A Multi-Center, Multi-National Retrospective and Prospective Natural History Study of Canavan Disease

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Canavan Disease

Background

- Canavan disease is a serious and ultra-rare autosomal recessive leukodystrophy[1-4] (1:100,000 births/year in the United States and European Union[5])
- Mutation on chromosome-17 in the *ASPA* gene results in a deficiency of aspartoacylase, which catalyzes breakdown of N-acetylaspartate (NAA) into aspartate and acetate[6]
- The accumulation of NAA interferes with growth of the myelin sheath of the nerve fibers of the brain. Canavan disease is characterized by degeneration of myelin, resulting in disruption of the phospholipid layer insulating the axon of a neuron (Figure 1)

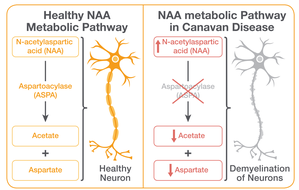


Figure 1: ASPA Enzyme Deficiency and NAA Accumulation Leads to Demyelination in Canavan disease

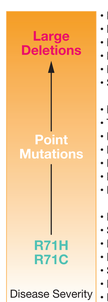
Genetic Testing

- The diagnosis of Canavan disease is established in a proband with typical clinical findings and elevated NAA in urine and/or with biallelic pathogenic variants in the *ASPA* gene identified by molecular genetic testing

ASPA Gene Test Method		% of Pathogenic Variants Detectable
Targeted testing[a]	p.Glu285Ala p.Tyr231Ter	Ashkenazi Jewish: 98%[7] Non-Ashkenazi Jewish: 3%[7]
	p.Ala305Glu	Ashkenazi Jewish: 1%[8] Non-Ashkenazi Jewish: 30%-60%[8,9]
Sequence analysis[b]		~99%[9,10-13]
Gene-targeted deletion/ duplication analysis[c]		9 reported[9,14-17]

[a] Various molecular methods may be used to detect targeted variants.
[b] Sequence analysis detects variants that are benign, likely benign, of uncertain significance, likely pathogenic, or pathogenic. Pathogenic variants may include small intragenic deletions/insertions and missense, nonsense, and splice site variants; typically, exon or whole-gene deletions/duplications are not detected.
[c] Gene-targeted deletion/duplication analysis detects intragenic deletions or duplications. Methods that may be used include: quantitative polymerase chain reaction (PCR), long-range PCR, multiplex ligation-dependent probe amplification, and a gene-targeted microarray designed to detect single-exon deletions or duplications.

Phenotypic Spectrum of Canavan Disease



- Earlier onset and rapid progression of symptoms in the neonatal period
- Hypotonia, marked irritability and seizures
- Nystagmus, blindness
- Failure to develop
- Macrocephaly
- Spasticity

- Normal development during 1st month of life
- Truncal hypotonia, persistent head lag, and progressive macrocephaly at 3-5 months
- Developmental delays and loss of early milestones
- Poor visual tracking and difficulties with sucking and swallowing
- Leukodystrophy on neuroimaging
- Hypertonia, spasticity
- Decerebrate / decorticate

- Early development is normal
- Speech, language, and motor functions are intact
- Poor attention and learning disabilities
- Incoordination, then progressive motor impairments (late-onset)
- Seizures (rare)
- Hypomyelination is localized to the basal ganglia
- Developmental delays tend not to regress

Differential Diagnosis

- The clinical triad of macrocephaly, truncal hypotonia, and visual decline should initially suggest Canavan disease
- Distinguishing factor of Canavan disease is presence of NAA in urine and elevation of NAA peak on magnetic resonance spectroscopy (MRS) (Figure 4)
- Canavan disease can be mistaken for the following well documented and studied diseases, but is distinguished by elevated NAA:
  - Neurodegenerative disorders
    - Alexander disease
    - Tay-Sachs disease
    - Metachromatic leukodystrophy
    - Glutaric acidemia type 1
  - Spongy degeneration of the brain
    - Leigh syndrome
    - Glycine encephalopathy
    - Other mitochondrial disorders and viral infections[6]

Pathophysiology of Classical Canavan Disease

- NAA Metabolism
  - NAA is one of the most abundant metabolites in the mammalian brain and it is a widely used biomarker of neuronal health on MRS
  - NAA metabolism is compartmentalized: synthesized in neurons and degraded in oligodendrocytes. Astrocytes are thought to play a role in osmoregulation
  - NAA is important in myelination (using NAA-derived acetate), osmoregulation, as well as maintaining energy and pH homeostasis
  - In Canavan disease, the accumulation of NAA is thought to lead to demyelination as well as derangements in energy metabolism, and osmoregulation leading to vacuolization[18]
- Histopathology
  - Hallmarks: white matter loss, vacuolation, and cellular edema[19]
  - On the ultrastructural level, swollen astrocytic mitochondria and disruption of the myelin sheath order[19]
- MRS of affected white matter demonstrates an elevated peak of NAA, elevated NAA-to-creatinine ratio, and elevated NAA-to-choline ratio with normal creatinine (Cr) and choline (Cho) peaks (Figure 3)
- Magnet resonance imaging (MRI) T2 sequences demonstrate bilaterally symmetrical, confluent hyperintensity of white matter with involvement of the subcortical arcuate fibers[20,21]
  - Axial T2-weighted imaging (Figure 4) shows abnormal elevated T2 signal in large bilateral areas including the globus pallidus and thalamus at 1 month of age

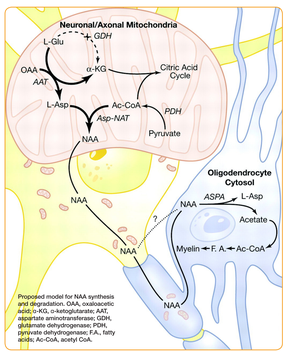


Figure 2: NAA Metabolism[22]

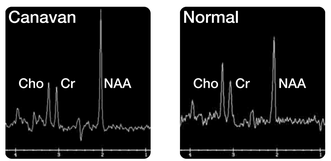


Figure 3: NAA Peak (MRS)

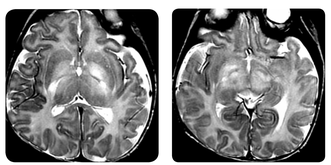


Figure 4: MRIs showing Infantile Onset Canavan Disease

Progression of Classical Canavan Disease

- Progressive, neurologic decline with the velocity of change more pronounced in the earliest months of the patient's life
- Acquisition of new developmental milestones typically halts and regresses within the first 1 to 2 years of life and often before, resulting in severe neurological deficit from an early age (Figure 5)[23]
  - Each line represents a patient with Canavan disease, with Function scores summed over time: hearing, visual tracking, head control, rolling over, reaching for an object, transfer of an item hand to hand, sitting without support, standing with support, speaking single words, and scrawling
- Many children generally do not live past the age of 10 years but those who do are typically severely cognitively impaired, nonverbal, and wheelchair bound
- There are currently no approved therapies for Canavan disease and current standards of care are aimed primarily at palliating disease burden and maximizing quality of life[24,25]

References: 1) Hoshino 2014 *Pediatr Int* 2) Gordon 2001 *Eur J Paediatr Neurol* 3) Pastores 2010 *Dementia* 4) Surendran 2003 *J Child Neurol* 5) Orphanet 2019 6) Matalon 2018 NCBI Bookshelf 7) Michals 2011 *Leukodystrophies* 8) Kaul 1994 *Genomics* 9) Elpeleg 1999 *J Inherit Metab Dis* 10) Kaul 1994 *Am J Hum Genet* 11) Olsen 2002 *J Med Genet* 12) Zeng 2002 *J Inherit Metab Dis* 13) Michals 2011 *Leukodystrophies* 14) Shaag 1995 *Am J Hum Genet* 15) Zeng 2006 *Mol Genet Metab* 16) Caliebe 2010 *Mol Genet Metab* 17) Cozzolino 2011 *Mol Genet Metab* 18) von Jonquieres 2018 *Acta Neuropathol* 19) Adachi 1973 *Hum Pathol* 20) Sreenivasan 2012 *Indian J Pediatr* 21) Pradhan 2011 *Neurology* 22) Madhavarao 2005 *Proc Natl Acad Sci USA* 23) Hamburg database, Bley et al, under submission 24) NORD 2019 25) Traeger 1998 *Pediatr Neuro*

Natural History Study

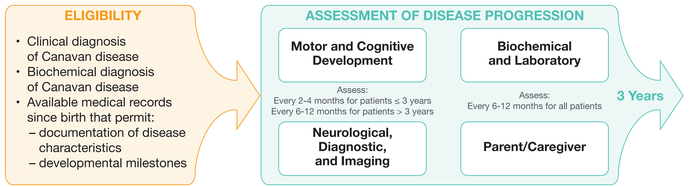
- There is a paucity of published longitudinal data on the progression of Canavan disease, and no approved therapies exist. Advancing the understanding of Canavan disease and facilitating the development of therapeutic options requires a comprehensive, precise delineation of Canavan disease natural history. Therefore, a collaboration between established Canavan patient treatment sites in the United States and Germany will be supported by Aspa Therapeutics as the sponsor of a natural history study of patients with Canavan disease.
- The objective of the study is to rigorously collect natural history data from Canavan disease patients and caregivers to be used to define endpoints for interventional trials, and to identify gaps in disease management across ranges of ages and disease severity.

Study Design

Multi-center, conducted at centers in New York, Boston, and Hamburg, Germany to enroll Canavan disease patients for prospective, longitudinal collection of clinical data using scales and intervals on an observational basis and for retrospective collection of medical record data for both deceased and living patients

Study Endpoint and Assessment Selection

- No instruments for data collection or disease assessment are validated for Canavan disease
- Critical input for selection of assessment instruments was sought from:
  - Expert neurologists at treatment centers in the United States and Germany, who diagnose and treat patients with Canavan disease and counsel families with children affected by Canavan disease
  - Parents and caregivers of patients with Canavan disease
  - Patient advocacy groups



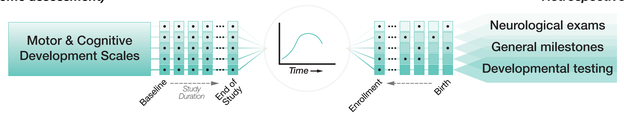
Motor and Cognitive Development Assessments

- Test of Infant Motor Performance Screening Items (TIMPSI) [motor]: Measures postural and selective control of movement needed for functional motor performance in early infancy
- Gross Motor Function Measure (GMFM) [motor]: Measures gross motor function in children between 5 months and 16 years
- Bayley Scales of Infant Development (BSID)-III [motor, cognitive, language]: Measures gross motor and fine motor developmental functioning as well as expressive and receptive communication and cognition development in children between 1 and 42 months
- Hammersmith Infant Neurological Examination (HINE)-2 [motor]: Measures head control, sitting, voluntary grasp, ability to kick in a supine position, rolling, crawling or bottom shuffling, standing, and walking between 2 and 24 months
- Denver-II: Measures developmental progress in infants and children up to 6 years of age in gross motor, language, fine motor-adaptive, and personal-social functions
- Canavan Disease Assessments: Measures disability/ability in several common and important domains in CD derived from the elements of a previous natural history study in Canavan disease[23]

Prospective Data Collection (via at-home assessment)

- Performed at the patient's home by a highly trained Rater
  - Raters with particular skills, expertise, education, and knowledge will be rigorously trained through a prospectively defined certification program
  - Administration of the scales in the patient's home relieves travel/expense burden on the patient and family

UNDERSTANDING OF FUNCTIONAL/DEVELOPMENTAL DECLINE



Retrospective Data Collection (via medical records)

- A specialized medical records retrieval service will relieve the burden from consenting, opted-in families by collecting, reviewing, and extracting relevant data from the medical records of patients with Canavan disease
- Relevant data will populate fields that correspond to the prospective scales

Biochemical and Laboratory Assessments

- Urinary NAA Levels: NAA is the direct substrate for the ASPA enzyme, and NAA levels accumulate to pathologically elevated levels in Canavan disease
- Biomarker and Genetic Mutation Analysis: Measurement of ASPA enzyme levels in blood cells and characterization of genetic mutations and variability among patients with Canavan disease may contextually frame genotype, pathophysiology, and disease severity
- Routine Laboratory Tests: Measurements may reveal other trends in patients' physiology

Neurological, Diagnostic, and Imaging Assessments

- Neurological Exams: Level of consciousness, expression/reception of language; temperament; ocular function; truncal and appendicular tone (including active/passive, and gross/fine motor function, reflexes [including deep tendon reflexes], plantar response, presence of clonus, primitive reflexes; withdrawal to touch, presence of adventitious movements, ability to bear weight, and gait, if ambulatory); muscle bulk; and presence of contractures
- Diagnostic Tests: Nerve conduction velocities; visual evoked potential
- Standard of Care (Historical Records): Imaging (MRI), hearing, and neural conduction assessments

Parent/Caregiver Assessments (Parents as Respondents)

- Pediatric Quality of Life Inventory (Family Impact Module) (PedsQL-FIM): Measures parent self-reported physical, emotional, social, and cognitive functioning; communication; and worry
- Vineland Adaptive Behavior Scale: Measures the level of a patient's personal and social skills required for everyday living, including communication, daily living skill, socialization, motor skills, and maladaptive behavior
- Canavan Disease Questionnaire: Measures aspects of life and functioning specifically in patients with Canavan disease

Summary

- This multi-center, multi-national retrospective and prospective natural history study will collect and assess data of disease characteristics that are caused by the neurodegenerative disorder of Canavan disease
- Clinical sites are actively enrolling participants
- The study is expected to continue for 3 years with no maximum on enrollment so that sufficient data can be collected to characterize the natural history of Canavan disease
- The natural history database will be available to physicians and scientists for meaningful research towards treatment of Canavan disease

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