Temple University

Statement of Work

Study Title: Serum PINCH Levels as a Biomarker for Severity of Epilepsy

Langford, T. Dianne

Specific Aim 1: To compare serum PINCH levels among the three groups: control patients, patients with medically controlled epilepsy and patients with medically resistant epilepsy.

Specific Aim 2: To demonstrate an association between permeability of the blood brain barrier (BBB) and serum PINCH levels.

For experiments associated with AIMS 1 and 2, Dr. Langford will obtain serum from patients from USC from all three groups (control patients, patients with medically controlled epilepsy and patients with medically resistant epilepsy) and conduct ELISA or standard western analyses to compare PINCH levels among the three groups. For western analyses, equal volumes of serum will be loaded per well. The expression levels of PINCH from Western analyses will be conducted using ImageJ and statistical analyses will be conducted using the Prism GraphPad program. ELISA results will be determined according to manufacturer’s instructions via standard plate reader assessment. Levels of PINCH protein from each sample will be reported to USC in a blind coded fashion and the sample code will be broken to determine if PINCH levels are different across the three groups and if levels associate with BBB permeability.
Budget Justification

Katz School of Medicine at Temple University
Personnel:
T. Dianne Langford, Ph.D., Significant Contributor/PI at Temple, (effort = 0 calendar months). Dr. Langford will act as a significant contributor on this grant with the University of Southern California. Dr. Langford will oversee experiments performed by Ms. Ferrero and analyze data with the investigators at USC. Dr. Langford is not requesting salary as her supervision of Ms. Ferrero is within the course of daily laboratory management.

Kimberly Ferrero, Technician, will contribute 5% (0.6 calendar months) to this project. Ms. Ferrero is a highly trained biomedical research technician with several years of experience with ELISA and western analyses. She will process and run all of the serum samples collected for AIMS 1 and 2. She will report all results to Dr. Langford who will then forward results to USC.

Materials and Supplies
Western analyses will be conducted on serum as described in AIMS 1 and 2 for PINCH and for Tau.

PINCH and Tau antibodies will be used to assess changes in levels of PINCH and Tau in Western blotting. PINCH antibody 2 at $415/each, p-Tau antibody 1 at $398/each, T-Tau antibody 1 at $376 each

PINCH antibody 2 at $415/each, p-Tau antibody 1 at $398/each, T-Tau antibody 1 at $376 each $1,604

PAGE (polyacrylamide gel electrophoresis) gels will be used to run serum samples to assess changes in levels of PINCH and Tau. 2 kits at $170/each

PAGE (polyacrylamide gel electrophoresis) gels will be used to run serum samples to assess changes in levels of PINCH and Tau. 2 kits at $170/each $340

Transfer membranes will be used to transfer proteins from PAGE gels to as is standard for Western blotting. 1 at $161/each

Transfer membranes will be used to transfer proteins from PAGE gels to as is standard for Western blotting. 1 at $161/each $161

ELISA analyses will be conducted on serum as described in AIMS 1 and 2 for PINCH and for Tau.

PINCH ELISA 2 at $870/each and T-Tau ELISA 2 at $530/each

PINCH ELISA 2 at $870/each and T-Tau ELISA 2 at $530/each $2,800

TOTAL SUPPLIES $4,905
Proposal Being Submitted by the
Temple University - Of The Commonwealth System of

Principal Investigator
Name: T. DIANNE LANGFORD
Department: TUSM:NEUROSCIENCE (04400)
Address: Dept of Neuroscience
3500 N. Broad Street
Philadelphia Pennsylvania 19140-4106

Phone: 215-707-5487
Fax: 215-707-4888

Proposal Title: Serum PINCH levels as a biomarker for severity of epilepsy

Total Funds Requested: 7,950
Project Period Dates: 01-Jul-2017 to 30-Jun-2018

Submitted To
Name: UNIVERSITY OF SOUTHERN CALIFORNIA

Awardee Institution
Name: Temple University - Of The Commonwealth System of
Attention: CARRIE FARMER
Address: 3340 N. Broad Street
Student Faculty Center Suite 427
Philadelphia PA 19140-5104

Phone: (215)707-7547
Fax: (215)707-8387
Email: grantsmanagment@temple.edu
Entity Identification Number: 1231365971A1
DUNS: 05-712-3192
Congressional District: PA-002

Principal Investigator Date
Authorized Institutional Officer Date

Institution Number (Internal use Only):

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Langford, T. Dianne

POSITION TITLE
Associate Professor

eRA COMMONS USER NAME (credential, e.g., agency login)

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>MM/YY</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troy State University</td>
<td>BS</td>
<td>05/92</td>
<td>Marine Biology</td>
</tr>
<tr>
<td>University of Alabama, Tuscaloosa, AL</td>
<td>PhD</td>
<td>12/96</td>
<td>Cell/Molecular Biology</td>
</tr>
<tr>
<td>University of California San Diego, CA</td>
<td>Post-Doctoral Fellow</td>
<td>04/02</td>
<td>Neuropathology, Infectious Diseases</td>
</tr>
</tbody>
</table>

NOTE: The Biographical Sketch may not exceed four pages. Follow the formats and instructions below.

A. Personal Statement

Briefly describe why your experience and qualifications make you particularly well-suited for your role (e.g., PD/PI, mentor, participating faculty) in the project that is the subject of the application. Within this section you may, if you choose, briefly describe factors such as family care responsibilities, illness, disability, and active duty military service that may have affected your scientific advancement or productivity.

I have investigated the PINCH protein for over 10 years during which time I discovered that it is expressed in the central nervous system. The PINCH protein is important for the maintenance of neuronal polarity as well as for cell migration and attachment to the extracellular matrix. In the healthy adult brain, PINCH is nearly undetectable, however in pathologies involving hyperphosphorylated Tau (hpTau), PINCH is found in neurons and some astrocytes and can be detected in the cerebrospinal fluid (CSF) and blood. In fact, we have discovered that PINCH levels in the brain reflect those of hpTau and that hpTau and PINCH bind one another. In a recent publication, (Charles Liu, Jon Russin, Christianne Heck, Keisuke Kawata, Radhika Adiga, William Yen, Jonathan Lambert, Meng Law, Yvette Marquez, Peter Crino, David Millett and Dianne Langford Dysregulation of PINCH Signaling in Mesial Temporal Epilepsy. Journal Clinical Neuroscience 2016) we reported that PINCH was detectable in hippocampal tissues from MTLE patients compared to controls. PINCH and Tau were co-localized in some neurons in MTLE tissues. While PINCH was expressed by both neurons and astrocytes in MTLE tissues, hpTau was primarily extracellular or associated with neurons. Finally, PINCH was readily detectable in the serum from patients with chronic epilepsy but absent from the serum of control subjects. Our study describes the expression of PINCH and points to AKT/GSK3β signaling dysregulation as a possible pathway in hpTau formation in MTLE. In view of the interactions between hpTau and PINCH, understanding the role of PINCH in MTLE may provide increased understanding of mechanisms leading to inflammation and MTLE epileptogenesis and a potential biomarker for drug resistant epilepsy. With over 10 years of experience working with PINCH in the context of neurodegenerative disease, our lab is the leader in PINCH CNS dysfunction.
B. Positions and Honors

List in chronological order previous positions, concluding with the present position. List any honors.

Positions and Employment

<table>
<thead>
<tr>
<th>Year</th>
<th>Position/Title</th>
<th>Institution</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>1992-1996</td>
<td>Doctoral Candidate</td>
<td>Univ. of Alabama</td>
<td>Tuscaloosa, AL</td>
</tr>
<tr>
<td>1996-1997</td>
<td>Postdoctoral Fellow</td>
<td>San Diego State</td>
<td>San Diego, CA</td>
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<tr>
<td>1997-2000</td>
<td>Postdoctoral Fellow</td>
<td>UC, San Diego</td>
<td>San Diego, CA</td>
</tr>
<tr>
<td>1997-2002</td>
<td>Post-grad Res.</td>
<td>UC, San Diego</td>
<td>San Diego, CA</td>
</tr>
<tr>
<td>2002-2003</td>
<td>Program Project Sci</td>
<td>UC, San Diego</td>
<td>San Diego, CA</td>
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<tr>
<td>2004-2008</td>
<td>Asst. Adj. Professor</td>
<td>UC, San Diego</td>
<td>San Diego, CA</td>
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<tr>
<td>2007-2011</td>
<td>Asst. Professor</td>
<td>Temple University</td>
<td>Philadelphia, PA</td>
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<tr>
<td>2011-present</td>
<td>Assoc. Professor</td>
<td>Temple University</td>
<td>Philadelphia, PA</td>
</tr>
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Other Experience and Professional Memberships

- 2008-present: International Society of NeuroVirology, Editor NewsLetter, Chair Communications committee
- 2012-present: Editorial Board, Journal of Biological Chemistry
- 2012-present: Editorial Board, Journal for Neurovirology
- 2013-present: Special Society Reviewer, Alzheimer’s & Dementia: the Journal of the Alzheimer’s Association
- 2013-present: Board of Directors, ISNV

Honors and Awards (selected)

- 2006: National Center of Leadership in Academic Medicine, UCSD SOM
- 2006: Honorary Faculty Member of the Addis Ababa University School of Medicine, Department of Neurology, Addis Ababa, Ethiopia

C. Selected Peer-reviewed Publications

Do not include manuscripts submitted or in preparation. The individual may choose to include selected publications based on recency, importance to the field, and/or relevance to the proposed research.

   http://dx.doi.org/10.1016/j.jocn.2016.10.012

   DOI information: 10.1016/j.neubiorev.2016.05.009

   PMCID: PMC3595241

   PubMed NIHMS ID: NIHMS162502


D. Research Support

List both selected ongoing and completed research projects for the past three years (Federal or non-Federally-supported). Begin with the projects that are most relevant to the research proposed in the application. Briefly indicate the overall goals of the projects and responsibilities of the key person identified on the Biographical Sketch. Do not include number of person months or direct costs.

**Active NIH Support**

1R01MH107340 (Langford, Dianne) 08/25/2016-05/31/2021
PINCH-mediated CNS cell dysfunction and tauopathy in HIV
Major goals: uncover the molecular signaling pathways among HIV Tat and TNF-a that regulate PINCH leading to hpTau accumulation.

NCAA-DoD Grand Alliance Clinical Study Core (Langford, Dianne) 06/01/16-05/31/18
Temple University will serve as a partnering institution in the CSC that will develop and implement a multi-year, multi-institution prospective, longitudinal phased-in research protocol whose aim will be to study the natural history of concussion.

1P01DA037830-01A1 (Khalili, K) 02/15/2015 – 01/31/2020
Dysregulation of metabolic and bioenergetic pathways by cocaine and HIV-1 in CNS
Major Goals: Investigate the impact of cocaine and HIV on metabolism in oligodendrocyte and neuron crosstalk.

United States Army ACC-APG-RTP W911NF (Langford, T.D.) 09/01/2016-08/31/2018
Advanced Ballistics Technology Material development, characterization and computational modeling: High rate deformation and failure materials
Major Goals: Increase understanding of traumatic brain injury, resilience and injury prevention

P30MH092177 (P.I.: K. Khalili) 08/05/11 - 05/31/16
NIH/NIMH
Comprehensive NeuroAIDS Core Center Grant
Basic Science Core
Goals: To provide mammalian cell culture and virology services to investigators performing biomedical research on AIDS and the nervous system.

No Overlap

Previous NIH Support:
P30 MH092177 (Khalili, K) 07/01/11 - 06/30/16
Comprehensive NeuroAIDS Core Center Grant
Major Goals: To provide mammalian cell culture and virology services to investigators performing biomedical research on AIDS and the nervous system.

R01 MH085602 (Langford, TD) 05/01/09 – 01-31-14
Role of PINCH in neuronal response to HIV infection of the CNS
Major Goals: Determine the level(s) at which neuronal expression of PINCH is regulated in response to factors present in the brain during CNS HIV infection and identify the biological consequences of PINCH expression in neuronal response to host and viral factors produced during HIV CNS disease

R21DA029523-01A1 (Langford, TD) 07/01/10 – 04/30/12
Cocaine and HIV-mediated disruptions of hypothalamic signaling in hypothyroidism
Major Goals: Investigate the effects of cocaine and HIV infection on hypothalamic signaling in the euthyroid and hypothyroid mouse and the molecular level(s) at which cocaine and/or HIV affect hypothalamic thyroid signaling

R21 NS055639-01 (Langford, TD) 09/06/06 – 05/31/11
CNS Involvement in the HIV Population in Ethiopia: A Post-mortem Forensic Study
Major Goals: Determine and characterize the patterns of HIV-related CNS complications in the Ethiopian population and potential interactions with Tuberculosis. Define unique pathological patterns in this population.

K01MH071206 (Langford, TD) 04/01/04 – 03/31/09
Effects of HIV PIs on CNS endothelial cells in NeuroAIDS
Major Goals: To determine the effects of chronic HIV protease inhibitor treatment on cerebral endothelial cell fitness and signaling mediated by FGF2 via interactions with P-glycoprotein
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