

PI: **Mustafa al'Absi**, Professor and Max & Mary LaDue Pickworth Chair, University of Minnesota Medical School (U.S.A.)

Foreign collaborators:

Molham Al Habori, Professor and Dean, College of Medicine, Sana'a University, Yemen

Mohammed alSoofi, Professor and President, Taiz University, Yemen

Stephan Bongard, Professor, Fankfurt University, Germany

Anisa Dokam, Assistant Professor, Taiz University, Yemen

Saba Kassim, St Mary London University, U.K.

Najat Khalil, Associate Professor, Sana'a University, Yemen

Grant number: DA024626

Grant title: Khat Research Program: Neurobehavioral Impact of Long-Term Use

This program addresses the growing use of khat and its harmful effects on health in many countries in Africa and the Middle East and among immigrant communities in Western Europe and North America. Khat (*Catha edulis*) is a flowering plant native to tropical East Africa and the Arabian Peninsula. It contains cathinone, an amphetamine-like stimulant. It is a controlled substance in many countries, but is legally available in many others. Little is known about its addictive liability and its effects on brain functions. A partnership between the University of Minnesota and two universities in Yemen will establish the first ever multidisciplinary research and training program focusing on this substance. We will use this exploratory/developmental grant to achieve two primary goals. The first is to develop collaborative relationships and provide needed capacity-building resources that will include a series of research training workshops, establishing an Institutional Review Board, conducting relevant training on ethical standards of research, and organizing semi-annual meetings to develop future programmatic research. The second goal is to complete preliminary research to determine cognitive, affective, and biobehavioral consequences of long-term khat use. Khat users and nonusers will be compared on their performance on tests of response inhibition, attention, concentration, memory, and hormonal and cardiovascular responses to acute stress. The research is geared towards maximizing the potential for advancing khat-related research in this and surrounding countries, and will facilitate later development of research that will guide efforts to develop methods to reduce the harm caused by khat and the concurrent use of tobacco. We have assembled a multidisciplinary, international team with distinguished records and experience in all relevant topics. Both the training and research arms of the program will emphasize this breadth of expertise. This program will form a core part of our conceptualization of future research initiatives to further identify brain effects of khat and develop means for effective intervention strategies. Combating this problem will have significant benefits on the health conditions in many countries in the world.

PI: Rhoda Au, Ph.D.; Abu Abdullah, M.D., Ph.D.; USA

Foreign Collaborators: Jin-ling Tang, M.D., Ph.D., Chinese University of Hong Kong; Jia-ji Wang, M.D., Guangzhou Medical College; Li Yang, M.D., Ph.D., Guangxi, Medical University; Zhen-xin Zhang, M.D., Peking Union Medical College Hospital; China

Presenter: **Rhoda Au**

Grant Number: 1R21TW008855

Grant Title: Epidemiology of Mild Cognitive Impairment and Dementia in China

Abstract

The pilot study, Epidemiology of Mild Cognitive Impairment and Dementia in China, explored the feasibility of establishing a cohort study to identify prevalent and incident cases of mild cognitive impairment (MCI) and Alzheimer's disease (AD) and other types of dementia in rural and urban regions of southern China. Using the Framingham Heart Study of preclinical and clinical dementia as a model, a basic health examination and cognitive test battery have been field-tested for appropriate use in urban regions of Southern China. Additionally, relationships formed between Chinese collaborators were important for facilitating the next stage of project development. Several issues that were confronted included finding appropriate translators of Mandarin Chinese in the U.S., the variable research experiences of the China based teams, individual differences in work style (e.g., authoritarian versus a consensus building approach), and restricted resources to confront and overcome these issues. Further, the low literacy rate of rural residents did not make possible adaptation of cognitive tests. The lessons learned are now being applied to achieve the long term goal of establishing a longitudinal cohort study. With China's rapidly growing elderly population, a well-designed incident study will greatly enhance the understanding of AD/dementia in China, as well as provide important comparative data for identifying the role that environment and genes play in development of dementing illnesses

PI: Gretchen L. Birbeck; USA and Zambia

Foreign Investigators: zukanje Sikazwe, Omar Siddiqi, Lisa Kalungwana

Other US Investigators: *Michigan State University:* Michael J. Potchen & Melissa Elafros. *Harvard BIDMC:* Igor Koralnik & Omar Siddiqi. *US NIH William Theodore. Greater Lawrence Family Health Center:* Christopher Bositis

R21NS073509 A Cohort Study of Seizures and Epilepsy in HIV+ Zambian Adults

Study Sites: The University Teaching Hospital for University of Zambia and Chreso Clinics, Lusaka, Zambia

Background: The risk for recurrent seizure(s) among people with HIV/AIDS who experience new onset seizure is not known and extrapolation of data from HIV negative populations is likely inappropriate. Given the potential for drug interactions between antiepileptic drugs (AEDs) and antiretroviral therapies (ART), the decision to start an HIV+ individual on AEDs is critical. We are conducting an epidemiologic study of seizure outcomes among HIV+ Zambian adults with new onset seizure.

Objective: To conduct a prospective cohort study of HIV+ adult Zambians presenting with new onset seizure and Identify risk factors for recurrent seizures among those without a history of epilepsy.

Methods: Between 8/8/11–9/1/12, we enrolled HIV+ adults presenting with new onset seizure (within 2 weeks) who had a Karnofsky score of >50. Serum sodium, glucose, CD4 count, and malaria rapid diagnostic test, RPR, were obtained. CSF evaluation included cell count, differential, total protein, glucose, CRAG, and, in the case of positive serum RPR, VDRL. We also performed PCR on CSF to detect DNA from JCV, VZV, CMV, EBV, HSV 1 and 2, mycobacterium tuberculosis (MTB), and toxoplasma gondii (TG). Each patient had a routine EEG. We obtained neuroimaging if the initial evaluation did not reveal an underlying etiology. Patients were followed during their routine ART visits to ascertain if they experienced recurrent seizures.

Results: Of 180 HIV+ adults presenting with seizure, 61 (33.8%) met inclusion criteria and 37 (60.6%) consented to enrollment. Karnofsky < 50 and prior seizure were the commonest reasons for exclusion. Refusal of lumbar puncture was the commonest reason to refuse consent. Patient characteristics were median age 35 years, 21 females (56.8%), median CD4 124 cells/μl, with 7(33.3%) on ARVs. 10 (47.6%) had some focal aspects to their seizures and/or examination. Complete lab data was available on 34 patients. CSF PCR revealed 7 (20.5%) EBV DNA. One sample each was (+) for both in TB/EBV, JCV/EBV, and EBV/serum RPR. There were 2 (5.9%) cases of isolated JCV DNA and 4 (11.8%) cases of cryptococcal infection, 2 with VZV co-infection. 5 (13.5%) patients suffered recurrent seizures and another 7 (18.9%) died. 1 of these deaths occurred in a patient who died prior to completion of the full evaluation. CT scans were completed on 15 patients. There were 5 normal studies, 3 cases of multifocal and 2 cases of diffuse white matter abnormalities, 2 focal lesions with associated vasogenic edema and one case each of calcified granuloma, meningitis, and chronic stroke findings.

Conclusions: Seizures in HIV+ adults in Zambia are associated with advanced disease as well as significant morbidity and mortality. An infectious etiology can often be identified. Study

enrollment is currently ongoing and inclusion criteria have been liberalized to capture very ill patients (with no Karnofsky cut-off) as well as patients refusing lumbar puncture, who tend to be less symptomatic than those who agree. A K23 application expanding further upon diagnostic evaluations and aimed at developing point-of care CSF diagnostic options has been submitted to NINDS. Data from this work will also be used to develop an R01 epidemiologic which will include rural regions and children.

Capacity Building: Professional development opportunities for investigators have been provided including Masters' level training and good progress has been made. This work has also provided resources and infrastructure for an MD PhD thesis, a clinical research fellowship from the American Academy of Neurology and a Seed Grant from the American Academy of Neurology.

Publications to date: OK Siddiqi, M Elafros, I Sikazwe, C Bositis, MJ Potchen, IJ Korolnik, W Theodore, GL Birbeck. Seizure Etiology and Outcome in HIV⁺ Zambian Adults. 2012 Annual Meeting for the American Neurologic Association. Boston, 7-9 October 2012. Abstract M1224, abstract book page 91.

Co-PIs: Gretchen L. Birbeck and Michael J. Potchen; USA and Zambia
Foreign Investigators: Sam D. Kampondeni, Macpherson Mallewa, Cowles Chilingulo; Malawi
Other US Investigators: Terrie E. Taylor, Khalid Ibrahim, Joseph Bonner

R21NS069228

The Malawi-MSU-MRI Project: An Epidemiologic Study of CNS Abnormalities

Study Site: Queen Elizabeth Central Hospital and the Blantyre Community

Objective: MRI became available in Malawi in 2008. As with any newly available technology, normative MRI data are needed for effective clinical and research applications.

Methods: We identified a representative, community-based sample of children 9-14 years old. Children were screened for neurodevelopmental problems. Demographic data, medical history and environmental exposures were ascertained. Eligible children underwent the Neurologic Examination for Subtle Signs (NESS) and a brain MRI. Descriptive findings and analyses to identify risk factors for MRI abnormalities are detailed.

Results: 102/170 households screened had age-appropriate children. 2/102 children had neurologic problems—one each with cerebral palsy and epilepsy. A total of 96/100 eligible children were enrolled. Mean age was 11.9 years (SD 1.5), 43 (45%) male. No acute MRI abnormalities were seen. NESS abnormalities were identified in 6/96 (6%). Radiographic evidence of sinusitis 29 (30%) was the most common MRI finding. Brain abnormalities were found in 16 (23%) including mild diffuse atrophy in 4 (4%), periventricular white matter changes/gliosis in 6 (6%), multifocal punctuate subcortical white matter changes in 2 (2%), vermian atrophy in 1 (1%), empty sella in 3 (3%), and multifocal granulomas with surrounding gliosis in 1 (1%). Having an abnormal MRI was not associated with age, sex, antenatal problems, early malnutrition, febrile seizures, an abnormal neurologic examination or housing quality (all p's > 0.05). No predictors of radiographic sinusitis were identified.

Conclusion: Incidental brain MRI abnormalities are common in normal Malawian children and radiographic findings of sinusitis are seen in almost a third of healthy, symptom-free children.

Capacity Building: To date, images and findings obtained from this healthy pediatric population on Malawi's 0.35T MRI have provided critical data for comparative analysis and four R01 submissions. Professional development, including Masters-level training for the principal MRI technician, has been supported. As part of this work, NeuroInterp was developed. NeuroInterp is a computer-based data entry, work flow and database development application that provides MRI findings in a quantitative, searchable format greatly facilitating quantitative analyses. NeuroInterp is now used for interpretation of virtually all research MRIs in Malawi.

Project Status: In year 1, no cost extension for dissemination and completion of capacity building activities.

Future Work: Based upon the infrastructure developed for NeuroInterp, an R01 is under consideration that would establish a comprehensive database for the Blantyre Malaria Project which would include acute clinical data, neurophysiologic assessments, ophthalmologic evaluations, autopsy data, cognitive and neuropsychiatric outcomes. These data presently exist in unlinked dataset of varying sophistication and a unified database would greatly enhance further investigation of pediatric cerebral malaria.

PI: H el ene Carabin, United States

Foreign Collaborator: Humberto Foyaca-Sibat, South Africa

Presenters: H el ene Carabin (US)

Grant no. 5R21TW008434

Grant title: "HIV-CNS Diseases and Parasitic Zoonoses in the Eastern Cape, South Africa"

Significance: Both HIV and parasitic zoonoses infecting the brain are considered endemic in the Eastern Cape Province of South Africa. A pilot study conducted by our group demonstrated that 37% of people with epilepsy in one rural hospital had lesions of NCC in their brain. HIV infection may modify the severity, frequency, and type of neurological manifestations associated with brain infections with the larval stages of *Taenia solium* and *Toxocara* spp. Such interaction has not been explored to date. The purposes of this pilot study are to: 1) continue collaboration with Walter Sisulu University (WSU) researchers to develop local research capacity and 2) conduct a pilot study to evaluate the interaction of cysticercosis and toxocariasis with HIV infection on the development of neurological manifestations in people living in the Eastern Cape of South Africa.

Progress of research / preliminary results: The funding for this grant ended in April 2012. Since then, we have finalized all the serological analyses and have been trying to track down a few missing CT-scan results. Available results showed that if the Garcia and Del Brutto (2005) criteria are applied, there were no cases classified as definitive or probable NCC among the HIV positive group without neurological symptoms. Among that group, that were nearly 43% of those patients had definite or likely lesions of NCC at the CT-scan none are classified as a definite or probable case. This shows that this classification may not be appropriate in the context of HIV. Among patients with lower levels of CD4 counts (<250), the proportion of NCC among people with manifestations was lower than among people without manifestations. Among people with higher levels of CD4, there was a larger proportion of NCC lesions among people with neurological manifestations than among those without manifestations. This confirms our initial hypothesis that some level of CD4 counts may be required for patients to have sufficient inflammation in the brain to result in neurological manifestations such as seizures and epilepsy.

Capacity building: Until all the data have been finalized, we are not able to write manuscripts. We have agreed on a list of publications to result from the project and identified lead author for each of these.

Barriers: The project encountered numerous barriers. First, the laboratory staff who was in charge of conducting the serological analyses failed to do so. This resulted in the loss of monoclonals which are essential to the conduct of the ELISA test to detect antigens to the larval stages of *Taenia solium*. We are also concerned about the storing of the Western blot test kits. We were able to afford replacing the monoclonals, but not the Western blot tests. Dr. Korsman, the co-investigator who is also the director of the laboratory, had to come to Mthatha to run all the tests, despite the fact that all laboratory staff had been trained in these techniques in March 2011. Also, one of the radiologist who was in charge of reviewing the CT-scans left the Walter Sisulu university, leaving only one radiologist, busy with clinical responsibilities, to finalize the reviews of the CT-scan images. This has resulted in several delays in obtaining the final data, and has unfortunately led to concerns about the quality of the results of the laboratory tests.

Sustainability: There is a strong faculty and clinical turnover at Walter Sisulu University and at the Mthatha hospitals. In addition, there is no culture of research at these institutions, and we found it very challenging to start establishing one, although the research director lived in Mthatha for two years. WSU may need to invest in the recruitment of strong researchers to build a culture of research.

PI: H el ene Carabin, United States

Foreign Collaborator: Athanase Millogo, Burkina Faso

Presenters: **H el ene Carabin**

Grant no.R01NS064901

Grant title: "EFECAB: Improving pig management to prevent epilepsy in Burkina Faso"

Significance of research: Burkina Faso ranked 181st out of 187 countries according to the Health Development Index in 2011. Access to improved sanitation was estimated at 6% in rural areas in 2010. This results in a very high burden of disease associated with environmental contamination such as *Taenia solium* cysticercosis. Cysticercosis in humans may result in neurological disease when eggs present in the environment are ingested and the larvae establish in the brain (neurocysticercosis). The most common manifestation of neurocysticercosis is epilepsy. Our R21 found that in the two villages where pigs were raised and pork was consumed, 45% of people with epilepsy showed lesions of NCC at the CT-scan. In spite of the high prevalence of NCC in Sub-Saharan Africa, the effectiveness of sustainable interventions in reducing the incidence of both human and porcine infection has never been tested. There has also never been a cohort study to estimate the incidence rate of cysticercosis infection in humans and to understand how it is linked to manifestations of NCC.

Progress of research: We have now completed the baseline phase of our randomized controlled community trial and are finalizing the analysis of the sera for the detection of circulating antigens of cysticerci in 3600 human samples and nearly 2400 pig samples. Our last meeting between collaborators took place in July 2012. The goal of this meeting was to finalize the intervention strategy following numerous meetings with staff from WaterAid and the "Water and Sanitation for Africa (WSA)" in Burkina Faso. This led us to choose, instead of the Community-Led Total Sanitation (CLTS) approach, which is very time consuming and requires multiple follow-up visits, the Participatory Hygiene and Sanitation Transformation approach (PHAST), a method aimed at empowering the community to improving sanitation. The educational package for the intervention includes a movie and accompanying comic book, discussion of the movie, and PHAST. We have asked a renowned screenwriter to produce a 50 minutes comedy which summarizes the life cycle of the parasite and its impact on the communities, as well as the steps which can be taken to reduce the infection. The movie will be presented to the intervention villages (n=30) followed by discussion of the key messages with the field staff, and implementation of PHAST. The 60 villages have now been randomized to the intervention and control groups and the educational package is ready for use. The first follow-up visit took place in August 2012.

Capacity building: We are training one PhD in epidemiology in Oklahoma and one PhD in immunology at the ITM in Belgium. Both students are making progress, although some issues have arisen (see barriers). Our field team has been trained in the use of PHAST, which was modified by WSA to better represent the life cycle of cysticercosis.

Barriers: We have faced three major barriers this year. The first was the difficulty to find partners willing to work with us on the development and implementation of the CLTS for the intervention. Both organizations that we contacted were demanding budgets that we could not afford, and, even after several meetings where the benefits of our collaboration were emphasized, we had to change the method to be used. The second barrier has been the difficulty of students to become highly motivated to become independent researchers. The third barrier has been communication issues between laboratories which are performing lab analysis for our study (IRSS lab in Burkina Faso and ITM lab in Belgium). These barriers are now being solved but have resulted in some delays.

Sustainability: We are making every effort possible to develop an intervention which will be sustainable. Our colleagues at WSA believe that PHAST may be a more sustainable approach than CLTS. The screenwriter is keen on having the film widely distributed in the country when the study will be finished. More efforts need to be made to select students who are motivated to make things better.

Nancy Carney, USA

Gustavo Petroni, Argentina

Grant number: R01 HD0605740

Grant title: Pediatric Traumatic Brain Injury in Latin America: Effects of Caregiver Intervention

A randomized, outcome-masked, clinical trial of an intervention to assist the family caregivers of pediatric traumatic brain injury (TBI) survivors in the use of community resources to aid the recovery of the patients and improve the post-injury adjustment of the family.

Objective

Worldwide, TBI is the leading cause of death and disability among children and adolescents. There is little post-discharge rehabilitation for TBI in Latin America, and follow-up care is rare. The objective of this project is to provide home-care skills and support to families with a child with moderate to severe TBI, and to test the influence of this intervention on mortality and functional outcomes. A secondary objective is to assess the influence of resource availability on outcomes.

Research Design & Methods

The primary study is a randomized controlled trial (RCT) with blinded evaluation of outcome. The project also includes a Prospective Observational Study. Seven Level 1 Trauma Centers in Argentina were selected. Patients meeting criteria whose families provide consent are enrolled into the Observational Study at hospital admission. Randomization into the RCT occurs two days before hospital discharge either to receive the home-care intervention (in addition to standard outpatient care) or to only the standard outpatient care.

The intervention consists of (1) a Caregiver's Manual containing information about caring for the injured child after discharge, and (2) interaction with a Community Resource Coordinator (CRC). Patients are evaluated at 3 and 6 months after injury; at 3 months using the Pediatric Overall Performance Category (POPC) and the Pediatric Cerebral Performance Category (PCPC); and at 6 months using the POPC and PCPC, the Pediatric Quality of Life Inventory Generic and Cognitive Scales (PedsQL); and the Family interview

We will recruit new patients for 26 months at 7 hospitals. We expect complete primary data for the study on a total of 274 cases, with 137 cases in each of the two comparison groups.

Results

From August 2011 to October 2012 in 7 Hospitals, we recruited 184 patients. 62 % male and 38 % female. Age: 25% < 2 years old, 37.5 % 3 - 8 years old, and 37.5% 9 - 18 years old .

Based in GCS at hospital admission 43% were severe, 12 % moderate and 45% complicated mild. 57% had LED II in Marshall Classification.

89% follow up at 3 months.

Professor Monique Chaaya, PI, American university of Beirut, Lebanon
Professor Gunhild Waldemar, Co-PI, Danish Dementia Research Center, Denmark
Dr. Kieu Phung, Project Coordinator, Danish Dementia Research Center, Denmark
Professor Martin Prince, Consultant, King's College London, United Kingdom

Presenter: Professor Monique Chaaya

R21 PAR-08-113

Dementia Prevalence in Lebanon: A Nationwide Community-Based Cohort Study

Abstract

The aims of the study are:

1. To validate the 10/66 DRG one-stage dementia diagnostic package in Arabic and compare its discriminating power with two brief screening tests for dementia, RUDAS and IQCODE.
2. To carry out a pilot study in two governorates, to field test the instruments, assess the feasibility of the subsequent cohort study, and generate preliminary data about dementia prevalence

The data collection for the validation study is almost completed with 160 controls and 82 cases. Preliminary analysis was done on 135 controls and 56 cases and already presented in two international conferences. The results show that the 10/66 instrument has good psychometric properties (sensitivity 87.5%, specificity 91.1%). The RUDAS (sensitivity 87.5%, specificity 84.2%) and IQCODE (sensitivity 89.2%, specificity 89.6%) have similar sensitivity but lower specificity compared to 10/66 instrument.

Preparation for the pilot study is underway. A random sample of around 500 participants will be recruited by systematic sampling of households. In addition to 10/66 diagnostic package, back ground and risk factor questionnaires will also be administered. The questionnaires will also inquire about barriers to diagnosis and care for people with dementia and the burden for their caregivers.

The validated 10/66 diagnostic instrument provides researchers in Lebanon and the Middle East and North Africa region with a new and valid method for case ascertainment in epidemiological studies. It is the first important step towards improving the **quality** of dementia research in the region. This pilot study will lead to a longitudinal community-based cohort study about dementia prevalence, incidence, risk and protective factors in Lebanon, the first of the kind in the region.

To secure the expertise and the collaborative framework for the subsequent cohort study, we have built capacity in dementia epidemiological research for medical doctors (residents in neurology, geriatrics, and family medicine) and public health graduates (MPH); and we have established multidisciplinary collaboration between public health researchers, clinicians (neurologists, geriatricians, old age psychiatrists), nurses and social workers across institutions (American University of Beirut and Medical Center, Balamand University, Ain w Zein Hospital, NGOs, Ministry of Social Affairs social centers, nursing homes).

The greatest obstacle for recruitment is the taboo about mental illnesses and cognitive problems that are considered to be shameful and should be hidden from public knowledge. It is impossible to recruit participants with dementia from the hospital-based clinics in the city, so this strategy was abandoned and replaced by recruitment from nursing homes and rural communities through local gate-keepers.

To sustain the study beyond our current NIH R21 grant, we are extending our collaboration to Qatar and Kingdom of Saudi Arabia to apply for funding from these countries. At the same time, we are looking at funding opportunities in Lebanon. We will also apply for an NIH R01 grant once we have completed the pilot study.

Randall M. Chesnut, MD FACS FCCM, United States of America

Carlos Rondina, MD, Rosario, Argentina

Randall M. Chesnut, MD

Grant Number: 5R01NS058302

Grant Title: Traumatic Brain Injury in Latin America: Lifespan Analysis

Abstract (summary of our work):

Trauma is the major cause of death and disability for people under 45, and traumatic brain injury (TBI) is the primary cause across all age groups. It is also very under-recognized by research groups and funding agencies. TBI in the developing world (DW) is particularly unfortunate in these respects, particularly as their medical, social, and economic situations are different enough that research from the “First World” (FW) is often of marginal relevance. The remedy is to perform high quality outcomes research in the developing world. Within TBI management, one of the most important, unanswered questions is the necessity of monitoring and treating intracranial pressure (ICP). Although frequently practiced in the FW, it remains controversial despite being seen as generally indicated. Incorporating it in the DW incurs marked practice changes and expenses and such decisions are best made on RCT data from DW centres. Such an RCT will also be extremely valuable to DW centres for establishing the indications and efficacy of such invasive monitoring.

The final phases of this study have been completed: A) a prospective outcomes study of all severe TBI patients admitted at seven centres in five Latin American cities; and B) an RCT of ICP-monitor-based management versus treatment without ICP monitoring at six centres in Bolivia and Ecuador. We collected outcome data through six months. Data analysis includes outcomes modeling (to compare it against models from the FW), process variables (ICU and hospital lengths of stay, number of CT scans, etc), the resource levels of the hospitals (prespecified, based on available technology) and, in the case of the RCT, we will also look at the impact of monitoring ICP on outcome and processes of care.

The medical impact of these studies will be to supply DW physicians, insurers, and healthcare administrators with a rigorous understanding of the outcome from this widespread, devastating disease as it occurs in their reality. Such an understanding of their own system should prove invaluable in developing and tuning their operations within the constraints that they uniquely face. The capacity building impact of these studies is to establish a core of physicians, nurses, hospital administrators, and support personnel, plus their institutions, that have been involved in the elaboration, performance, and analysis of these investigations. Having also formed the Latin American Brain Injury Consortium during the development process, there is now a system to sustain research interest and expertise following these projects. As well, the numerous educational offerings, both performed and planned, that are part of actualizing these studies have enlarged the clinical and research capabilities of a large group at the involved centres. We already have potential investigators inquiring into expanding our capacity building and research efforts.

**PROGRESS ABSTRACT FOR NETWORKING MEETING
BRAIN DISORDERS IN THE DEVELOPING WORLD AWARDEES**

PI name/ PI Country: Cohen, Alexander (United Kingdom)

Major foreign collaborator name/ Foreign Collaborating Country: Oye Gureje (Nigeria)

Grant number: 5R21MH093304-02

Grant : Scaling up services for people with psychosis in Nigeria: a pilot study

Research accomplishments

- Mapping of public and private health facilities, as well as traditional/spiritual healers, in two local government areas (LGAs) in Ibadan, Nigeria: Ibadan South East, an urban LGA with a population of ≈270,000; and Ona Ara a semi-rural LGA with a population of ≈270,000.
 - In Ona Ara we have identified a total of 55 facilities (36 private, 19 public) that have contact with persons with severe mental illness.
 - In Ibadan South East, we have identified a total of 45 facilities (32 private, 13 public) that have contact with persons with severe mental illness.
- One of the most interesting findings of the mapping exercise was the extent to which traditional/spiritual healers provide care for people with psychosis in both catchment areas.
- We have conducted a total of 54 interviews to assess the experiences of providers, persons with psychosis, and caregivers. See Table:

- To collect quantitative data on the needs of persons with psychosis, we have also administered the Camberwell Assessment of Needs instrument to a sub-group of persons with psychosis.

	Ibadan SE	Ona Ara	Males	Females	Total
Traditional healers	3	2	5	0	5
Faith healers	4	1	4	1	5
Primary care providers	2	3	1	4	5
Private health providers	3	2	5	0	5
Caregivers	5	5	2	8	10
Confined patients	2	2	4	0	4
Outpatients	7	5	5	7	12
Inpatients	5	3	5	3	8
	31	23	31	23	54

- Preliminary findings from the interviews:
 - Health providers in need of extensive training in the care and treatment of persons with psychosis;
 - Traditional healers may command large fees for their services and, thus, may be reluctant to make referrals to psychiatric services
 - Persons with psychosis may live with traditional healers for up to 3 months
- Will soon begin indepth analysis of the interviews.
- Currently conducting analysis of the Camberwell Assessment of Needs surveys.

PI name/ PI country: Leslie L. Davidson-USA,

Major foreign collaborator name/ Foreign Collaborating Country:

Shuaib Kauchali- PI, UKZN, South Africa

South African co-investigators – Meera Chhagan, Jane Kvalsvig, Myra Taylor

US co-investigators – Stephen Arpadi, Claude Mellins, Zena Stein, Ida Susser

Presenter: **Dr. Leslie L. Davidson**

Grant Title: The ASENZE Study: Health & Psychosocial Need: Children with Neurodevelopmental Disorders in a Time of HIV (R01 DA023697-04)

With the long term goal of intervening to promote better neurodevelopmental, physical and psychosocial functioning of children in South Africa, the Asenze study is investigating how the ability of children with neurodevelopmental disorders to function cognitively and socially is influenced by both health-related (HIV, anemia, other infection), contextual (socio-economic and environmental, access to care and therapeutic intervention) and psychosocial factors (caregiver characteristics including mental health and substance use, family functioning). Our current work has demonstrated that about one quarter of these children live with caregivers who are HIV positive and many have parents who have died from AIDS. Additional challenges such as poverty, inadequate access to health care and to education are common; many other risks to child health and well-being, including inadequate nutrition, infection and trauma, as well as caregiver depression and substance use affect many children. As a result, we are finding that many children do not function at optimal levels, and are at risk for developmental disabilities.

In Phase I we enrolled and assessed 1584 preschool children in five tribal areas in a highly HIV prevalent area of KwaZulu-Natal in South Africa and determined if they had neurodevelopmental disability. Recently we completed Phase II and succeeded in assessing 87% of the original cohort of children and caregivers approximately 24 months later after the child's school entry. The study design allows us to investigate cross sectionally and then longitudinally the relationships among neurodevelopmental disorders, the above mentioned risk factors. In the longitudinal analysis we will look into school functioning. Community ethnographic studies are being linked to the findings of the epidemiologic study to better understand the quantitative findings and to identify culturally appropriate interventions. Overall the study is working to identify factors open to intervention which are known to affect child risk and resilience and work with community leaders in a participatory approach to develop an effective community based intervention.

We presented interim findings at several international congresses in the past year and are currently focusing on publishing the findings from Phase I: The study has provided robust South African population based estimates of the prevalence of seizure disorders in preschool children surviving infancy. Preliminary analyses found that children who were HIV positive had a greater prevalence of both conductive and sensory hearing losses. The most common reasons for referral for non acute problems were hearing (22%), anemia (14%) and vision (12%) problems. 6.6% of referrals were for HIV infection. Referral compliance was high for HIV infection and anemia, but lower for vision problems, hearing or ear problems, anemia and developmental delay. Compliance with referrals for non-acute conditions such as hearing and vision problems, which may contribute to long-term functional and cognitive difficulties, is sub-optimal in this population. Though alcohol use is low in the caregiver population, with only 3 percent admitting to hazardous or harmful drinking, it was related to a positive caregiver HIV status and to food insecurity in the household but not significantly to depression (under review). A family asset index was related to the identification of neurodisability by the study physician.

In addition, training in research methods and enhancing research capacity is one of the key aims of this research. Epidemiology and ethnography students, pediatricians and psychologists in both the USA and in Africa are being trained as part of the study. Study team

members in South Africa are also enrolled in university courses to receive training in research methods and substantive areas related to child disability.

We plan to submit a proposal for a follow-up study when the children are 10-14 years old.

PI/Country: Nancy Fiedler/U.S.A.

Major Foreign Collaborator: Wattasit Siriwong/Thailand

Grant Number: R21ES018722-02

Grant Title: Neurobehavioral effects of pesticide exposure among children in rural Thailand

Exposure to pesticides is a growing problem in Thailand due to the exponential increase in pesticide importation for agricultural purposes during the last decade. Despite the increased vulnerability of the developing organism, few studies have evaluated the neurobehavioral effects of pesticide exposure in children. Fifty-three 6 to 8 year old children from rice farming (n=24 exposed) and shrimp farming (n=29 control) districts outside of Bangkok, Thailand completed a repeated measures study to evaluate exposure to OP and pyrethroid pesticides and neurobehavioral performance. Preliminary analysis of a subset of DAPs reveal significantly greater urinary DAP concentration among children living on farms relative to their age matched controls and significantly greater DAP concentrations during the high (rainy) relative to the low (hot) seasons. Adjusting for age, we observed that increasing DAP levels predicted reduced speed of response on coding and visual memory (symbol digit: $r=0.35$, $p=0.11$; match to sample: $r=0.42$, $p=.06$). On a task of attention/vigilance, children with higher DAP concentrations were less accurate when the number of trials administered increased (continuous performance: percent correct: $r=-0.42$; $p=0.06$). These preliminary results lend support to our hypothesis of compromised attention with increasing exposure to organophosphates. In addition, increasing DAP levels predicted less improvement in memory and motor speed with repeated testing over a 6-month period (object memory delayed recall: $r=-0.37$, $p=0.10$; tapping dominant hand: $r=-0.40$, $p=0.05$). Consistent with these behavioral results, parents of children living where organophosphate use is greater (i.e., rice vs. shrimp farms) report that their children exhibit significantly more learning problems (Connors 3 Parent Short Form ($t=-2.86$, $p=0.008$)). Thus, expected improvements with practice or test repetition were not observed behaviorally, which appears consistent with parental reports of learning problems among children with higher exposure to organophosphates.

Capacity building activities include an experiential workshop on questionnaire development, statistical analysis, and cultural adaptation of questionnaires for 26 students from the Chulalongkorn School of Public Health. Psychology and public health students participated in two separate, one month intensive fellowships providing training in behavioral assessment (UMDNJ) and laboratory methods for analysis of pesticides (Emory University). Ongoing pilot studies using the HOME and neurobehavioral measures for younger Thai children are being conducted in preparation for further studies in Thailand.

Annette L. Fitzpatrick, PhD / USA

Quang Van Ngo, MD / Viet Nam

Annette L. Fitzpatrick

1 R21 TW008431-01A1

Neurological Manifestations of Cerebrovascular Disease in Da Nang, Viet Nam

Low and middle income countries have the largest burden of stroke, accounting for more than 85% of stroke deaths globally. Developing countries such as Viet Nam are currently experiencing a health transition from infectious to chronic diseases in which the impact on the health of the population from chronic diseases will be especially devastating. The primary aims of this project were to 1) DEVELOP collaborations to conduct research; 2) IMPLEMENT a research study to demonstrate feasibility and provide preliminary data on cerebrovascular disease in the region; 3) INTEGRATE capacity building and training into the collaboration. Of utmost importance in this first US-Da Nang collaboration was the building of trust between investigators and administration in order to facilitate both training and conduct of research in a communist country. Specific tasks that were completed included a) development of a US-Vietnamese Advisory Board to provide guidance for activities; b) provision of six short (2-5 days each) training courses on clinical and research topics including diagnosis/treatment/prevention of stroke, brain imaging, epidemiology, biostatistics, and data management/analysis using Epi-Info; c) A Computer Resource Center was created with six stations, software, and texts/manuals to allow research to be done on site at Da Nang Hospital; d) Support for three Vietnamese investigators to develop abstracts and present them at the International Stroke Conference 2011 in Los Angeles was provided; e) Travel was also provided for trips to the University of Washington for tours and seminars in several departments including Neurology, Epidemiology, and Global Health. Development of protocols for cultural sensitivity and training of community health workers was also done as we developed protocols for two separate studies, a community-based risk factor surveillance study and a hospital-based stroke registry. The community study of adults age 35 and older in six districts of Da Nang province included collection of demographics, medical history, blood pressure, anthropometrics, and health behaviors using World Health Organization guidelines. The study resulted in evaluation of 1,621 adults, mean age 52.0 years (\pm 12.5) and 56.1% women. Over 27.3% were found to have hypertension, 26.2% used tobacco, and 16.1% were overweight. More than two-thirds of participants with measured hypertension were unaware of their condition. Almost one-fourth of participants were identified as having previously experienced at least one stroke symptom. The study highlighted the critical need for education on cardiovascular risk factors as well as information on stroke symptoms and the need for responding to them. Stroke registry data was collected by physician and nurses at Da Nang Hospital on all strokes admitted between March 2010 and February 2011. Mean age of patients was 65 years, and 39% were female. Nearly 50% of strokes were hemorrhagic. Crude mortality rates were 51.0% and 20.3% for hemorrhagic and ischemic strokes, respectively. A number of factors were independently associated with 28-day mortality, the two strongest being depressed level of consciousness on presentation and hemorrhagic stroke type. While all patients completed a CT during the admission, evidence-based processes of care, such as antithrombotic therapy and carotid ultrasound for ischemic stroke patients, were underutilized. This project highlighted the high early mortality rate of stroke due in part to the large proportion of hemorrhagic strokes in Viet Nam but also documented lack of awareness of hypertension and limited standards of care available. Vietnamese co-investigators participated in analysis and development of manuscripts from these data. Two publications have resulted thus far with another under review, and seven abstracts were presented at major conferences. The Da Nang DOH is eager to continue this collaboration as their skills and capacity to do research improves. The need to develop strategies for awareness, screening, and treatment of cerebrovascular disease in this country is critical.

PI: Paul Florsheim
5R21TW007803
Abstract
05/01/2006 to 04/30/2009

Development of an Intervention for Palauan Youth at Genetic Risk for Psychosis

P. Florsheim, S. Kuartei, L. Phillips, L.M. Ord, F. Blailes, J. Tiobech

The primary goals of this grant were to build capacity in the Republic of Palau to conduct indigenously-generated research on the development and treatment of psychotic disorders, including schizophrenia and to conduct a pilot study of a culturally appropriate preventive-intervention for adolescents at high risk for psychotic disorders. Our rationale for this project was based on evidence that Palauans are at increased genetic risk for schizophrenia and other psychotic disorders, and that the mental health system is not equipped to address this problem. This project involved the following activities:

(1) Research Training Program. Palauan nationals with an interest in research and capacity for working with adolescents were selected to receive basic research training in the behavioral and neuro-development sciences. The team worked with trainees to develop research projects using existing datasets previously collected from Palauan youth at-risk for psychotic disorders.

(2) Clinical Training Program. Two workshops were conducted in Palau, the first focusing on theoretical and methodological issues pertinent to preventive intervention research for youth at-risk for psychotic disorders, and the second focusing on the PACE model of preventive intervention for youth at-risk for psychotic disorders.

(3) Following the training, the PIs and the Palau Team modified the PACE program to address the particular, localized issues, concerns, and needs of Palauan youth and their families, resulting in a manual for conducting cognitive behavior therapy with Palauan youth at risk for psychosis, based on the PACE model.

(4) A randomized pilot study to test the effectiveness of the Palau-adapted PACE based preventive intervention (PAP) was conducted, with trainee involvement in all aspects of the pilot, including data collection, program administration, therapy sessions, data management and analyses. Pilot study participants were 30 adolescents between the ages of 14-21 ($M=17.33$), who were: (a) predetermined to be at genetic risk for schizophrenia¹, and (b) mildly or moderately symptomatic. Fifteen participants were randomly assigned to the PAP with the average number of sessions being 7.43 (S.D. 5.54). Although all participants were first degree relatives of people previously identified as having a psychotic disorder, and all participants reported some signs of psychological distress or psychosocial dysfunction, we were surprised to find less psychopathology than we expected. Results indicated the PAP program did not effectively reduce symptoms. However, we successfully engaged youth in the counseling process. Because youth participants were not particularly distressed or disturbed at T1, it is not surprising that positive changes (reductions in symptomology) were modest. The culturally adapted program might work more effectively with a more distressed/symptomatic group of adolescent participants. To date, the project has resulted in two published papers (with Palauan trainees as the lead authors):

Ierago L, Malsol C, Singeo T, Kishigawa Y, Blailes C, Ord L, Florsheim P, Phillips L, Kuartei S, Tiobech J, Watson B, Ngiralmu H. (2010). Adoption, Family Relations, and Psychosis among

¹ We defined genetic risk in the following terms: (a) one first-degree relative with schizophrenia or (b) two second degree relatives with schizophrenia

Palauan Adolescents who are Genetically at Risk for Developing Schizophrenia. *Social Psychiatry and Psychiatric Epidemiology*, 45, 1105-1114.

Madraisau S, Tomoichi U, Ord L, Florsheim P, Phillips L, Blailes C, Basilius M, Kuartei S, Tiobech J, Myles-Worsley M, Ngiralmu H. (2010). Early Signs and Symptoms of Psychosis in Palauan Adolescents. *Early Intervention in Psychiatry*, 4(2), 153-161.

PI: Judith Grether, PhD/ USA

CO/PI-Major Foreign Collaborator: Angelina Kakooza, MD/ Uganda

Presenter: Judith Grether

Grant number: 5R21TW008222-02

Neuro-Developmental Disorders Screening and Assessment in Uganda

SUMMARY

Neuro-developmental disorders (NDDs) are recognized to be relatively common in developing countries but little data exist for planning effective prevention and intervention strategies. In particular, data on autism spectrum disorders (ASD) are lacking. For application in Uganda, we developed a 23-question screener (23Q) that includes the Ten Questions (TQ) screener and additional questions on ASD behaviours. We then conducted household screening of 1,169 children 2-9 years of age, followed by clinical assessment of children who screened positive and a sample of those who screened negative to evaluate the validity of the screener. We found that 27% of children screened positive and 68 received a clinical diagnosis of one or more moderate-severe NDDs (ASD, cerebral palsy, epilepsy, cognitive, speech and language, hearing, or vision impairment), including 8 children with ASD. Prevalence and validity of the screener were evaluated under different statistical assumptions. The combination of screening positive on both ASD and TQ items was successful in identifying a subgroup of children at especially high risk of ASD. We recommend that ASD and related behavioural disorders be included in studies of NDDs in low resource settings to obtain essential data for planning local and global public health responses.

PUBLICATION LIST:

Angelina Kakooza-Mwesige, Keron Ssebyala, Charles Karamagi, Sarah Kiguli, Karen Smith, Meredith C Anderson, Lisa A Croen, Edwin Trevathan, Robin Hansen, Daniel Smith, Judith K Grether, Adaptation of the 'ten questions' to screen for autism and other neuro-developmental disorders in Uganda, (Accepted for publication *Autism: International Journal of Research and Practice*)

BIOSKETCH: Dr. Grether is a perinatal epidemiologist who conducted research on developmental disabilities, primarily cerebral palsy; now retired from the California Department of Public Health and serving as PI on this grant through the Sequoia Foundation. Dr. Kakooza is a pediatrician with many years of experience working with children with developmental disabilities at Mulago Hospital-Makerere University in Kampala, Uganda.

PI Name: Elena Grigorenko, Ph. D. / USA

Major Foreign Collaborator: Phil Thuma, M.D. / Zambia

Grant No: 5 R01 TW008274-5

Grant Title: "Reading Disabilities in Zambian Children"

Presenter: **Thuma PE**, Sinamwenda P, Reich J, Tan M, Krivulskaya S, Grigorenko EL

Abstract: The challenges and rewards of carrying out a screening program for reading disabilities in a rural part of Zambia are ones not easily anticipated until the project is well underway. The presentation will describe the methodology employed in setting up the project in schools that have never participated in this type of research, with some of the lessons learned from collecting data of different types (behavioral, biological, and anthropological), and the progress and rewards to date.

PI name/ PI Country: Richard L. Guerrant, USA

Major foreign collaborator name/ Foreign Collaborating Country: Aldo A. M. Lima and Reinaldo B. Oriá, Brazil

Grant number: (1 R01 HD053131)

Grant title: APOE and the Effects of Malnutrition on Cognitive and Intestinal Development

APOE and the Effects of Malnutrition on Cognitive and Intestinal Development Report Summary

SUMMARY

In our studies, we have addressed the effects of under nutrition alone or compounded with enteric pathogens to study the vicious cycle of enteric infections and under nutrition during early post-natal development using murine models. This unique approach of studying components of the intestinal and brain adaptations against early challenges of undernutrition and enteric infections has established a sustained model to evaluate key interventions to ameliorate the short and long-term deficits we have seen with environmental enteropathy (tropical enteropathy). Environmental enteropathy (with recurrent episodes of enteric infections/malnutrition) afflicts young children living in impoverished areas of the developing world and causing a syndrome of chronic intestinal malabsorption, that ultimately leads to growth stunting and cognitive decline in times of important developmental milestones, therefore jeopardizing one's full genetic potential. In this report, we have shown data from controlled-models of under nutrition (alone or combined with enteric infections, e.g. *Cryptosporidium parvum* and *Clostridium difficile* infections) in the first post-natal (by litter size clustering or by maternal-offspring separation) and post-weaning (with a low protein diet or a Brazilian northeastern-regional diet) days of life. In addition, we have addressed nutritional interventions with glutamine (or alanyl-glutamine), zinc or arginine in these selected models to evaluate their role during intestinal and brain adaptations after the challenge. Finally, in collaboration with Dr. Michael Vitek and Patrick Sullivan (Duke University, North Carolina), we have assessed different apoE genetic backgrounds, including apoE deficient and apoE target replacement mice carrying either E4 or E3 human genes, therefore challenging our findings from our cohort studies with Brazilian shantytown children where we could find a protective effect to APOE4 carriers with history of heavy diarrhea burdens early in life. We are now applying for an NIH Director's transformative grant to extend these findings to address the pathogenesis and biomarkers of the environmental enteropathy, EE, ("impoverished gut") and the role of key nutritional interventions alone or in combination with antimicrobial therapy in ameliorating the developmental deleterious effects seen with EE. In new collaborations, we are teaming-up with Duke University and Cincinnati's Children Hospital's researchers to study the signaling pathways regulating crypt stem cells and T-cell function to build functional villi capable of absorbing key gut and brain trophic nutrients using in vitro stem cell models. We will also include interventions with apoE COG133 mimetic peptides, kindly provided by Dr. Michael Vitek (Duke University) to our in vitro intestinal cell line models of wound healing and cell migration in addition to the model of malnutrition and refeeding described above. This apoE COG133 mimetic peptide has been found by our group to be anti-inflammatory in a murine model of 5-fluorouracil-induced intestinal mucositis and now we have promising data, as described above, with a murine model of *Clostridium difficile* infection. In addition, there is an interest to examine the role of the blood brain barrier as a gate for regulating the gut-brain axis and changes with circulating bacterial products and pro-inflammatory cytokines with microglial activation and cognitive performance during and after malnutrition and infection challenges.

PI: Chandy John

Grant Title: Pathogenesis of Cognitive/Neurologic Deficits in Central Nervous System Malaria

Grant Number: R01 NS055349

Presenter: Michael Boivin

Title: **Child neuropsychological assessment from infancy to middle childhood: a preliminary evaluation**

Authors: Paul Bangirana¹, Chandy C. John², Robert O. Opoka³, Richard Idro³, Michael J. Boivin⁴

Affiliations: ¹Department of Psychiatry, Makerere University; ²Department of Pediatrics, University of Minnesota; ³Department of Paediatrics and Child Health, Makerere University;

⁴Department of Psychiatry and Neurology/Ophthalmology, Michigan State University

Introduction. Assessment of cognition from infancy to middle childhood requires different tests for different age groups. These tests should measure the same outcome to monitor development consistently from infancy to adolescence. This poster presents preliminary analyses comparing early childhood cognitive outcomes with corresponding scores in school-age children.

Methods. 75 children between 3 and 5 years (38 males, 50.7%) were assessed for cognition using the Colour Object Association Test (COAT), Mullen Scales for Early Learning and the Early Childhood Vigilance Test (ECVT) of attention. Repeat testing was done approximately 12 months later using the Kaufman Assessment Battery for Children second edition (KABC-II) and the visual Test of Variables of Attention (TOVA). Correlations and linear regressions were run between corresponding scores from both batteries.

Results. Composite scores from the Mullen and KABC-II were highly correlated ($r=0.74$, $p<0.0001$). Correlations were observed between TOVA and ECVT attention ($r=0.49$, $p<0.0001$) and between the Mullen and KABC-II Visual Processing ($r=0.54$, $p<0.0001$). Regression coefficients showing which early tests predicted older age tests are presented in Table 1.

Table 1. Regression coefficients showing the association between similar abilities in the different age groups

	Visual reception	Vigilance	Early learning composite
Simultaneous processing	0.06 (0.03 to 0.08), <0.001		
Learning			0.01 (0.01 to 0.02), <0.001
Planning	0.04 (0.0005 to 0.08), 0.05		
Knowledge			0.01 (0.003 to 0.01), <0.002
Attention		2.78 (1.41 to 4.16), <0.001	
Fluid crystallized index			1.29 (0.94 to 1.64), <0.001

Conclusions. ECVT and Mullen test scores in early childhood predict corresponding outcomes in the KABC-II and TOVA in middle childhood. These tests batteries can be used to assess neurodevelopment from infancy to middle childhood in Ugandan children.

Acknowledgements. This study was funded by an NIH award (5R01NS055349) to Chandy C. John. Author correspondence: Paul Bangirana, PhD, Department of Psychiatry, Makerere University P. O. Box 7072, Kampala, Uganda; e-mail: pbangirana@yahoo.com

PI: Krister Kristensson, Sweden

Major foreign collaborators:

Alfred K Njamnshi, Cameroon (Presenter)

Marina Bentivoglio, Italy

Grant number: 1R21NS064888-01A1

Grant title: Neural dysfunction and neuroinflammation in African brain disorders

The project (April 2010 to May 2012) has had the research objectives to explore in animal models pathogenetic mechanisms of nervous system involvement in the neglected disease human African trypanosomiasis (HAT) or sleeping sickness caused by *Trypanosoma brucei* (*Tb*) parasites, and to monitor in HAT patients disease severity by means of a non-invasive technique. The project has had the specific capacity-building goal to solidify collaborative relationships leading to durable capacity building of African investigators. In particular, concerning capacity building the project focused on training in molecular and cell biology protocols and in the analysis of rest/activity rhythm and sleep structure in laboratory rodents, assessing also the feasibility to carry out the proposed research at the University of Yaoundé I (Yaoundé, Cameroon). Furthermore, the project has aimed at fostering the establishment in this University of a research center in basic and translational neuroscience which could also contribute to regional networking and partnerships with a broader international perspective.

Concerning the research objectives, molecular mechanisms for *Tb* brain invasion have been analyzed experimentally in rodent models of *Tb* infection. The main results have shown that the cytokine interferon-gamma and the chemokine CXCL10 regulate the passage of the parasites across the blood-brain barrier and that these molecules in body fluids can provide biomarkers for this event. The adapter molecule MyD88 has been shown to control trypanosome growth in the brain parenchyma. In the study of functional parameters related to *Tb* brain invasion, the results have documented in infected rats that the onset of functional changes revealed by anomalies of the sleep-wake cycle and sleep structure precede the parasite invasion of the brain parenchyma and that their severity parallels disease progression. Concerning the translational research part of the project, the study has been performed in collaboration also with the “*Institut National de Recherche Biomédicale*” (INRB, Kinshasa, Democratic Republic of Congo). The results have assessed the feasibility of wrist actigraphy as a tool for an objective evaluation of sleep disturbances (characteristic of HAT) in patients at different stages of disease and disease severity monitoring after therapy. Further analyses of actigrams obtained from HAT patients are ongoing.

Capacity building efforts saw the defense of the PhD thesis of a Cameroonian student (Paul F. Seke Etet) at the University of Verona, and administrative procedures for his recruitment to the University of Yaoundé I have been initiated. The neuroscience laboratory created in the Faculty of Medicine of The University of Yaoundé I is being equipped and pilot experiments are ongoing. The co-PI in Yaoundé, Alfred K. Njamnshi, has been appointed head of this new laboratory, which will serve as the starting point for the future Brain Research Center. In Cameroon, research collaborations have been established with the Universities of Ngaoundéré and Buea on epilepsy and leprosy, respectively. Networking is ongoing with several African institutions, and in particular with INRB.

Built on the results and accomplishments of this R21 project, an R01 application entitled “Neural dysfunction and neuroinflammation in African brain disorders” has been submitted in January 2012. This application involves all the three partners of the R21 project, but foresees coordination by Partner 2 (University of Yaoundé I) instead of Partner 1 (Karolinska Institutet) to emphasize that the capacity building has progressed effectively for coordination transfer. This project focuses on specific pathogenetic questions on nervous system involvement in HAT and its comparison with that in toxoplasmic encephalitis.

Brain Disorders Network Meeting 10/22/2012 to 10/23/2012

Contact PI/PD name: Patrick Kwan; country: Hong Kong
Other PI/PDs: Larry Baum, Stacey Cherny, Ding Ding, Josemir Sander, Wenzhi Wang
Major foreign collaborator name: Beijing Neurosurgical Institute, Fudan University
Major foreign collaborator contry: China

Presenter name: **Patrick Kwan**

Grant number: 1R21NS069223

Grant title: **Development and validation of clinical assessment tools for population genetic studies of epilepsy in rural Chin**

Purpose

Research and application of findings in epilepsy genetics in resource poor settings are hampered by lack of validated clinical assessment tools for phenotyping at the primary care level, agreed investigation protocols, established logistics and network, and trained personnel. This study aims to overcome these barriers with emphasis on building research infrastructure and capacity in rural China.

Method

Patients with epilepsy receiving treatment in the national epilepsy control program were eligible for inclusion. In stage 1, rural primary care doctors performed phenotyping using clinical assessment tools (questionnaires). Patients attended the provincial hospitals for independent phenotyping by the “gold standard”, consisting of neurologist assessment, EEG and brain MRI. In stage 2, patients were phenotyped by rural doctors. Blood samples were collected from patients in both stages for DNA extraction. To develop locally valid clinical assessment tools, reference was made to screening questionnaires previously employed for seizure classification and provincial neurologists were consulted. Their feasibility was pilot tested in 80 patients in the rural areas. Common EEG and brain MRI protocols were checked for compatibility with local equipment and practice.

Results

After piloting testing and revision, clinical assessment tools covering epilepsy history and seizure semiology were developed. Common EEG and brain MRI protocols were employed in the provincial hospitals. Provincial neurologists and rural doctors were trained in clinical and research skills and ethics. A total of 2,071 people with epilepsy were recruited from rural areas of four provinces in China (n=610 in Henan, 341 in Shanxi, 638 in Ningxia, 509 in Hebei). All patients were evaluated by neurologists in provincial centres; 637 also underwent brain MRI and EEG. Genotyping for candidate polymorphisms associated with increased susceptibility for epilepsy is underway.

Conclusion

Results will help overcome barriers in epilepsy genetics research and application in rural China. The model developed may be adopted in other low- and middle-income countries where 80% of the world's people with epilepsy live.

Publication

Kwan P, Wang W, Ding D, et al. Development and validation of clinical assessment tools for population genetic studies of epilepsy in rural China: a GREAT study. *Epilepsia* 2011;52(Suppl 6):92-3. [abstract]

PI name/ PI Country: Maselko/ USA

Foreign collaborator name/ Country: Bilesha Perera/ Sri Lanka

Presenter: Joanna Maselko and Bilesha Perera

Grant number: 5R21TW009151-02

Grant title: The Sri Lanka Healthy Minds Study

ABSTRACT:

The current project lays down the foundation for the Sri Lanka Healthy Minds Study (SLHMS), a new collaborative research project between Duke University and the Faculty of Medicine at the University of Ruhuna that aims to describe the epidemiology of depression and cognitive impairment among elderly Sri Lankans. The SLHMS will be a population based study where individual, caregiver, and other family level factors will be examined using a lifecourse perspective to better understand depression risk and its influence on cognitive impairment. An important goal of our current proposal is to build sustained research capacity and technical expertise in epidemiological research at the University of Ruhuna in order to facilitate the SLHMS as well as other long term collaborative projects. While Sri Lanka is one of Asia's most rapidly aging countries, there is a lack of epidemiological data on the most common neuropsychiatric conditions among the elderly in Sri Lanka and it is this gap that our research program plans to address. A recent estimate of 28% depression among those over 60 is especially troubling, as the condition is associated with significantly worse prognosis of most other health problems, including cognitive impairment and its associated disability. The overarching goal is to both describe the epidemiology of depression and cognitive impairment and to identify modifiable psychosocial factors, which can be utilized in future health promoting efforts as well as healthcare resource allocation.

The specific aims of this proposal are to: (1) use qualitative methods to examine local understanding of depression and cognitive decline among elderly in Sri Lanka, concepts such as successful aging and attitudes towards mental illness; (2) validate measures of depression and other constructs to be used in a household survey; (3) pilot a household survey to establish feasibility and generate preliminary data for R01 submission, and (4) develop the research capacities at the University of Ruhuna which will lead to long term research collaborations. Our long term research goal is to examine *prospectively* the role of the caregiver and other family members in relation to the depression and cognitive impairment status of the elderly family member. Ultimately, we hope that our findings will provide concrete evidence to guide cost-effective, community based interventions to prevent, or ameliorate the impact of, depression and cognitive impairment among Sri Lankan elderly.

Progress to date: We have made significant progress towards achieving the goals of this study. Incorporating capacity building activities, we have completed both the qualitative and household survey data collection phases and are now in the data analysis phase. Analysis of the qualitative data has resulted in 3 conference presentations and 3 manuscripts, one of which is under review. We also conducted a qualitative research methods training workshop and a workshop on community based household survey based research. These activities have incorporated student projects while involving a growing number of faculty at both of our institutions.

PI: MKC Nair

Grant Title: Neuro-developmental Disorders in India – An INCLEN Study.

Grant Number: R21 HD053057

MKC Nair¹, S. Gulati², D. Silberberg³, J. Pinto-Martin⁴, N. Arora⁵, (1. Child Development Centre, Medical College Campus, Kerala, India; 2. Department of Pediatrics, AIMS, New Delhi, India; 3. Department of Neurology, University of Pennsylvania School of Medicine; 4. Center for Autism and Developmental Disabilities Research and Epidemiology, University of Pennsylvania School of Nursing; 5. International Clinical Epidemiologic Network, New Delhi)

Objectives: The objectives of our research are: 1) to estimate the prevalence of NDDs among children aged 2-9 years in India; 2) to gather information on risk factors, including those that are potentially modifiable; 3) and to develop and disseminate methodology that includes culturally-sensitive screening and assessment tools that may be useful in other low income countries. The domains that we have included are: Attention Deficit Hyperactivity Disorder (ADHD), Autism Spectrum Disorders, Intellectual Disability, Epilepsy, Learning Disability, Neuromuscular Disorders including cerebral palsy, Speech and Language Disorders, and Hearing and Vision Impairment.

Methods and Progress to date:

Phase 1:

Development of a Neuro-developmental Disorders Screening Tool (NDST): A 39 question NDST was developed in English and Hindi, translated into 8 regional languages, back-translated into Hindi and English, tested for understanding, and validated among 593 subjects in 9 regions of India

Development of Consensus Clinical Criteria (CCC): CCC's for the 9 NDD's to be surveyed were developed and tested in 2 phases in Delhi and Trivandrum, on 1,250 subjects. 10 training modules have been developed (9 CCC modules and one Family Counseling Module).

Validation of the Neuro-developmental disorders screening tool (NDST): The test-retest and inter-rater **reliability** of the NDST was assessed at two centers in New Delhi, and at the Child Development Center in Thiruvananthapuram, testing 192 children. The test re-test reliability coefficient was above 0.8 for 35 of the 39 questions (89.7%) among both doctors and research assistants. The inter-rater reliability correlation co-efficient was above 0.8 for 21 questions (53.8%). The **content** validity of the refined tool was then validated in four different strata: rural, urban, hilly areas and tribal areas at five field sites. Conventional clinical diagnostic methods, including expert opinion were used to establish or rule out the presence of NDD's. A total of 593 subjects participated in this piloting

Phase 2:

Estimation of prevalence and description of the spectrum of neuro-developmental disorders: The prevalence of neuro-developmental disorders is being assessed among a total of 4,000 households in 5 zones via a population-based cluster survey using the Probability Proportionate to Size (PPS) technique. A one-time survey was conducted over a period of six months, from December 2011, to May 2012. First, the NDST was applied at the community level. Following this, all of the children were taken to operating bases that had been set-up in each region, for diagnosis, using the CCC for the ten NDDs. After diagnosis, the children were referred for appropriate care to the attached institute or hospital.

Subject to extensive ongoing analysis, it appears that 7.5% to 18.5% of children ages 2-9 years suffer from one or more NDD, including those who exhibited more than one NDD. Importantly, in Northern India, 14.2 to 17.5% exhibited possible hearing impairment. This includes some with impacted wax or middle ear pathology. Hearing impairment as an isolated problem occurred in 15.5% of children in the same region. If one excludes isolated hearing impairment, the overall prevalence of NDD's seems to be from 4.7 to 13.7% (3).

Quantification of potentially modifiable known risk factors for neuro-developmental disorders: A questionnaire was designed based on the initial research data that will quantify potentially modifiable risk factors. For this part of our survey, we included children ages 2-14 years, in order to gain a more complete picture of risk factors, attitudes and coping strategies. 100 interviews were conducted at each of three sites (50 with NDD and 50 without NDD) and an additional 50 interviews were conducted for each of the remaining two sites. The qualitative data obtained from these in-depth interviews will be analyzed systematically. The significantly associated risk factors associated with NDDs will be identified and a multiple logistic regression analysis will be conducted.

Conclusions

This INCLLEN study appears to be the first comprehensive national determination of neurodevelopmental disorders that includes screening followed by verification and diagnostic determination by health professionals. The test instruments and training manuals that have been developed should be useful in many low-income countries, with suitable modifications of language and cultural references. The success to date in India, a large culturally, linguistically and economically diverse country, supports this suggestion.

This project was supported by:

NIH (USA) Grant R21 HD53057, MKC Nair (PI), J. Pinto-Martin and D. Silberberg (Co-PI's), S Gulati, Network Coordinator); Autism Speaks (USA); The National Trust (Government of India) and INCLLEN. NK Arora served as the Team/project leader.

Title of Project: Multi-pronged genetic studies of schizophrenia in an inbred population, 1R01MH093246-01A1

Principal Investigators: Vishwajit L Nimgaonkar MD, PhD (USA); Hader Mansour MD, PhD (Egypt)

Presenter: Hader Mansour MD PhD (Mansoura University, Egypt)

SPECIFIC AIMS

Across the world, schizophrenia (SZ) is a common, severe and debilitating disorder. Its etiology and pathogenesis are unknown, but genetic factors are a likely source of risk. Because SZ treatment remains symptomatic and unsatisfactory, etiological studies are important. Ongoing efforts have confirmed several genetic risk variants, but many remain to be discovered. With new tools and designs we expect to identify far more of the genes involved.

In response to an increasing desire in the Middle East to reap the benefits of genomics, we initiated SZ genetics research in Egypt through a Fogarty R21 grant. We found that inbreeding substantially increases SZ risk (odds ratio, OR ~ 3.53). We have improved the research infrastructure at Mansoura University (MU), our Egyptian collaborating institution, by establishing a productive molecular genetics lab. We have also trained laboratory/clinical investigators, published peer reviewed papers and spawned research in several related areas. In consultation with our collaborators, we propose to exploit novel research leads and further expand the research infrastructure at MU.

Aim 1. Homozygosity by descent (HBD) analysis to identify schizophrenia risk polymorphisms

Hypothesis: HBD analysis can identify a portion of SZ recessive risk polymorphisms.

It is reasonable to hypothesize that certain risk variants for SZ act recessively and that a fraction of these variants can be detected in Egypt, where inbreeding is not uncommon. We will attempt to identify these variants by HBD analysis, which uses the fact that chromosomal fragments flanking a recessive risk allele can be passed down from the same ancestor to cases through multiple lines of inheritance in inbred populations. Population-level HBD analysis has been successful for several complex genetic disorders such as intellectual disability and autism, but has not been tested for SZ. Our preliminary analyses have identified several HBD regions among SZ cases that are much rarer in controls. To take these results further, we will identify and evaluate additional participants. We will genotype them in conjunction with our R21 samples using SNP arrays to enable HBD analysis (total 400 SZ cases, 300 controls, 60 affected relatives of cases).

Aim 2. Follow up sequencing and bioinformatics analysis

Hypothesis: Genetic variables contributing to SZ risk can be identified in HBD regions from Aim 1.

To further localize SZ susceptibility variants, we will prioritize HBD chromosomal segments analyzed in Aim 1 and select individuals harboring the HBD segments (estimated N = 10 regions, N = 200 cases/region). Through targeted capture and Next Generation sequencing, we will evaluate the HBD segments intensively. Using state of the art bioinformatics tools, we will identify SZ risk variants and their functional relationships.

Aim 3. Phenotypic expression of identified SZ risk variants among multi-generational families:

Hypothesis: The SZ risk variants from Aim 2 exhibit variable expressivity and incomplete fidelity to SZ.

To understand the range of expression of the identified risk variants, we will select patients with SZ from Aim 3 who are HBD for SZ risk mutations. We will consent and assess all of their available relatives (estimate 5 families, 100 members total). We will genotype these participants and evaluate their psychiatric diagnoses, cognitive function and physical abnormalities.

Aim 4. Continue sustainable research infrastructure and capacity building: We will build research capacity for bioinformatics and statistical analysis through training and purchase of software/hardware, and extend clinical and laboratory expertise. Egyptian collaborators will lead the entire research by grant end.

STUDIES AND RESULTS

We received the Notice of Grant Award in September 2010. We have obtained ethical approval from MU and the University of Pittsburgh. Refresher training, along with a review of the proposed design and methods are ongoing.

PEER REVIEWED PUBLICATIONS: None to date.

ABSTRACT

***Age-Related Loss in the Elderly in Mumbai, India: Research development and Collaborative Project of Nair Hospital/TN Medical College, Mumbai and Mount Sinai School of Medicine, New York**

PI: Dushyant Purohit, M.D, Mount Sinai School of Medicine, New York, USA

(Grants: 1R21 AG024065 (2004-2006) and 1R01 AG- 028188 (3006-2012) awards from the Fogarty International Center and the National Institute for Aging)

Introduction: Under the initiative titled “Brain Disorders in the Developing World: Research Across the Lifespan”, this R21 and the follow up R01 funded research development project has succeeded in carrying out capacity building for research on dementia at an academic center in India, a Low/Middle Income Country. We developed research infrastructure including setting up of the research site (memory clinic and a brain bank/ research laboratory) and training to the Mumbai collaborators in research methods which has led to research studies and sustained collaboration. In this work, we comprehensively employed the elements of the Clinical, Neuropathology, Database and Education and Information Transfer Cores of the Mount Sinai Alzheimer’s Disease Research Center, which is an established, effective and highly productive NIA-funded ADRC.

Specific Aims Fulfilled at the Conclusion of the Project in 2012:

Specific Aim #1: Study Cohort Building: We recruited 290 study subjects (more than the original study target of 150). The cases were clinically evaluated and longitudinally assessed for cognitive status using appropriate portions of UDS (NACC) based Mount Sinai ADRC research protocol including clinical examination, the Clinical Assessment Measures (Geriatric Depression Scale, Mini-Mental Status Exam, Clinical Dementia Rating and Neuropsychiatric Inventory) and Neuropsychological Battery (CERAD Word List Immediate and Delay, Logical Memory I & II -Story A, Boston Naming Test, adapted for this population, Letter Fluency –FAS, Category Fluency - Animals and Fruits, TMX and Shape Cancellation tests - Numeric cancellation replaced “TMX” cancellation test with participants of low literacy, Constructional praxis; Trail Making Test A and B -Colored Trails were used with participants of low literacy. We provided continual training in the research protocols to the research staff. This helped us to characterize the profile of cognitive loss in dementia in this large urban center in the developing world. A study paper was published (Nair G et al.). **Study Finding:** Our observations indicated that memory loss was an initial and prominent symptom among the subject, but the rate of progression was indeterminate since the longer follow up was not achieved in a large sample.

Specific Aim #2: Normal Controls: We also recruited from the Nair Hospital clinic as well through a community outreach clinic a cohort of 154 cognitively intact controls, far exceeding our target of 100 subjects. This cohort was evaluated for cognitive function and followed up. The follow up period was sufficiently long to identify conversion of any subjects to the diagnosis of MCI or AD-dementia.

Specific Aim #3 and 4: Brain Bank facility and neuropathology: This was achieved with training to junior research staff in methods of neuropathological study of Alzheimer’s disease and other dementias, setting up of laboratory for neuropathological investigation and training of technical staff. Over 170 brain specimens were collected and studied for Alzheimer’s disease and other dementia related pathology.

Study findings: The study did not reveal any difference in the rate and extent of Alzheimer’s related lesions as compared to the age matched cases in the Mount Sinai ADRC brain bank. This finding was reported in a paper (Purohit DP, et al.). Pathological lesions of other less common dementing diseases were rare. This was likely due to selection bias, as the autopsy cases were recruited in an acute hospital from the decedent pool of general hospital autopsy cases. Applying the newer NIA/AA guidelines for neuropathological diagnosis of AD (2011), there were five cases with high or intermediate level of AD-neuropathological changes. We carried out clinicopathological consensus conferences, conducted by Mumbai and New York collaborators on-site or by conference call on these cases, about which we concluded that retrospective family (informants) interviews were not satisfactory, probably because cognitive loss in old age as a disease phenomenon is an unknown concept and sparsely observed or noticed in the low income community from where the autopsy cases were derived (unpublished findings).

Specific Aim #5: This specific aim (to facility development and train local personnel at the TNMC/NH to create and maintain database capabilities for data entry, storage, and retrieval, including data auditing and preparation of summary documents and statistical analysis) was largely achieved. This led to collection of the National Alzheimer's Coordinating Center's (NACC) Uniform Data Set (UDS) on these subjects.

Specific Aim #6: Develop an education and information transfer function (EITF): Formalizing the training for TNMC/NH project staff was an impressive achievement: Two of the research staff have continued work in Cognitive Neurology Clinics in different hospitals, and are carrying out impressive work in outreach to physicians and psychologists in lectures and seminars. One research staff (Ms. S. Shankar) went to UK for further career. The Memory Clinic continues clinical and research work directed by one of the Co-PI who has remained at Nair Hospital as Adjunct faculty after his retirement. There are plans for continuing grant funded research on dementia and to continue research collaboration, develop new research focus and continue knowledge exchange between investigators in New York and Mumbai.

Summary of Results:

- **Our** study (both clinical and pathological) indicates that burden of cognitive deficits in older people in an urban center in India is not different from that encountered in New York. The societal approach on dementia is improving and there is increasing awareness of dementia from research project like ours.
- **Our** project demonstrated it well that research development with active/dynamic partnership between developed and developing countries can be achieved and sustained research collaboration established.
- **Our** study also showed that adaptation and transfer of study methods and protocols can be implemented and pursued successfully through dedicated partnership between the scientists of developed and developing countries.
- **Long** term research development with collaborative projects between the high income countries and low/ middle income countries is an effective way of knowledge and skill transfer, leading to research development and in the low and middle income countries in a cost effective way and establish sustained collaboration with high income countries.

List of publications:

1. Purohit DP, Batheja NO, Sano M, Jashnani KD, Kalaria RN, Karunamurthy A, Kaur S, Shenoy AS, Van Dyk K, Schmeidler J, Perl DP. Profiles of Alzheimer's disease-related pathology in an aging urban population. *J Alzheimers Dis.* 2010 Dec 27. [Epub ahead of print] PMID: 21187583
2. Nair G, Van Dyk K, Shah U, Purohit DP, Pinto C, Shah AB, Grossman H, Perl D, Ganwir V, Shanker S, Sano M. Characterizing cognitive deficits and dementia in an aging urban population in India. *Int J Alzheimers Dis.* 2012; 2012:673849. Epub 2012 Jun 27

*** Prepared for the Networking Meeting of The Fogarty International Center on 23rd October, 2012.**

Principal Investigator: Mohammad Hossein Rahbar, PhD / USA
Professor of Epidemiology & Biostatistics, The University of Texas School of Public Health at Houston

Presenter: **Mohammad Hossein Rahbar, PhD**

Maureen Samms-Vaughan, MD, PhD / Jamaica

Professor of Child Health, The University of the West Indies

Grant Number: 1R21HD057808-01A1

Epidemiological Research on Autism in Jamaica

Autism Spectrum Disorders (ASDs) are complicated neurodevelopmental and behavioral disorders that manifest in early childhood and continue into later life. Although the causes of ASD are unknown, the evidence suggests that the origins are likely the result of gene-environment interaction occurring in *utero* or very early in infancy. The prevalence of ASD appears to be on the rise in developed countries, and has become a serious public health concern. In most developing countries, however, the nature and prevalence of factors associated with ASDs are unknown. The long-term goal of this project is to develop capacity for conducting large-scale population-based ASD studies in Jamaica.

The Jamaican Autism study is an age- and sex-matched case-control study with expected enrollment of (150 pairs of ASD cases and controls and their parents) to investigate whether environmental exposures to mercury, lead, arsenic, manganese and cadmium play a role in ASD. In addition, we plan to assess the role of select polymorphisms in glutathione-S-transferase genes (GSTM1, GSTT1, GSTP1), and their potential interactions with these heavy metals in relation to ASD. Enrollment started in December 1, 2009 and ended on March 31, 2012. To enroll 150 ASD cases, we screened 332 children from the Jamaica Autism Database, at the University of the West Indies' (UWI), that included children previously identified as potentially having an ASD based on Diagnostic Statistical Manual of Mental Disorder, 4th Edition (DSM-IV-TR) criteria and the Childhood Autism Rating Scale (CARS). These children were invited to be reassessed for ASD using the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview-Revised (ADI-R). In addition, to enroll 150 age- and sex-matched controls we screened 351 children from schools and well child clinics, and their parents/guardians were administered the Social Communication Questionnaire (SCQ) to ascertain that their behavioral ratings were not suggestive of significant ASD symptomatology.

For both cases and controls, we administered a questionnaire to assess the demographic and socioeconomic status, occupation, smoking of the parents, medical history of children and potential exposure to lead, mercury, arsenic, cadmium, and manganese through food, with a particular focus on the types and amount of vegetables and seafood consumed by children. At the end of interview, 2 mL of saliva and about 5 mL of whole blood were collected from the case and control children.

In interim analyses based on data from 65 match paired (130 children), we did not find a significant association between ASD and blood mercury, arsenic, cadmium, manganese, and lead concentrations. Overall, we have made 7 presentations at scientific meetings and conferences. So far, we have published 3 papers in peer-reviewed journals and one manuscript is under review by Journal of Autism and Developmental Disorders (JADD). However, we have not yet tested the role of gene-environment interactions because of recent challenges for transportation of specimens to the US. The Jamaican government has imposed new licensing requirement for shipment of human blood from Jamaica to the US. We have submitted a request for approval of

the Jamaican government and waiting for their response. We expect to receive the specimens before the no-cost extension period ends on December 31, 2012.

In summary, we have developed a very strong collaboration with our colleagues at the UWI. We have focused on capacity building activities that included conducting a week-long biostatistics workshop at the UWI. Our R01 grant that attempts to build upon on our current work by expanding our environmental exposures to include PCBs was submitted to NIEHS in January 2012. The Council's decision is expected by end of October 2012.

Diane S. Rohlman/ USA,
Gaafar Abdel Rasoul & Ahmed Ismail/ Egypt,

Diane Rohlman

R21 ES017223,

Assessing Vulnerability of the Adolescent Brain to Organophosphorus Pesticides

Organophosphorus (OP) pesticides are used extensively in agriculture throughout the world. There is compelling evidence that repeated (chronic) low-level occupational and environmental pesticide exposures are associated with neurobehavioral performance deficits in adults. Adolescents working in agriculture are exposed to the same risks as adults but it is unknown whether their risk is equivalent to or greater than that of adults. Experimental animal studies indicate that the developing brain is more susceptible to the neurotoxic effects of OPs than the adult brain, and low-level exposures to OP pesticides cause significant neurobehavioral deficits in animal research. Adolescents in Egypt are legally hired as seasonal workers to apply pesticides to the cotton crop. The pesticide application to the cotton crop is highly regulated and standardized across Egypt, and is limited primarily to OP pesticides generally chlorpyrifos, and pyrethrins. This provides a unique opportunity to examine the impact of a highly consistent known OP pesticide exposure on the adolescent and their developing nervous system.

During April 2010, we initiated a pilot study to address Aim 1 of our R21 grant: to collect preliminary data to examine the dose-related response of the adolescent nervous system to chlorpyrifos, an OP pesticide, to determine if repeated exposures produce a progressive deficit and to determine if this deficit is reversible. With support from local Ministry of Agriculture officials, adolescent pesticide applicators (N=58) were recruited from field stations in villages in the Menoufia Governorate. A non-applicator (environmentally exposed) cohort (n=40) was recruited from the same villages. The mean age of all participants was 15.5 years. Applicators reported applying pesticides for an average of 3 years, typically working 4-5 days per week during the application period (June-August). Participants completed 35 test sessions before, during and after the application period, which is standardized across Egypt. As expected, chlorpyrifos (CPF) was the primary OP pesticide applied to cotton crops in the Menoufia Governorate, with daily applications extending for up to 30 days. Internal dose was assessed by measuring urinary 3,5,6-trichloro-2-pyridinol (TCPy) levels, a CPF-specific metabolite, which serves as a biomarker of exposure to CPF. Urinary TCPy levels (exposure biomarker) indicate that CPF exposure increased during the application season followed by a decrease once the application stopped. Blood acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities (effect biomarkers) decreased during the application season, more in applicators compared to non-applicators and showed recovery to baseline levels after application. A preliminary look at ~8 time points across the pilot study indicates that neurobehavioral performance shows a similar pattern. This pattern is also observed in self-reported neurological symptoms, showing an increase in symptoms in applicators during the time of pesticide application and a decline once the season is over.

Aim 2 of the R21 grant was also successfully addressed by expanding research capacity at Menoufia University through both training and education. Equipment and training for biomarker analysis and behavioral testing was provided to Egyptian researchers. Staff were trained and certified on both the analysis of cholinesterase (ChE) activity in blood samples and administration of the neurobehavioral test battery and a Neurobehavioral Testing Laboratory was formed. To date, these methods have been incorporated into 5 additional projects. Cholinesterase data presented above were generated entirely by our Egyptian colleagues. During our initial collaborations with Menoufia we identified a need to provide training on developing fundable research ideas and writing grant applications that express those ideas

effectively. A needs assessment revealed that although faculty are interested in applying for funding (93%) or training opportunities (83%), the majority are not aware of available opportunities, much less what is required to apply for these opportunities. A series of seminars and videoconferences were presented to over 120 researchers to provide an overview of grant funding. Each of these included an evaluation component.

SUMMARY

Dr. MaryAnn Romski/UNITED STATES

Dr. Juan Bornman/SOUTH AFRICA

Dr. MARY ANN ROMSKI

Grant Number: 1R21TW008999-01A1

Grant Title: Speech and Language Delays in Children with Neurodevelopmental Disorders in South Africa

Speech and language skills provide a child with the ability to express wants and needs, interact socially, and gain information about the complex world in which we live. A significant impairment in the development of speech and language has far reaching consequences for a child's long-term development including education and employment. Children with neurodevelopmental disorders are at extremely high risk for speech and language disorders secondary to their primary condition. This project brings together researchers from the United States and South Africa to examine the patterns of language disorders in children with neurodevelopmental disorders. Post-apartheid South Africa provides a unique setting for the study of this issue. It is a country where 11 national languages are immersed within a range of socio-economic contexts. This study will extend the knowledge related to speech and language delays in children with neurodevelopmental disorders from different language backgrounds. Adaptation of assessment tools to this context must be informed by observation and rigorous information about the parent's perception of language development. This planning grant's aim is to develop measurement tools to identify speech and language delays in children with neurodevelopmental disorders within specific language contexts so that appropriate behavioral interventions can be provided to the children. During the first year, we began work on the cross-sectional pilot study to examine the speech and language delays in children, ages 3-8 years, who come from the four predominant language groups (i.e., Afrikaans, English, Sotho, and Zulu) in northern South Africa. To date, we have culturally and linguistically translated the assessments into the four languages. We have developed Institutional partnerships between Pretoria three government hospitals that will participate in the study and are in the process of training the staff who will administer the assessments. Given the substantial need to develop qualified behavioral investigators in South Africa, to date, we have provided statistical and methodological training to staff. We expect to contribute to the long term goal of building sustainable capacity in behavioral research on speech and language delays at the University of Pretoria and other universities to enhance participant recruitment and research training with respect to measurement design and methodology for neurodevelopment disorders.

PI: Ned Sacktor/USA

Collaborator: Elly Katabira and Noeline Nakasujja/Uganda

Presenter: Ned Sacktor, MD

Grant#: MH 083465

Title: HIV DEMENTIA AND SENSORY NEUROPATHY IN UGANDA

HIV dementia (HIV-D) and HIV-associated sensory neuropathy (HIV-SN) are the most common neurological manifestations of advanced HIV infection. The prevalence of HIV-D and HIV-SN in Sub-Saharan Africa where the majority of HIV cases reside globally is largely unknown. The project assembled a cohort of 125 HIV+ individuals in Uganda: 1) to determine the prevalence of and risk factors associated with HIV-D and HIV-SN among untreated HIV+ individuals with moderate-advanced immunosuppression, 2) to determine whether untreated HIV+ individuals decline from baseline in neuropsychological (NP) test performance, and peripheral nerve function, and 3) to obtain preliminary data to determine whether HIV subtypes differ with respect to the risk of HIV-D and HIV-SN, and progression of HIV-associated cognitive impairment and peripheral nerve function.

At baseline 42% of the cohort (52 of 125) had HIV-D. In multivariable analyses, advanced age (OR=1.09, $p=.003$), female sex (OR=4.33, $p=.002$), and low education (OR=1.16, $p=.010$) were associated with an increased odds of HIV-D. 77 HIV+ individuals received follow-up evaluations after 12 months. 31 individuals initiated antiretroviral therapy (ART) after their baseline visit ("ART+" group) due to having CD4 counts < 200 cells/ μ L whereas the other 46 individuals did not require ART initiation ("ART-" group). The ART+ group showed significant improvements, between baseline and 12 months, on several NP tests including the World Health Organization –Auditory Verbal Learning test total score ($p=.003$) and short delay ($p=.008$), Color Trails 1 ($p=.015$) and 2 ($p<.001$), Timed Gait ($p=.012$), and Grooved Pegboard dominant ($p=.003$) and non-dominant hand test ($p=.003$). However, these improvements were no higher for the ART+ group than for the ART- group. Supplementary analyses showed that the ART+ group had significant improvements in CD4 count over the 12 months ($p<.001$) whereas the ART- group did not ($p=.132$). However, both groups had improvements in depression symptomatology ($p=.036$ for ART+ group, $p=.003$ for the ART- group).

A previous study by our group demonstrated that among HIV+ individuals with advanced immunosuppression (Mean CD4 count = 127) in Uganda, HIV subtype D was associated with an increased risk for dementia compared to HIV subtype A. As part of the R21 we examined the association of HIV subtype with dementia among individuals with moderate immunosuppression (Mean CD4 count = 254), and we found no association between HIV subtype and dementia.

We also performed phylogenetic analysis for HIV subtype in both blood and cerebrospinal fluid (CSF). For most individuals the HIV subtype matched between blood and CSF. However, for 3 HIV+ individuals there were discordant results, suggesting the possibility that HIV subtype compartmentalization could contribute to the risk for dementia.

These results demonstrate that HIV-D is common in this Sub-Saharan. ART use is associated with improvements in cognitive functioning among HIV+ individuals in Uganda. However, these improvements did not appear to be higher than those seen among HIV+ individuals who did not initiate ART. Possible reasons for this include practice effects as well as improvements in mood and overall quality of life among the ART- group. In addition, HIV subtype D may have an increased risk for dementia compared to HIV subtype A only among individuals with advanced immunosuppression suggesting that the severe immunodeficiency is a contributing factor to the development of the dementia. Future studies are planned to evaluate the epidemiology of HIV-associated neurocognitive disorders among HIV+ individuals with both moderate and advanced immunosuppression in a large population based study of 400 HIV+ individuals in Uganda and to examine differences in virological compartmentalization within the CSF and blood among HIV+ individuals with and without dementia.

PI: Stephen Schroeder

Grant Title: Early Prevention of Aberrant Behavior in Neurodevelopmental Disorders in Peru

Grant Number: R21HD060500

FIC HD 060500

ABSTRACT

Early Prevention of Aggression, Self-Injury, and Stereotypy among Infants and Toddlers in Peru

Steve Schroeder and an interdisciplinary team of 15 researchers at KU, Texas Tech, CDC, and their Peruvian colleagues at the Centro Ann Sullivan del Peru (CASP), have been conducting a study of the development of severe behavior problems, such as self-injurious behavior (SIB), Aggression, and stereotyped behavior, among infants and toddlers in Peru. Their hope is to decrease or eliminate them before they become deeply ingrained and intransigent in later life.

Screening, Evaluations, and Follow-up. The team first screened for these aberrant behaviors across the country in Peru by advertising in the national media, and they were deluged with phone calls. After 1000 calls, CASP invited 341 for screening interviews and then selected 262 children aged 4-48 mos.

(mean=27 mos.) for in-depth interdisciplinary evaluations. First eight U.S. expert consultants went to CASP to train teams of evaluators. Under the consultants' supervision, children received evaluations involving Developmental and Behavioral Pediatrics, Genetics, Neurology, Psychiatry, Cognition, Language/ Communication, Hearing, Nutrition, Dentistry, Aberrant Behavior, Medical and Social History. Children then were followed with monthly telephone calls to each family, with bi-monthly training workshops on the ABCs of raising a child with disabilities. They were evaluated every six months for a year with an interdisciplinary evaluation at CASP, to see what happened to their aberrant behaviors.

Results. The mountains of data produced in this project have all been analyzed at LSI, except for the genetic s data. The major findings up till now are:

1. Over 90% of these children exhibited some form of SIB, aggression, and stereotyped behavior at an age much earlier and in more complex patterns than previously found in the literature. Over half of them were already doing all of them by six months of age.
2. After a year of follow-up, 57% decreased their aberrant behaviors by over 50%, but 43% were still increasing their aberrant behaviors.
3. The risk factors related to these trends differed for aggression, SIB, and stereotyped behavior. For instance, for the eight risk factors assessed, the main risk factors for aggression were genetic diagnosis, mother education, family income, visual impairment, and gender; but for SIB, they were family income, genetic diagnosis, and gender; for stereotyped behavior, they were genetic diagnosis, gender, family income, and age.
4. We also found large interactions among age, diagnosis, gender, IQ, and communication scores for the different aberrant behaviors. For instance, younger children with Down Syndrome and more stereotyped behaviors, had lower IQs and tended to be increasing stereotyped behavior and SIB by the end of one year, whereas older children at risk for autism and more aggression tended to have higher IQs, and have mothers with more education and higher incomes, and their aggression decreased markedly over one year. These are new findings that have not been demonstrated before among this population at this early age.
5. Not all of the DNA analyses of the saliva from these children have been completed as yet, but we have found some genetic disorders with microarray analysis that were not found by clinical genetic examination. Few of the rare genetic diseases previously found in the literature to be associated with SIB, aggression, and stereotyped were found via clinical diagnosis in our study. So a genomic approach, as well as a clinical syndromal approach may be very valuable in assessing genetic contributions to these children's aberrant behaviors.

The Future. We are currently considering a long-term early intervention program, to follow these valuable children in Peru, as well a similar project to assess children of Hispanic and other ethnic origins in the U.S.

PI: Desire Tshala-Katumbay, MD PhD (Oregon Health & Science University, USA)
Major US collaborator: Michael Boivin, MD MPH (Michigan State University, USA)
Major foreign collaborator: Tamfum Myembe, MD PhD (National Institute for Biomedical Research, Democratic Republic of Congo, DRC)

Presenter: Desire Tshala-Katumbay, MD PhD

Grant number: R01ES01984102

Grant title: Toxicodietary and genetic determinants of susceptibility to neurodegeneration

Background: The food (cassava) associated syndrome also known as konzo is a permanent upper motor neuron disease (paralysis) that affects thousands of children and women of child-bearing age mostly in Sub-Sahara Africa. Whether risk factors include genetics, or nutritional toxicity, or their interaction; or whether the neurodegenerative process extends into cognitive domains remains unknown

Goals: (1) Research Goals: Elucidate the neuropsychological profile of konzo-affected children and determine the role of toxicant exposure, genetic polymorphisms, or their interaction, in modulating risk for cassava-associated neurodegeneration; and (2) Capacity Building Goals: Enhance research capabilities for neuroepidemiology, neuropsychology-neurotoxicology, and genetic testing in the DRC

Research Outcomes: Children relying on insufficiently processed cassava display dramatic cognitive impairments indicating that the overall burden associated with cassava-related diseases has been underestimated. Genetic polymorphisms in thiosulfate sulfur transferase *a.k.a* rhodanese seem to modulate risk for neurocognitive impairments suggesting novel mechanisms for the disease pathogenesis. Findings have profound global health impact as risks for cassava-associated neurodegeneration should be reassessed among millions of those relying on cassava as staple food. The proposed pathogenetic mechanisms will help design strategies to prevent the nervous system consequences associated with the aforementioned diet, toxins, and genetic susceptibility. New collaborations were established to develop an experimental model for further investigation on mechanisms underlying cassava-associated neurological diseases.

Capacity Building Outcomes: In addition to enhancing biochemical capabilities (R21 achievement), a neuropsychology unit has been put in place at the neurology center of Kinshasa University. Research collaboration between the university system and the ministry of health, and between physicians and basic scientists, has been enhanced. Efforts are undergoing to build a "complex morbidity unit" with storage capabilities (cryobank) at the National Institute for Biomedical Research where an ethical IRB has already been put in place. Training in neuropsychological testing have been extended to Rwanda, a DRC neighboring country that is desperately in need for research capacity building thanks to a small training grant from the International Brain Research Organization, the umbrella organization for the Societies for Neurosciences. A multidisciplinary team of 8 young investigators (one MD PhD biologist and postdoc; three MD neurologists, one MD public health specialist, one MD nutritionist, and one Biochemist, all PhD candidates) is being trained to conduct neuroepidemiological research, neuropsychological testing, and genetic analyses as per needs for career and manpower development.

Future directions: (1) Elucidate the neurodevelopmental trajectory (longitudinal assessment) of children relying on cassava as staple food and (2) Develop a research-based intervention to test the proposed mechanistic hypotheses while, simultaneously, introduce cassava processing techniques to reduce exposure to culpable cassava cyanogenic compounds. Research program will be tied to the needs for continuing capacity building.

PI: Jasmin Vassileva, Ph.D., University of Illinois at Chicago, USA

Foreign Collaborator: Georgi Vasilev, MD MPH, Bulgarian Addictions Institute, Sofia, Bulgaria

Presenter name: **Jasmin Vassileva**

Grant number: R01DA021421

Grant title: Varieties of Impulsivity in Opiate and Stimulant Users

The overall aim of this project is to better understand the role of impulsivity in drug addiction and to determine whether some of its neurocognitive manifestations are common across addictions, whereas others are unique to specific classes of drugs. This type of research is significantly complicated by the high rates of polysubstance use among drug users, which preclude investigations aimed at dissociating the unique effects of different classes of drugs on neurocognitive functioning. To address this difficulty, the current study was conducted in Bulgaria, where polysubstance dependence is relatively uncommon and we have access to a unique population of relatively “pure” heroin and amphetamine users. To date, we have tested 97 opiate (heroin) users, 88 stimulant (amphetamine) users, and 105 non-drug using control participants on an extensive battery of neurocognitive, personality, and psychiatric indices of impulsivity. Most opiate and stimulant users were in protracted abstinence at the time of testing (~29 months post discontinuation of drug use), therefore, our findings could increase our understanding of the brain’s recovery of function after discontinuation of drug use.

Our preliminary findings have begun to challenge the predominant unitary account of addiction and reveal that the addiction to opiates and that to stimulants are behaviorally and neurocognitively distinct and may be driven by different underlying mechanisms. Specifically, our findings reveal that stimulants and opiates had common residual effects on neurocognitive indices of “motor” impulsivity, evidenced by increased errors of commission on a task of response inhibition. In contrast, stimulants and opiates were associated with unique deficits on various dimensions of “cognitive” impulsivity, with increased discounting of delayed rewards being uniquely associated with stimulant use, and increased delay aversion on a probabilistic decision-making task being uniquely associated with opiate use. Further, to better characterize the decision-making performance of these two groups of drug users, we partnered with the research group of Dr. Jerome Busemeyer, who applied a computational modeling approach to the decision-making data of our participants. Results revealed that stimulant and opiate users were characterized by distinct decision-making profiles. Specifically, stimulant users showed greater sensitivity to rewards, which was related to duration of drug use, whereas opiate users evidenced reduced aversion to loss, which was related to personality characteristics such as impulsivity and psychopathy. To our knowledge, this is the first study applying a computational modeling approach to investigate the neurocognitive functioning of opiate and stimulant users, and our findings support a “unique” rather than “unitary” account of drug addiction, or rather drug addictions.

Another goal of the study was to determine whether individual differences in risk factors such as externalizing spectrum personality traits or psychopathology would increase vulnerability to neurocognitive deficits in drug users. Our findings indicate that whereas the personality trait of impulsivity is associated with neurocognitive deficits in “motor” impulsivity in stimulant users, that same personality trait is associated with improved “motor” impulsivity performance in opiate users. These findings underscore the complex relationship between personality, psychiatric, and neurocognitive risk factors and indicate that their effects are not

always deleterious and could be potentially beneficial for the neurocognitive functioning of certain types of drug users.

Finally, given that the neurocognitive deficits were observed years after discontinuation of drug use, we hypothesized that deficits in neurocognitive impulsivity could be a viable endophenotype for drug addiction, possibly shared with other externalizing disorders. To investigate this further, we established collaboration with colleagues at the Medical University in Sofia and obtained prior approval from NIDA to collect DNA from our participants. To date, we have obtained DNA from 199 participants (61 opiate users, 61 stimulant users, and 77 control participants) and have genotyped 45 SNPs on 88 of these participants. Preliminary association analyses show trends for differential associations of stimulant and opiate addiction with brain reward and anti-reward systems, such as polymorphisms in the OPRM1, CRH and TPH2 genes.

Together, these findings contribute to a growing body of literature indicating that there are important differences between addictions to different classes of drugs, which are clearly observable in protracted abstinence. These results could have important implications for the development of targeted interventions that focus on the specific type of impulsivity displayed by users of different types of drugs.