiform delamination of existing or developing myelin sheaths. A mouse model of CD has been cured by redirecting the catabolism of NAA to astrocytes, and a human cure appears to be possible if an appropriate adenovirus-human gene construct *targeted to astrocytes* is introduced via the *vascular system* [23] within a short time after birth.

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