

Enabling Technologies 2002 Workshop Summary

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Introduction

This report summarizes discussions at the second workshop on Enabling Technologies for Alzheimer's Disease (AD), held in August 2002 in Bar Harbor, Maine. Participants included academic and industry scientists from both inside and outside the field of Alzheimer's disease research, foundation representatives, and a program officer from the National Institute on Aging. The goals were to identify major knowledge gaps that are limiting progress in AD diagnosis and therapy, and to generate and share ideas about how new technologies and strategies might help bridge those gaps.

The recommendations presented here emerged during the discussion and do not necessarily reflect an order of priority or the unanimous views of all participants.

Gene Discovery

One major impediment has been the slow pace at which genomic screens and subsequent association studies have identified new risk genes. Participants discussed several strategies to accelerate this process. One involved broadening association studies to include all genes under the strongest linkage peaks. Participants also expressed enthusiasm for developing a comprehensive systems-biology study-analogous to that used to identify critical gene clusters in hypertensive rats-to identify AD-related gene clusters and the relationships between them.

Recommendations:

1. Several dozen human diseases map to the chromosome 10 region. All the genes in this region should be analyzed with multivariate analysis to detect subtle interactions.
2. Brains of people with Mendelian diseases mapping to an AD linkage region should be examined for AD pathology.
3. Consider comprehensive systems biology study to find new risk genes for neurodegeneration, including AD, using biomarkers validated in the National Coordinating Center series.
4. Use yeast, fly, and worm to discover novel genes and potential targets in neurodegeneration-related pathways.
5. Develop screens to look for loss-of-function mutations (independent and upstream of A β PP/tau) in aging cohorts (of mouse mutants) assembled by three large neurology centers nationwide.

Pathways and Target Discovery

A majority of investigators agree that A β PP processing, A β generation, A β degradation, and A β aggregation play a major role in Alzheimer's disease. There is a growing consensus on the potential importance of low-n oligomeric species of A β and protofibrils. Investigation of the pathways surrounding A β PP processing must broaden to include all fragments generated during A β PP cleavage, as well as other targets and downstream pathways of Presenilin1. Bold approaches are warranted in the discovery of novel disease-modifying genes. The processes that lead to synaptic dysfunction and neuronal loss remain unclear and must be a high priority.

Recommendations:

1. Study mechanism of synaptic dysfunction/loss prior to neuron loss.
2. Elucidate function of A β PP and its metabolic products.
3. Support development of antibodies specific for A β in β -sheet conformation to use in screen for compounds that perturb the formation of nascent oligomers or protofibrils. Support development of long-red fluorophores and in-vivo multiphoton imaging of humans.
4. Support study addressing how β -sheet structures of aggregation-prone proteins form in vivo. Which are good drug targets to avoid stabilizing toxic forms?
5. By analogy to recent discoveries of the neurotoxic effects of cytoplasmic misfolding of prion protein, express cytoplasmic misfolding of A β in mouse neurons to examine impact on neurodegeneration.
6. Use yeast to study tau pathways and find genes regulated by AICD and A β -binding proteins.
7. Support study of nuclear transfer from AD cells into existing ES lines and/or the culture of neurogenic cells from AD brain. Consider feasibility of nuclear transfer from cells of people with familial Alzheimer's disease.
8. Discover other unknown factors that cause both amyloid and tau pathologies.
9. Develop novel strategies for neuroprotective drugs (other than NMDA antagonists).
10. Develop neuroimaging markers to image synaptic function and deficits. Identify best markers among current technologies and pursue them in parallel, including measurements of A β /plaque, as well as metabolic, functional, structural, and volumetric changes in the hippocampal region and the whole brain.

Biomarker Development

Key gaps impeding the progress of clinical trials are the absence of one or more biomarkers to permit enrollment of enriched trial populations and the lack of good endpoints or surrogate endpoints endorsed by clinicians and the Food and Drug Administration. Participants were enthusiastic about the development of an A β challenge test involving the use of antibody to block A β clearance in the periphery, thus allowing measurement of A β efflux from the brain; this could lead to inferences about the dynamics of A β load in the brain. Inexpensive, effective, and objective behavioral tests or imaging tests could serve as surrogate markers. Neuroimaging methods have been developed sufficiently to create a robust measure.

Recommendations:

1. Test sensitivity and specificity of potential biomarkers in available blood and DNA samples from deceased patients who were tracked by the National Coordinating Center. Do that prior to using those biomarkers in current series/new trials.
2. In cell lines of human samples, assay oxidative markers, plasma A β , presenilin axis, CSF phosphorylated tau, CSF GFAP, cholesterol, homocysteine, lipoproteins, oxidative load, inflammatory markers, calcium, intranuclear APP-CTF. Test to determine whether a combination of the above has sufficient sensitivity and specificity to be predictive.
3. Refine mass spectrometry of serum/CSF supernatant for biomarker development.
4. A β challenge test: temporarily block degradation and deliver A β -antibody to measure efflux from brain.
5. Study A β metabolism/clearance/breakdown products for new biomarkers.
6. Facilitate researchers' access to samples of people who are still normal (in large prospective studies such as the Framingham Heart Study).
7. Take list of genes known to modify neurodegeneration in animal models and determine whether serum levels of associated proteins change in humans.
8. Study A β PP fragments other than A β to determine whether production rates are affected by A β PP and PS mutations. Study how PS mutations affect other γ -secretase substrates.
9. Look for blood- or fibroblast-based marker among peripheral proteins whose expression is regulated by AICD and Notch.
10. Identify other important A β -binding proteins and how they work. Determine effect on aggregation and clearance.
11. Study muscle as potential peripheral biomarker tissue for products associated with A β PP processing, e.g. cellular nucleic acid binding protein (CNBP) binds BACE and

affects A β production. Elevated BACE levels linked to AD and also inclusion body myositis.

12. Support in-vivo imaging of mice to test sensitivity of biomarker candidates.

13. Develop fMRI markers of alterations in cognitive processing that occur before detectable anatomical changes.

Lead Discovery

Lead discovery is hampered by the absence of good cell-based assays in which to screen libraries against A β PP secretases and other targets. One participant presented a new strategy for using fluorescent proteins in cell-based assays for drug screening. Application of this strategy to in vivo studies would require acceptable endpoints, as well as the refinement of fluorophores active in the long-red spectrum for imaging in live mammals. A bottleneck slowing down structure-based drug development is the paucity of BACE crystal structures and the complete absence of structures for presenilin-inhibitor complexes.

Recommendations:

1. Develop bioluminescence assays to screen drug libraries for compounds affecting tau/A β
2. Encourage qualified structural biologists to seek crystal structures of the presenilin complex, providing a foundation for structure-based drug design.
3. Investigate pathway around presenilin to look for components that are more suitable as drug targets.
4. Use worm and fly to study control of synthesis, assembly, trafficking, and specificity of β -secretase complex. Better understanding of presenilin biology will be essential to assess therapeutic index of γ -secretase inhibitors and overcome industry reservations.

Mouse Models

A key controversy revolved around the value of available mouse models. Current A β models are only partial models of AD; they recapitulate amyloid deposition, gliosis around neuritic plaques, subtle synaptic changes and neuritic damage, and subtle learning and behavioral deficits. Their key shortcomings are that most do not exhibit neurofibrillary pathology, and none show the massive neuronal loss seen in AD. Transgenic models of tau pathology also mimic only parts of the disease. Defenders of the models said that a large number of strains have independently produced similar data. Technical criticisms included the possibility of artifacts caused by overexpression of A β PP with physiologically irrelevant promoters, the possibility of variegated silencing of transgenes, and the use of cDNA transgenes rather than genomic DNA. Suggested

improvements included generating knock-in strains to express human genes under the control of endogenous mouse regulatory regions, and the creation of a series of mice on a common background that are deleted for different parts of suspected pathways. Suggestions made with regard to current strains involved setting up modifier screens to identify additional risk factor genes, and crossing them to strains deleted for other genes in AD linkage regions.

Recommendations:

1. Investigate existing resources in mouse genetics or set up a new program to systematically create a series of knockouts focusing on AD-relevant pathways using a common genetic background.
2. Cross the best A β PP mice to other knockouts to find modifiers (candidates might include the ER stress mouse or the oxidative stress mouse). Cross the best A β PP mice with strains deleted for genes in the AD linkage region.
3. Support knock-in strains of A β PP and other genes of interest.
4. Support creation of models for AD-relevant measures of plasticity: electrophysiology/LTP and synaptic markers.
5. Study how mouse models differ from humans to identify human AD modifier genes.
6. Support development of portal for repeated CSF withdrawal and drug delivery to mouse spinal cord.
7. Establish mechanisms to facilitate getting the needed strains into the hands of willing investigators.

Infrastructure Development

Recommendations:

1. Facilitate access to blood samples from people in prospective studies so that experimental biomarkers can be tested in stages prior to disease. Help develop standards for sample collection that preserve RNA and protein for further analysis.
2. Facilitate creation of lymphocyte cell lines, perhaps beginning with Down's syndrome cohort.
3. Fund feasibility of MRI-guided ultrasound delivery of imaging ligands and therapeutic compounds.

4. Set up working group to import additional information management tools into Alzforum website, to support mechanisms for sharing information on preliminary data, negative results, equipment, etc.
5. Help implement mechanisms to improve sharing of mouse strains, antibodies, cell lines, genetic constructs, and other research materials. Support housing/breeding of mouse strains, antibody preparation.
6. Investigate funding companies to produce and distribute sought-after antibodies faster and more efficiently than is possible in academic labs. Oblige everyone receiving foundation support to sign pledge to share information and reagents.
7. Facilitate forging of consensus among drug makers and clinicians on what efficacy endpoints/surrogate markers to measure and how, before taking any compound into clinic. For MRI/PET/SPECT: facilitate consensus on markers other than cognitive change. Begin now to develop consistent strategy vis-a-vis the FDA.
8. Facilitate establishment of accessible databases about Down's syndrome populations at brain centers to encourage more natural history studies in subpopulations.
9. Initiate communication with people who have conducted 10-year trials on other late-onset degenerative diseases, such as osteoporosis, for shared lessons.

Participants at the 2002 workshop

Susan L. Ackerman, Ph.D., Associate Staff Scientist, The Jackson Laboratory

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Gabriel Corfas, Ph.D., Children's Hospital, Boston

Peter Davies, Ph.D., Department of Pathology & Neuroscience, Albert Einstein College of Medicine

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