NEURODEGENERATION IN MICRONESIA

UCSD-UNIVERSITY OF GUAM COLLABORATIVE RESEARCH PROJECT – SUMMARY

OVERALL ORGANIZATION OF THE PROJECT (FUNDED BY A PO1 GRANT FROM THE NATIONAL INSTITUTE ON AGING (NIA)):

This report describes a P01 grant from NIA to UC San Diego (UCSD). The Project PI originally was Dr. Wigbert Wiederholt, and Dr. Douglas Galasko took over as PI in 2000 after Dr. Wiederholt retired as PI because of illness. The P01 involved a cooperative agreement between UCSD and the following organizations:

1. University of Guam, where a clinical core was maintained (Director Douglas Galasko, MD, Site coordinator Ulla-Katrina Craig, DrPH). Clinical Core support and Data Core activities were maintained at UCSD (Wigbert Wiederholt, PI, and later Douglas Galasko, MD)
2. University of Washington, Seattle, WA, (Gerard Schellenberg, PhD, PI) where a DNA Core was maintained and a Project investigated genetic risk factors for ALS-PDC and late-life dementia.
3. Mount Sinai School of Medicine, NY, (Daniel Perl-PI), Neuropathology Core, and a Project related to analysis of iron and free radical damage in brain tissue (Paul Good, PhD).
4. University of Pennsylvania Medical School, Philadelphia, PA (Virginia Lee-PI) - research project characterizing tau and other pathological forms of proteins in relation to neurodegeneration
5. Oregon Health and Sciences University, Portland, OR (Jeffrey Kaye-PI) - research Project involving volumetric analyses of MRI scans of research participants on Guam (years 6-10)
6. University of South Florida, Tampa, FLA (Amy Borenstein-PI) - research project on prevalence of ALS, PDC, and dementia among Chamorros, and associated risk factors (years 6-10).
7. University of Virginia, Charlottesville, VA (Davis Parker, MD, PhD), research project examining mitochondrial function in cybrid cells in relation to ALS-PDC (Years 1-5)

All aspects of the study required informed consent from study participants, and were monitored by the IRB at UCSD, and the IRB at University of Guam. Sub-studies or projects carried out at other institutions were also monitored by the local IRBs of each institution.

The project was coordinated by an Administrative Core at UCSD. This core maintained administrative and fiscal oversight over the project, coordinated a common database through a Data Core, and supervised Clinical research on Guam, including a population-based prevalence study in 2004-2005.

The Administrative structure also included an external Scientific Advisory Board, consisting of independent scientific investigators from 4 academic medical Centers in the US, who provided annual reviews and advice to the PO1 PIs through either an in-person meeting or a teleconference call.

A Guam Community Advisory Board provided feedback to investigators regarding aspects of conduct of the study on Guam.

RESEARCH PROJECTS CONDUCTED IN YEARS 1-5

**Project 1**: Epidemiology: of neurodegenerative diseases in Micronesia (Dr. W.C. Wiederholt, Dr. D. Galasko after 2000).

This project carried out assessments of patients with ALS and PDC and dementia, together with detailed family histories, medical and clinical characterization. Data from prior cohorts established on Guam in 1950-1970 were integrated into a computerized database. A risk factor questionnaire was developed. Case control studies were conducted. A cohort of subjects aged 75 and older was recruited to improve the match between cases of dementia and older controls.
Key research findings included:
1. Continued decline in the incidence of ALS. The incidence of PDC remained low, but new cases were consistently identified.
2. Clinical and psychometric comparisons were made between PDC and late life dementia in Chamorros. There were many areas of overlap including impairment of memory and slowing of cognition in both groups.
3. Follow-up of prior cohorts who underwent clinical assessment in the 1960s and 1970s did not reveal a clear familial influence on ALS and PDC.

Project 2: Genetic analysis of Guam ALS and PDC (PI: Gerard Schellenberg)
Pedigrees that were recorded during prior research projects on Guam (from 1950 – 1982) were integrated into databases, with the assistance of Dr. Chris Plato. Procedures were developed to collect blood samples on Guam for DNA preparation. These were shipped to Dr. Schellenberg and were banked. DNA was extracted for APOE genotyping. Genetic research continued throughout the entire funding period. DNA has been shared with ongoing independent studies, including a GWAS related to Progressive Supranuclear Palsy (PSP).

Key research findings included the following:
1. Low prevalence of the APOE 4 allele in Chamorros with ALS, PDC or late life dementia.
2. Polymorphisms related to the tau gene were associated with increased risk of ALS, PDC and late life dementia.
3. Refined mapping of the gene for the microtubule-associated protein tau (MAPTAU) was carried out. We found that novel genetic polymorphisms (SNPs) in the MAPTAU gene were associated with the risk of ALS, PDC or dementia among Chamorros on Guam. These SNPs overlapped with those that were risk factors for PSP in a Caucasian series of cases.
4. New statistical methodology was applied to improve pedigree analysis among Chamorros. This was complicated because of extensive inbreeding. Methods using MC/MC models were developed by Dr. Ellen Wijsman.
5. A genome-wide analysis of markers in familial cases as well as a case-control series revealed a number of new statistically significant associations. These are being pursued in further research studies, including comparisons with PSP.

Project 3: Neuropathology studies of preclinical ALS and PDC (PI: Dr. Daniel Perl)
This project aimed to determine the prevalence and distribution of neurofibrillary tangles in normal Chamorros, aged 50 or older, who were well-characterized clinically and whom brain specimens were obtained during autopsy. A series of tissue blocks and sections obtained on Guam in the 1960s and 70s, and previously stored in Perth, Australia, was obtained by Dr. Perl and moved to Mount Sinai Medical Center, NY. Tissue from that series was analyzed together with newly acquired brain samples.

Key research findings:
1. Amyloid deposition was more common in the newer series of patients than in the previous series. The prevalence of tangles increased with age, and was higher than would be expected in comparison with an age-matched group of brains selected from a brain bank at Mount Sinai Medical Center as part of a separate aging study in Caucasians.
2. The severity and distribution of tangle pathology in new cases of ALS and PDC on whom autopsies were obtained was identical to that of cases described in the 1950s.

Project 4: PHF-tau in neurodegenerative diseases of Micronesia (PI: Dr. V. M-Y Lee)
This project analyzed PHF-tau in relation to neurodegeneration in ALS/PDC. Varies extraction methods and biochemical analyses were performed on brain tissue from Chamorros. In addition, a transgenic
mouse model related to over expression of tau was developed. The transgenic mouse showed signs of motor neuron disease.

Key research findings:
1. Studies of human tissue showed that the abnormal forms of tau in ALS/PDC had a wide distribution but generally corresponded to cortical areas in patients with dementia, and motor neurons in patients with ALS. The biochemical nature of abnormal tau was similar in ALS/PDC as in Alzheimer’s disease.
2. A transgenic mouse that overexpressed tau and developed motor neuron degeneration was generated by Dr. Lee’s laboratory, with partial support through this PO1 funding.

Project 5: Oxidative stress in ALS and PDC (PI: Dr. Paul Good)
Dr. Good carried out a number of histological examinations for markers of oxidative damage in autopsy material in cases of ALS/PDC. No publications resulted from this project. This project was not considered for continuation in the renewal of the Guam PPG.

Project 6: Mitochondrial defects in Guam ALS/PDC (Dr. W.D Parker)
Dr. Parker’s project involved preparing cybrid cells from patients on Guam, to study markers related to mitochondrial electron transport. Initial findings showed differences between PDC patients and controls. However the long distances involved in shipping samples from Guam to the U.S. Mainland were problematic. This led to technical difficulties in interpreting the results of studies. No publications resulted from this project. This project was not continued when the grant was renewed in 2002.

In years 4-5, Dr. Ralph Garruto, State University of New York, Binghamton, NY, was awarded an Administrative Supplement by NIA to reorganize biological samples (brain tissue, plasma and DNA samples) that had been collected during 1959-1982, when the NINDS Field Station conducted research on Guam related to ALS and PDC. He developed a catalog of biosamples and provided brain and blood samples to Drs Schellenberg and Lee to support research Projects.

RESEARCH PROJECTS CONDUCTED IN YRS 6-10:

A grant renewal application was re-funded by NIA, and research continued in years 6-10. In addition to continuation of Projects 1, 2 and 4, the following new projects were included during years 6-10:

New Project 1: The prevalence of ALS, PDC, and dementia among Chamorros, and associated risk factors. (Amy Borenstein, PhD, University of South Florida-Tampa, PI)

New Project 3: Volumetric analyses of MRI scans of research participants on Guam, comparing controls and subjects with PDC and late-life dementia (Jeffrey Kaye, MD, Oregon Health and Sciences University, PI).

Progress during years 6-10

Project 1: Determine the prevalence of ALS, PDC and Late Life Dementia among older Chamorros on Guam, and risk factors associated with these disorders.

A planned prevalence study was delayed because 2 super typhoons struck Guam in 2002, leading to flooding and damage. We began the prevalence survey in 2003, targeting all Chamorros aged 65 and older on January 1, 2003. We generated publicity, met with village and community leaders, trained research staff, and finalized assessment instruments, questionnaires and procedures. According to the
2000 U.S. Census, there were 2770 Chamorros aged 65 or older on Guam. By using voters’ rolls, working with village mayors, and making telephone calls and home visits, we identified 2789 eligible Chamorros. From January 1, 2003 to March 31, 2005, we invited people to participate throughout the island. Subjects who consented were screened using the Cognitive Abilities Screening Instrument (CASI), followed by evaluation of screen failures and a percentage of those who passed the screen. A CASI cutoff of below 75, with sensitivity of 96% and specificity of 90% for dementia was used as a threshold for screen failures. Research assistants fluent in English and Chamorro contacted subjects and provided an initial home visit. Complete assessment included questionnaires for demographic information, cognitive symptoms, medical history and current medications. A brief examination of vital signs and a motor screen were conducted. The CASI was administered, together with the CERAD word list as a test of episodic memory. Questionnaires about risk factors, physical and mental activities and social interactions were administered. Family history was obtained. Blood was drawn for routine tests and DNA was prepared and banked. Evaluations were performed by 1 of 3 neurologists from the U.S., or a geriatrician on Guam who evaluated subjects at their homes or at a senior center, day care center or residential facility. Additional evaluation included neuropsychological testing on a subset of people who were normal or only mildly impaired, obtaining medical records from requesting physicians, and research MRI imaging on a subset. Two neurologists and a neuropsychologist reviewed all information to assign consensus diagnoses. Of 2789 eligible subjects, 2029 were screen and enrolled. Seven hundred and sixty (27%) could not be enrolled because 430 refused to participate, 280 died before they could be screened, and 48 were off-island. Another 45 were enrolled but died before February 1, 2004, which was defined as prevalence day.

Key Research findings:

1. We determined the prevalence of PDC and Dementia in Chamorros aged 65 or older (Galasko et al 2007). We found an overall prevalence of dementia of 12.3% in the elderly. Late-life Dementia was strongly associated with age, low education was a risk factor, but the APOE e4 allele was not associated with PDC or late-life Dementia.

2. We analyzed dietary reports of cycad and other traditional food usage in detail (Borenstein et al, 2007). Preparation or eating fadang, a starch obtained from cycad nuts that grow on Guam, which used to be a traditional food source, during early adulthood was significantly associated with PDC and Guam Dementia. This revives interest in the cycad hypothesis for ALS-PDC of Guam.

Project 2: Genetic analysis of Guam ALS and PDC (PI: Gerard Schellenberg)

Dr Schellenberg’s laboratory continued to carry out analyses of single nucleotide polymorphisms (SNPs) within and around the TAU gene.

Key research findings:

1. Three SNPs in the TAU gene were associated with increased risk of ALS, PDC and Late-life dementia. One of these was also associated with Progressive Suprauclear Palsy (PSP) in a series of DNA samples obtained from Caucasian patients with PSP. This suggests that while there are no causative mutations in the TAU gene, there could be subtle genetic variants that alter TAU mRNA levels or splicing and alter risk of Guam ALS-PDC as well as PSP.

2. Statistical techniques to deal with the complex pedigrees on Guam (with high ates f inbreeding) were developed, and used to analyze a genome-wide association study. This study yielded several markers with significant association with ALS/PDC vs controls.

Project 3: Volumetric analyses of MRI scans of research participants on Guam (PI: Dr. Jeffrey Kaye)
A total of 245 MRI scans were collected on 114 subjects, using a 1.5 T MRI located at a Radiology practice near Guam Memorial Hospital. Researchers from Dr. Kaye’s group helped to develop procedures to obtain reliable MRIs of research quality. Of the total 245 scans, 32 had technical issues, leaving 213 scans available for analysis. Of these, 185 scans (from 97 subjects) have complete volumetric analysis for the following regions: total intracranial volume, total CNS, subarachnoid space, Traced, ventricular CSF volumes, lobar, basilar region, hippocampus, high signal intensity volumes (deep, periventricular). Structures that are bilateral have volumes also divided by side.

Of the 97 subjects with completed regional volumetric analysis subjects have 1 to 5 scans analyzed. The number of scans per subject was as follows: 51 (of the 97 subjects) had one scan analyzed, 21 had 2 scans analyzed, 12 had 3 scans analyzed, 9 had 4 scans analyzed, and 4 subjects had 5 scans analyzed. The subjects scanned at baseline fell into the following 10 diagnostic categories (from the clinical database): Controls, MCI, Alzheimer’s disease, Probable AD, Guam AD (“Guam dementia”), Parkinson’ disease, Dementia, Alcoholic Dementia, Vascular Dementia, Diagnosed OD, and No Diagnosis.

Key research findings:
2. Control subjects with a positive family history of ALS/PDC had smaller brain volumes than those that lacked a positive family history, supporting our hypothesis that genetic factors may underpin susceptibility to ALS/PDC.

Project 4: PHF-tau in neurodegenerative diseases of Micronesia (PI: Dr. V. M-Y Lee)

Dr Lee’s laboratory identified the protein TDP43 as part of ubiquitin-positive inclusions in Fronto-Temporal Dementia and ALS. They extended this work to Guam (Geser et al 2008) by showing immunostaining of motor neurons and neurons in the frontal neocortex of patients with ALS and PDC of Guam. This suggests that there are common sets of proteins involved in neurodegenerative disorders where ubiquitin inclusions are formed.

CLOSE-OUT OF THE PO1, AND ARCHIVING AND STORAGE OF STUDY DATA AND MATERIALS:

After a new grant, submitted in 2008, was reviewed and not funded, we discussed options with the NIA and decided to close down the research program on Guam.

In March 2009, Dr. Ulla Craig (UOG) oversaw the disposition and secure storage of the clinical research data collected under this study. Administrative records were shredded and disposed of along with old survey forms, logbooks, etc. Records from the grant periods 1997-2002 and 2002-2009 were copied on microfiche and the cartridges have been kept for the record. Any remaining files have been de-identified (approx 2000) and are stored in locked file cabinets, and maintained in a locked and secured room at UOG. Old NINCDS files from the earlier NIH studies (1958-1986) are also stored in this secured location. While the files were generated specifically for these studies and are not for general release to participants, in the event of a medical emergency, the following process has been developed to allow family members access to this information. A family member must provide: a) reason for requesting access; b) show identification and relationship; c) provide power-of-attorney to act on behalf of subject. A copy of the pertinent information will then be provided to the requestor, and the request will be documented and become part of the record. The file will then be returned its secured location.
A newsletter describing the close-out of the study and procedures to store data and materials was sent to all study participants. In addition, a letter from Drs. Galasko and Craig was sent to all study participants informing them about the end of the Project and the efforts to store and make available study data and materials.

**DNA samples** are under the control of Dr. Gerard Schellenberg, now located at the University of Pennsylvania. These samples are limited in quantity as they represent DNA extracted from blood - transformed cells were not made.

**MRI Data:** All data exists as raw image data stored on CDs, and backed up to physically remote external drives. All data are available on request from Dr Jeffrey Kaye at Oregon Health and Sciences University, Portland, OR.

An extensive **brain tissue** repository is maintained under the control of Dr. Daniel Perl. This includes fixed and frozen brain tissue procured between 1999 and 2008. In addition, there are fixed samples retrieved from Perth, Australia, obtained in the 1970’s under the NINCDS project, as well as some fixed tissue obtained from Dr. Ralph Garruto. Dr. Perl has moved to the Armed Forces Institute of Pathology, Bethesda, MD, and maintains the Guam brain bank there.

An electronic database is maintained at UCSD in a secure server under the control of Dr. Douglas Galasko. It contains data from 1997-2009 as well as earlier datasets that were developed under the NINDCS field station on Guam. In addition, approximately 400 Plasma samples are stored at UCSD under the control of Dr. Galasko.

**AVAILABILITY OF DATA AND SAMPLES:**

UCSD maintains a website describing the Guam research studies. This lists the materials that are available and how to contact Dr. Galasko or other investigators to request access to the data, MRI images or biological samples. In addition, links to this information will be available through the National Institute on Aging.

**PUBLICATIONS:**

1996

1997


1998


1999


Nimchinsky EA, Gilissen E, allman JM, Perl DP, Erwin JM, Hof PR. A neuronal morphologic type unique to the hominids. Proc Natl Acad Sci USA 1999;96:5268-5273. PMID: 10220455


2000


2001


2002


2003


2004


2005

Montine TJ, Li K, Perl DP, Galasko D. Lack of BMAA in brain from controls, AD, or Chamorros with PDC. Neurology 2005; 65: 768-769. PMID: 16157919


2006


2007


2008


2009


2010
