The National Prion Research Program (NPRP) Congressionally Directed Medical Research Programs

History: The Congressionally Directed Medical Research Programs (CDMRP) emerged from a powerful grassroots effort led by the breast cancer advocacy community that convinced Congress to appropriate funds for breast cancer research. A unique partnership, forged among the public, Congress, and the military, CDMRP was established within the US Army Medical Research and Materiel Command (USAMRMC) in fiscal year 1993 (FY93) to manage these funds. CDMRP has grown to encompass multiple targeted programs and has received almost $4 billion (B) in appropriations through FY06. Funds for CDMRP are added to the Department of Defense (DOD) budget where support for individual programs, such as the National Prion Research Program, is allocated with specific guidance from Congress.

Background: Transmissible spongiform encephalopathies (TSEs) are a group of related diseases that include Creutzfeldt-Jakob disease (CJD) and its new variant (vCJD), kuru, scrapie, bovine spongiform encephalopathy (BSE; "mad cow disease"), chronic wasting disease (CWD) in deer and elk, and others. These diseases have incubation periods of years or decades, cause progressive neurological degeneration, evoke no obvious immune response, and are invariably fatal. It is theorized that TSEs are caused by prions, normal cell membrane proteins that form atypical, three-dimensional configurations. These misfolded prion proteins trigger a cascade of events causing additional normal prion proteins to misfold and aggregate in central nervous system tissues, resulting in the symptoms of prion diseases. Although a Nobel Prize was awarded for the work underlying this proposed mechanism (Prusiner, 1997), the theory is controversial, because some scientists question whether a protein by itself can cause disease and believe that another infectious agent may induce the changes in the prion protein.

Prion disease in sheep, known as scrapie, has been recognized for centuries and controlled by removing infected animals from herds (culling). When BSE was identified in cattle in the 1980s, it was also controlled by culling infected animals. Prion diseases in humans are relatively rare. The incidence of CJD in humans is about 1 in 1 million; about 10% of such cases are inherited. However, the potential impact of prion diseases on human health was greatly magnified by the recognition that the transfer of BSE in cows to humans by beef ingestion resulted in vCJD. Changes in animal feed constituents and slaughter practices appear to have curtailed vCJD, but there is concern that CWD of free-ranging deer in the United States might be transferred from deer to humans through venison consumption. Whether BSE and CWD represent a transfer of scrapie in sheep to cows and deer or are newly arisen prion diseases is unknown. The possible transmission of prion disease through other food animals also cannot be ruled out. There is evidence that vCJD can be transmitted through blood transfusion. It is possible that an unknown number of asymptomatic individuals are infected with vCJD from BSE, CWD, or scrapie. This potential threat to blood and plasma supplies is of great concern to both civilian and military health professionals.

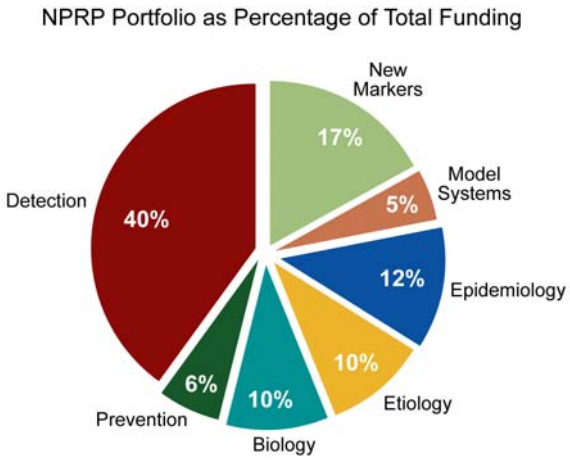
Threats to the food and blood supplies are serious because the incubation time for prion diseases is so long and there is no reliable diagnostic test for the presence of prion disease in living animals or humans (an antemortem test). Definitive diagnosis can be made only by examining central nervous system tissue at autopsy. Detection by measuring an immune response in affected animals or humans has not been possible. The development of a diagnostic test to detect misfolded prions in peripheral tissues such as blood depends on surmounting two major obstacles. First, the misfolded prion protein (PrP^{Sc}) is so structurally similar to the normal prion protein (PrP), that it is difficult to differentiate between the two. Secondly, PrP^{Sc} exists in extremely low concentration in blood or other peripheral tissues that could be used in antemortem detection assays, as compared to central nervous system tissue.

National Prion Research Program Vision:

To eliminate the occurrence of human transmissible spongiform encephalopathies.

History

Health threats posed by prion disease appear to involve the food and blood supplies and put military beneficiaries in affected areas overseas at risk. Such areas include any location in which cattle have been diagnosed with prion disease and anywhere beef may have been shipped from these areas for human consumption. People who have ingested contaminated beef may unknowingly transmit prions via blood donations. Concerns over potential contamination of the food and blood supply prompted the US Congress to provide the largest single appropriation in history for prion-related research – $42.5M – to establish the NPRP in FY02. The primary goal of the FY02 NPRP was to develop a rapid, sensitive, and reproducible test for the detection of misfolded prions suitable for use as an antemortem diagnostic test as well as a screening assay. In support of this goal, proposals also were solicited to better understand the prevention, transmission, and pathogenesis of prion disease to include chronic wasting disease. Proposals with military relevance were specifically sought. Award recipients began research in mid-2003 with performance periods ranging from 3 to 5 years. Congress has not provided any follow-up appropriations for the NPRP.



The NPRP Portfolio

The 38 proposals funded by the NPRP represent an exceptionally broad range of projects that bring new methodologies and additional expertise to the detection, prevention, and treatment of prion diseases.

The improvement of diagnostics is supported by 23 NPRP awards. Nineteen of these awards have the direct development of sensitive and reproducible antemortem diagnostics and/or the discovery and development of new disease markers as their primary focus. These proposals use a wide range of detection platforms and methodologies including development of new immunological, physical, and chemical detection methods, as well as optimization and standardization of existing methods. An additional four awards are directed toward developing experimental models to facilitate more sensitive and rapid detection of the disease agent in living systems. Assessing risk to the military is supported by development of tests that can detect prions in blood or tissues. Basic research is supported by 15 NPRP awards. Of these awards, 11 are focused on the biology, epidemiology, and etiology of prion diseases. These awards are expected to provide important insight into the further development of methods for the detection, treatment, and prevention of prion diseases. An additional four awards are designed to develop potential

methods to prevent infection or progression of prion diseases. These studies include the development of prion-resistant instruments, vaccines, inhibitory RNA molecules, monoclonal antibodies, and traditional drug screens.

Prion research infrastructure is supported by the funding of 15 new investigators and 40 pre- and post-doctoral trainees, expanding the intellectual foundation of the prion research field. An important research resource supported by the NPRP is a flock of 100 sheep bred to be highly susceptible to scrapie – most of these animals become symptomatic at 2 to 3 years of age. This flock serves as a source of large volumes of pooled brain homogenate and plasma, which can be used for standardized reference materials.

NPRP Research Highlights

Individual Success Stories

Dr. Stanley Prusiner of the University of California San Francisco has made important advances towards developing a sensitive and specific diagnostic test for prion disease by clarifying the biology of disease progression, elucidating chemical conditions for precipitation of pathogenic prion protein PrP^{Sc}, and establishing and validating new diagnostic protocols. Dr. Prusiner and colleagues developed a reliable fluorescence-activated cell sorting protocol to isolate subpopulations of human white blood cells from which they can measure PrP^{Sc} using a conformation-dependent immunoassay (CDI). They validated the CDI for diagnosis of human prion diseases and demonstrated 100% sensitivity for PrP^{Sc} in brain tissue and 58% sensitivity for PrP^{Sc} in muscle tissue of CJD patients. Using surface-enhanced laser desorption ionization – time of flight mass spectroscopy and protein microarrays in mouse models of prion disease, Dr. Prusiner's team identified 53 unique putative biomarkers for CJD. In parallel work aimed to increase the sensitivity of the CDI by precipitation of prions, Keggin-type polyoxometalate (POM) complexes demonstrated superior ability to selectively precipitate disease-causing PrP^{Sc}. The researchers propose that prion aggregation may involve multivalent electrostatic interactions between the POM anions and positively charged prion cleft sites.

- Safar JG, Geschwind MD, Deering C, et al. 2005. Diagnosis of human prion disease. *Proceedings of the National Academy of Science USA* 102:3501-3506.
- Lee IS, Long JR, Prusiner SB, et al. 2005. Selective precipitation of prions by polyoxometalate complexes. *Journal of the American Chemical Society* 127:13802-13803.

Dr. Shu Chen of Case Western Reserve University is developing improved immunochemical methods for antemortem detection of PrP^{Sc}. Based on a report that PrP forms complexes with nucleic acids, Dr. Chen and colleagues generated a panel of monoclonal antibodies to nuclear DNA, then screened the anti-DNA antibodies for their ability to capture PrP^{Sc} from homogenates of diseased brain. They identified the monoclonal antibody OCD4, which specifically binds PrP^{Sc} with high affinity but does not bind PrP. OCD4 can be used for immunocapture of PrP^{Sc} from brains of patients with sporadic, familial, or vCJD, as well as animal brains infected with BSE and scrapie. Dr. Chen's group then showed that the gene 5 protein from the Ff bacteriophage, a widely available and well-characterized single-stranded DNA binding protein, can also bind PrP^{Sc} with high sensitivity and specificity. These findings suggest that anti-DNA antibodies may be used to develop new strategies for the study, detection, and treatment of prion diseases.

- Zhou Q, Zheng J, Gray DM, et al. 2004. Antibody to DNA detects scrapie but not normal prion protein. *Proceedings of the National Academy of Science USA* 101:1380-1385.

Dr. Laura Manuelidis of Yale University has focused on identifying patterns of gene expression caused by CJD infection rather than on PrP misfolding that occurs late in disease. Myeloid microglia purified from the brains of mice experimentally infected by intracerebral inoculation with CJD were first used to identify a set of genes that showed increased expression (upregulation) versus controls. These genes were then used to screen CJD infected brain. A subset of these genes were detected as early as 20-30 days after inoculation, well before misfolded PrP and neurodegenerative changes appear. These upregulated genes, including L-selectin, myeloid cell recruitment factors, and pro-inflammatory activators, are all involved in inflammation and represent innate immune responses to the foreign CJD agent. Dr. Manuelidis and colleagues also developed a co-culture system using a mouse hypothalmic cell line to explore the infection process in the absence of immune cells. In this system, infection with one strain of CJD or scrapie can protect a cell from superinfection with a second TSE strain. Interestingly, this interference phenomenon is not related to the presence or absence of misfolded PrP.

- Nishida N, Katamine S, and Manuelidis L. 2005. Reciprocal interference between specific CJD and scrapie agents in neural cell cultures. *Science* 310:493-496.
- Lu ZY, Baker CA, and Manuelidis L. 2004. New molecular markers of early and progressive CJD brain infection. *Journal of Cellular Biochemistry* 93:644-652.

Dr. Byron Caughey and Dr. David Kocisko of the Laboratory of Persistent Viral Diseases of the National Institute of Allergy and Infectious Diseases are seeking to identify prophylactic or therapeutic compounds for prion diseases. Their strategy for high throughput screening is based on interfering with the conversion of PrP to PrP^{Sc} in a model system using neuroblastoma cells chronically infected with scrapie. The cells were grown in 96-well plates and assayed for PrP conversion by dot blot. In screening thousands of compounds, they identified numerous new classes of inhibitors including polyphenols, phenothiazines, statins, antihistamines, porphyrins, antimalarial compounds, and phosphorothioate oligonucleotides (PS-ONs). Inhibitory compounds identified by screening were administered to transgenic mice that had received intracerebral or intraperitoneal challenge with hamster scrapie. The porphyrin Fe(III)meso-tetra(4-sulfonatophenyl) porphine and the PS-ONs greatly increase survival times in these mice.

- Kocisko DA, Vaillant A, Lee KS, et al. 2006. Potent antiscrapie activities of degenerate phosphorothioate oligonucleotides. *Antimicrobial Agents and Chemotherapy* 50:1034-1044.
- Kocisko DA, Caughey WS, Race RE, et al. 2006. A porphyrin increases survival time of mice after intracerebral prion infection. *Antimicrobial Agents and Chemotherapy* 50:759-761.

http://cdmrp.army.mil/nprp