

SYMPOSIUM**NEUROGENOMICS: FROM CLONE TO CLINIC****Saturday, November 10, 2001****3:30–7:00pm****San Diego Marriott Hotel and Marina, Ballroom C
San Diego, CA, USA**

This preview presents speaker abstracts for the 2001 Promega Neuroscience Satellite Symposium at the Society for Neurosciences meetings in San Diego.

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Introduction

Stem cell research, an aging population, and the devastating effects of neurodegenerative disease have brought neuroscience research to the forefront of science news. However, the complexity of the mammalian nervous system provides significant challenges to the life science researcher. Dissecting the genetic, environmental and biochemical events that converge to create the vastly complex nervous system requires expertise from a variety of disciplines and technologies. How does science proceed from clinically presented pathologies to isolating and cloning the contributing genetic factors? And once the genes are identified, how does the information contained in these cloned pieces of DNA contribute to an understanding of biology in order to create workable therapies in the clinic?

In the Promega Neuroscience Symposium, *Neurogenomics: From Clone to Clinic*, researchers will present information describing the isolation of genes linked to neurological diseases such as Alzheimer's disease. They will show how work in model systems, such as *C. elegans* and *Drosophila*, is helping scientists understand the normal function of these genes in biological systems. Finally, presenters will describe current clinical research aimed at taking the exciting work of the research lab into the clinic for the development of novel therapies.

David Kaplan, Ph.D. from the Montreal Neurological Institute (MNI) will moderate the symposium. Kaplan is a professor in the Department of Neurology and Neurosurgery at McGill University and is Head of the Brain Tumour Research Center. Kaplan holds several patents as a result of his research and is a cofounder of Aegera Therapeutics, a company whose goal is the development of treatments for nervous system disorders.

SPEAKERS**David Kaplan, Ph.D. (Moderator)**

Montreal Neurological Institute

Donald L. Price, M.D. (Keynote speaker)

Johns Hopkins University School of Medicine

Peter St. George-Hyslop, Ph.D.

University of Toronto

Gabrielle L. Boulianne, Ph.D.

Hospital for Sick Children (Toronto)

Christopher A. Walsh, M.D., Ph.D.

Beth Israel Deaconess Medical Center

Harvard Institutes of Medicine

The Value of Transgenic and Gene-Targeted Models for Studies of Neurodegenerative Diseases

Donald L. Price, M.D.

The neurodegenerative diseases, including Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), are associated with characteristic clinical signs, specific genetic risk factors, dysfunction or death of specific neurons, and disease-defining pathological and biochemical abnormalities. Identification of gene products implicated in disease has led to the development of engineered models that have proven useful for understanding disease mechanisms and designing and testing novel therapies.

Some cases of autosomal dominant AD (FAD) are caused by mutant genes encoding the amyloid precursor protein (APP) or presenilins (PS1 and PS2). A β , a 4kD peptide generated by the cleavage of APP by β -secretase 1 (BACE 1) and by γ -secretase (influenced by PS1 and PS2), is deposited in the brains of individuals with AD. Mutations in APP and PS genes affect the levels or length of A β (A β _{1–40} or A β _{1–42}). Studies of transgenic (Tg) and gene-targeted model organisms can clarify the mechanisms of the cellular abnormalities.

Tg mice that overexpress Mo/Hu APP_{swe} show elevated levels of A β ₄₀ and A β ₄₂ in the brain and develop A β deposits in brain surrounded by enlarged dystrophic neurites and glial cells (neuritic plaques). Although not a model for AD, because, for the most part, the neurons lack tau pathology, these mice are excellent models of A β amyloidogenesis. The effects of introducing or ablating other genes can be examined in these animals. For example, Tg mice that co-express mutant A246EHuPS1 and Mo/Hu-APP_{swe} show an acceleration of the pathology. Gene targeting allows examination of the

consequences of ablation of specific genes on phenotype and pathogenic processes.

PS1^{-/-} mice fail to survive beyond the early postnatal period and exhibit severe perturbations in development, reflecting disrupted Notch-1 processing. Cultured PS1^{-/-} cells exhibit reduced levels of A β , indicating that PS1 influences APP processing events that lead to the formation of A β .

Recently, Price's group has targeted BACE 1. The major BACE 1 cleavage site generates +11 CTF and, with γ -secretase cleavage, A β _{11-40,42}. When BACE 1 null mice are mated to mutant APP/PS1 Tg mice, the progeny show no A β peptide in the brain. The enrichment of BACE 1 in brain (neurons), in concert with high levels of APP and low levels of anti-amyloidogenic alpha secretase and BACE 2 activities, determine, in large part, the vulnerability of the brain to A β amyloid pathology. Moreover, BACE 1 is an attractive target for therapeutic inhibition of A β formation in the brain. Similar Tg and gene-targeting strategies have been used to examine the mechanisms of other neurodegenerative diseases, including SOD1-linked ALS.

Genetics and Biology of Alzheimer's Disease

Peter St. George-Hyslop, Ph.D.

Genetic epidemiologic studies have indicated that about 40 percent of the population variance in risk for Alzheimer's disease (AD) is attributable to genetic factors. Molecular genetic studies focusing on pedigrees with autosomal dominant inheritance have identified four genes that confer susceptibility to AD: *presenilin 1* (PS1), *presenilin 2* (PS2), *β -amyloid precursor* (β APP), and *apolipoprotein E* (ApoE). Analysis of the biochemical roles of the proteins encoded by these genes indicates that they all influence the proteolytic processing of β APP, causing overproduction and accumulation of the neurotoxic derivative, amyloid β -peptide (A β), while one effect of the ApoE ϵ 4 allele is to reduce the clearance of A β . This commonality suggests that accumulation of neurotoxic A β is the central event in most forms of inherited and noninherited AD. A β accumulation then triggers a series of downstream events, including the misprocessing and accumulation of tau protein in neurofibrillary tangles (likely to be neurotoxic itself). Consequently, three therapeutic targets can be envisioned: i) blocking A β production by inhibiting one or both enzymes involved in cleaving A β from the β APP precursor (β -secretase and γ -secretase); ii) inhibiting the aggregation of A β monomers into toxic protofibrils; iii) augmenting the removal of A β . One such strategy is to induce an immune response to A β by active or passive

immunization. Early studies of two independent transgenic mouse models of AD reveal that immunization against A β does reduce the cognitive deficit [Janus *et al.* (2000) *Nature* **408**, 979–982] and the neuropathologic abnormalities. This suggests that vaccination and other strategies directed at producing and removing A β might be effective, alone or in combination, in blocking the disease process in humans.

Identifying Genetic Modifiers of Presenilins

Gabrielle L. Boulianne, Ph.D.

Alzheimer's disease (AD) is the most common cause of dementia in the aging population. Despite the significant advances that have been made over the past decade, it is increasingly clear that additional genes/factors must be involved in determining who is at risk for developing AD. A powerful method of identifying additional loci implicated in AD is to characterize genetic modifiers of genes such as presenilins or APP that have already been linked to AD. However, such modifier screens are currently difficult to perform in mice and impossible in humans. Boulianne's group uses the powerful genetic approaches available in *Drosophila* to systematically screen for genetic modifiers of presenilin. To date, they have identified over 70 modifiers of presenilins. To begin to prioritize these modifiers and test their relevance in AD, they have determined the ability of the modifiers to interact with the transmembrane receptor protein, Notch, and APP. Also Boulianne's group has identified human homologs of these modifiers and determined their chromosomal localization. Several of the modifiers map to chromosomes that are linked to AD, and at least two of the modifiers have recently been implicated as risk factors in AD. This demonstrates the validity of the *Drosophila* model to identify causative or risk factors in AD. The results of their genetic screen will be presented. Genetic modifiers will provide insight into the normal biological roles of presenilins during development and provide insight into potential causative and risk factors for late-onset AD. More importantly, these genes could provide additional therapeutic targets for this devastating disease.

Cerebral Cortical Development: Connecting Developmental Neuroscience and Human Genetics

Christopher A. Walsh, M.D., Ph.D.

While mice and zebrafish are perhaps the more familiar vertebrate systems used in genetic studies of the developing forebrain, the completion of the human genome sequence gives scientists a tremendous opportunity to learn more about normal brain development as well as human disease. Statistical analysis

suggests that any mutation that can cause an observable phenotype in the cerebral cortex is already present in humans somewhere in the world. The phenotypes of such mutations are easy to find because of the burden of disease they cause: autism, epilepsy, mental retardation and other learning disorders all seem to stem, in whole or in part, from disordered development of the cortex. Finally, MRI and other technologies have given us the capability to image the human brain, both its shape and its function, noninvasively in exquisite detail.

Research in the Walsh lab is aimed at identifying and studying genes that cause developmental disorders of the human brain. For example, an X-linked gene called filamin 1 (FLN1) encodes an actin-binding protein that is required for the initiation of migration of neurons out of the proliferative region where they are produced. FLN1 mutations block the migration of some neurons, causing some cortical neurons to develop in the wrong place (heterotopia). Surprisingly, affected patients usually have normal intelligence, although they usually suffer seizures. Mutations in another X-linked gene, *DCX*, allow neurons to leave the ventricle, but the neurons only migrate halfway to the cortex; these patients have severe cognitive and epileptic problems. *DCX* seems to regulate microtubule function by binding directly to $\alpha\beta$ tubulin. Other human genetic mutations (e.g., *Reelin*) allow neurons to migrate all the way to the cortex, but upon

their arrival in the cortex, the neurons do not organize themselves properly. Although *Reelin* encodes a large extracellular matrix protein, its mode of action is uncertain. Recent work in animal models shows that the Reelin protein acts as a stop signal for migrating neurons, causing them to dissociate from radial glial cells that normally guide them. Moreover, Reelin binds to $\alpha3\beta1$ integrin, protocadherins, and LDL superfamily receptors, but its antimigratory effect appears to depend upon $\alpha3$ integrin.

A remarkable variety of simple Mendelian disorders of human cortical development exist. Many of these loci have been mapped, and some others have been cloned. Human mutations can cause decreased cortical size (microcephaly), presumably reflecting defects in proliferation or survival, or can produce a cortex that is normal in certain regions but abnormal in others. Other mutations produce a brain of normal size and shape but appear to cause mental retardation by disrupting axonal or dendritic development (e.g., *PAK3*). Further genetic analysis of these disorders will help us understand how our brains developed, both in terms of ontogeny and phylogeny, and also will help us understand the causes of many common human neurological disorders. This research is supported by the National Institute of Neurological Disorders and Stroke and the March of Dimes.

