

Canavan Research Summit 2008 Synopsis



The first Canavan Research Summit was organized in an effort to bring together scientists and physicians from around the world that are currently working on defining the molecular, biochemical, pharmacological, developmental, and clinical correlates of this disease. The bringing together of individuals from diverse scientific backgrounds is intended to foster the sharing of information and collaborative efforts that will contribute to the identification of avenues of therapeutic intervention.

This initial meeting provided the opportunity to comment on the collective knowledge of Canavan disease. Although defined by mutations to a single known gene, *aspartoacylase* (*aspa*), the exact nature of the mechanism(s) causative for the disease phenotype remain elusive. Currently, elevated N-acetylaspartate (NAA) in the brain and the loss of ASPA catabolic activity remain the primary diagnostic indices of what is generally believed to be a homogenous phenotype. However, there are an increasing number of clinical case reports of atypical disease progression that are associated with specific mutational events, suggestive of a spectrum of phenotypic severity. Data presented by Dr. Kolodny at the summit documented the existence of a severe, early onset phenotype associated with large deletions in *aspa* (Zeng et.al. 2006. Mol Genet Metab 89:156-63), and a contrasting milder form of disease associated with a point mutation that converts an arginine to a histidine at position 71 of the ASPA protein (Janson et.al. 2006. Ann Neurol 59:428-431). This atypically mild form was noted to be associated with lower than expected levels of NAAG, with possible implications for the amount of glutamate present in the brains of Canavan patients. This clinical observation appears to be complimented somewhat by the reported increase in brain NAAG concurrent with NAA in the *aspa*-null tremor rat by Dr. Burlina, and the possibility of increased extracellular NAA resulting from the catabolism of axon-derived NAAG by NAAG-peptidase on the surface of astrocytes was proposed by Dr. Baslow. Although there is clearly some agreement as to the prominence of NAAG in both patients and the animal model, it is unclear at this stage to what degree, if any, either of NAA or NAAG contribute directly to pathology.

The absence of a definitive function for NAA is prominent in the field of Canavan research. A recurring theme in the field is a working hypothesis that defines NAA as a source of raw materials for myelin lipid synthesis. Data was presented by Dr. Ledeen that demonstrated a reduction in specific cerebroside and sulfatides in the tremor rat model (Wang et.al. 2008. Neurochem Res). Data from two age groups, 1 and 7 months, was presented, and an apparent increase in sulfatides with 2-hydroxyfatty acids was evident at 7 months and a transient increase in two phosphatidylcholine species evident at 1 month. The consequences of these changes for phenotype are unknown, but may compromise developmental myelination and/or myelin homeostasis. The possibility of reductions in free acetate being causative in Canavan disease was explored in Dr. Madhavarao's study in which glyceryltriacetate (GTA) was administered to tremor rats. GTA treatment was shown to improve the abundance of some lipid species and was associated with improved

motor performance. Toxicity studies have been performed by Dr. Namboodiri in tremor rats, demonstrating the absence of detectable adverse biochemical, histopathological or behavioral side effects consequent to GTA treatment. GTA has also been extended to the clinic with no apparent toxicity. Clinical efficacy data was not presented. The question of whether the biochemical and behavioral improvements in tremor rats consequent to GTA treatment improves global myelination is unknown.

A novel mouse model of Canavan disease was presented by Dr. Popko. This mouse harbors a nonsense point mutation in *aspa* (Q193X), and expresses no detectable ASPA protein. This model exhibits abnormally high CNS NAA and prominent vacuolization in grey matter. This model appears to exhibit regional differences in myelination throughout postnatal development that precede pronounced axonal degeneration, suggesting that developmental myelination and degeneration are separated in this model. The availability of this novel mouse model provides an opportunity to test intervention strategies, and to potentially standardize developmental and pathological end point measures that are poorly defined in currently utilized rodent models. Data on the analysis of developmental abnormalities in a pre-existing mouse model (Matalon et al., 200. *J Gene Med* 2:165-175) was presented by Dr. DeVellis that indicated abnormalities in the oligodendrocyte lineage. This data identifies a cellular deficit in this animal model and, together with the analytical end points presented for the novel Q193X mouse, provides a point of focus for the assessment of intervention strategies.

Modeling Canavan pathology in a systematic fashion is made difficult not only by the paucity of knowledge regarding NAA function, but also by how little is known about the characterization of ASPA. Data on structural modeling of the ASPA protein was presented by Drs. Bitto and Viola. Homology modeling of the protein suggests that ASPA is a member of the metalloproteinase family of enzymes, with the enzyme exhibiting a zinc-dependent mode of action. Studies performed using a stable intermediate analogue have assigned substrate binding groups within the protein that may provide targets for the analysis of function in biologically intact systems relevant to the Canavan phenotype, particularly with respect to specific clinical mutations.

Researchers outside of the established Canavan field presented data that could provide insights into Canavan pathology. Data generated in studies of transgenic mice harboring mutations in specific myelin proteins was presented by Dr. Nave that has been instrumental in the development of a hypothesis for a role for glia in the support of axonal function via the exchange of energy substrates. This role is independent of the established role of myelin in supporting impulse, and may be of relevance to degenerative mechanisms in animal models of Canavan disease. Studies on the relationship of NAA to cerebral metabolism were presented by Drs. Tavazzi and DiPietro that suggest changes in NAA in the traumatized brain reflect cerebral energy state. They showed that ASPA is a factor in regulating NAA levels under conditions of severe traumatic stress in a manner that may be linked to energy metabolism. A developmental perspective relevant to the oligodendrocyte lineage was presented by Dr. Casaccia-Bonnel that explored the regulation of genes involved in myelination by histone deacetylases. Perturbations to these regulatory mechanisms during the early stages of oligodendrogenesis was shown to

have consequences for developmental myelination, and in vivo studies of this nature may yield insights into oligodendrocyte deficiencies in Canavan disease.

The identification of targets for therapy was a major focus of this summit, and recent advances in stem/progenitor cell biology may be of relevance to Canavan therapeutic development. A selection of data on the characterization of human fetal neural stem cells was presented by Dr. De Filippis that summarized the possibilities for application to degenerative disorders of the central nervous system. The application of these cells to models of lysosomal storage disorders has demonstrated the potential of these cells to successfully engraft and correct a biochemical deficit in the brain. A prominent theme in stem cell biology is the ability of exogenous cells to support regeneration via trophic mechanisms that are independent of the differentiation of cells along a specific lineage pathway. This theme was expanded on by Dr. Snyder to support the idea of human neural stem cells specifically being suited to target areas of pathology in the host brain and provide trophic support. The possibility of repopulating oligodendrocytes within the brain is directly relevant to leukodystrophies, such as Canavan disease, and data generated in the application of oligodendrocyte progenitors derived from human embryonic stem cells to spinal cord injury was presented by Dr. Wirth. These cells have been through extensive pre-clinical characterization and evaluation, and present an opportunity to target remyelination in animal models.

The possible clinical application of stem cell therapy was discussed by Dr. Leone. Developmental studies performed in the tremor rat model appear to have highlighted abnormalities in the oligodendrocyte lineage that result in a significant reduction in late-stage cells that is associated with dysmyelination. Preliminary data demonstrating the successful engraftment of GFP-expressing oligodendrocyte progenitors was presented as a rationale for clinical development. The need for well-defined clinical end point measures is a requisite component of the assessment of therapeutic efficacy, and natural history data can provide a means of assessing efficacy in a statistically robust manner. The accumulation of natural history data in Krabbe disease patients and its application to mixed effects modeling was presented by Dr. Escolar. The natural history data generated was shown to be highly predictive of efficacy in patients receiving stem cell therapy, and can be considered a template for therapeutic design in Canavan disease.

Paola Leone, Ph.D.
Chair

Edwin Kolodny
Co-Chair