# Alzheimer's and Related Diseases Research Award Fund

## 2017-2018 FINAL PROJECT REPORT SUMMARIES

The Alzheimer's and Related Diseases Research Award Fund (ARDRAF) was established by the Virginia General Assembly in 1982 and is administered by the Virginia Center on Aging at Virginia Commonwealth University. The awards this year were enhanced by a $50,000 donation from Mrs. Russell Sullivan of Fredericksburg, in memory of her husband who died of dementia. Sullivan awards are indicated by an asterisk (*). Summaries of the final project reports submitted by investigators funded during the 2017-2018 round of competition are given below. To receive the full reports, please contact the investigators or the ARDRAF administrator, Dr. Constance Coogle (ccoogle@vcu.edu).

**VCU** **Heather Lucas, PhD**

***Developing an Expression Platform for Tetrameric Alpha-Synuclein to Advance Systemic Biochemical Studies***

The dynamic protein α-synuclein (αS) is universally known to be a key player in Parkinson's disease (PD) pathology and other synucleinopathies. In its monomeric form, αS is considered to be an intrinsically disordered protein that is known to aggregate into fibrillar strands that make up the major component of Lewy bodies, the pathological hallmarks of PD. Recent reports, based on studies investigating αS from human red blood cells and brain tissue, have suggested that a tetrameric form of αS also exists. Tetrameric αS has the potential to be a valuable PD drug target due to its aggregation resistance. Therefore, the focus of this project was to develop technology for the isolation and purification of tetrameric αS using a recombinant platform. Utilizing a mild purification technique that employs ammonium sulfate precipitation, in conjunction with sequential chromatography steps the investigators enabled streamlined access to this elusive protein. Improved access to the tetramer will provide a path to targeted drug discovery as well as a source for fundamental knowledge on the mechanisms linking αS biochemistry with PD etiology. The investigative group's expression platform represents the first reported method for accessing this elusive conformer without the addition of structure-modifying additives while also facilitating the installation of the N-terminal acetyl group present in the native human form of αS. Biophysical evaluation of their recombinant tetrameric αS confirms this multimeric conformer as aggregation-resistant, underpinning its therapeutic significance and importance as a scaffold for future biochemical and/or biophysical studies.

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**VA Tech** **Harald Sontheimer, PhD***

***Is Amyloid Toxic for Glial Cells?***

This project investigated the hypothesis that the gradual buildup of amyloid plaque, the pathological hallmark of Alzheimer disease (AD), may be toxic to glial support cells rather than neurons. More specifically the plaque may impair the interaction between astroglial cells and blood vessels, which is essential to maintaining the blood brain barrier and keeping harmful molecules and immune cells out of the brain. The investigators used a sophisticated imaging approach based on laser scanning microscopy through a glass window mounted in place of the skull above the brain of mice harboring human APP mutations. This allowed them to image the same blood vessels daily for many months and, indeed document a gradual buildup of plaque around many blood vessels. Using a contrast medium injected into the blood stream they could then show that leakage occurred only on vessels with amyloid plaque, indicating the focal and selective break down of the blood brain barrier by amyloid.

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**VCU** **Xuejun Wen, MD, PhD**

***An In Vitro Model for Alzheimer's Disease based upon 3D Self-Assembled Neurovascular Microtissues***

Conventional model systems that rely on *in vivo* transgenic animal models cannot capture the complexity and biology of human systems. As a result, therapeutic strategies that are efficacious in animal models fail in pre-clinical and clinical human trials. In order to improve the translational potential of experimental studies, establishing an in vitro humanized model for AD is imperative. The investigator previously established two induced pluripotent stem cell (iPSC) lines, one from an AD patient carrying a PSEN1 gene mutation and the other an AD patient carrying an APP gene mutation. For this study, the research team fabricated an *in vitr*o AD tissue model based upon 3D self-assembled neurovascular microtissues of primary AD cortical neurons and glia cells that are associated with microvasculatures. This project was intended to validate the model through testing of neurovasculature delivered drugs. To better mimic the native brain microenvironment, the lab group developed a new bioreactor to achieve creeping flow conditions which mimics the interstitial fluid flow in native brain tissue. They then tested several known compounds/drugs that either benefit AD patients or pose toxicity to neural tissues. Their experiments compared multiple models, such as 2D static culture model, 3D static/no-flow suspension culture, and 3D creeping flow model. The results showed that the 3D creeping flow model best represents the in vivo conditions in humans. These results then, define guidelines for the development of *in vitro* models of the specialized neurovascular tissue environment that will advance understanding of healthy states and pathologies, identifying therapeutic targets, and drug testing.

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**VCU-Shenandoah Family Practice Residency** **Jonathan Winter, MD***

*Changes in Physician Approaches to Behavioral and Psychological Symptoms of Dementia since CMS's National Partnership to Improve Dementia Care*

After CMS's 2012 initiative to reduce 'inappropriate' antipsychotic use in nursing homes, such prescribing decreased 27% in 4 years. Excluded from this calculation however were antipsychotics 'appropriately' prescribed for schizophrenia, Tourette's, and Huntington's. Over this same period, CMS has described a greater than 20 percent increase in the reporting of these diagnoses. Exactly how this might exaggerate apparent reductions in inappropriate antipsychotic use for dementia patients is unknown. In addition, since the initiatives debut, CMS has been careful to trend the prescribing of other psychiatric medications commonly used for dementia symptoms including anxiolytics, antidepressants, and sedative-hypnotics to ensure that medication substitution is not occurring as the use of antipsychotics decreases. Our earlier ARDRAF funded pilot study hinted that the use of lithium and anticonvulsant mood stabilizers - risky medications also used off-label for dementia symptoms in nursing homes – have increased since 2012. Unlike the other classes of psychotropics used for behavioral and psychological symptoms of dementia, utilization of this group of medications in the long-stay setting is not being trended by CMS, and exactly how such prescribing has changed since 2012 is similarly unknown. We propose to retrospectively query de-identified data from the Virginia Department of Medical Assistance Service's data warehouse for rates of these diagnoses and medications in Virginia's long-stay residents since 2011. Our objective is to better clarify how reactionary changes in diagnosing and prescribing distort the apparent reduction in pharmacologic solutions to dementia symptoms since CMS's 2012 *National Initiative*.

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**VA Tech** **Ling Wu, MD, PhD and Bin Xu, PhD***

***Drug Repurposing for Tau Aggregation Inhibitors as Neuroprotective Agents for Alzheimer's Disease***

The goal of this project was to apply an interdisciplinary approach involving cellular, biochemical, biophysical, drug screening and neurobiological methods to discover and validate novel candidates, from the National Institutes of Health Clinical Collection repurposing library, that will inhibit human tau aggregates and their neurotoxicity. The outcomes from this pilot project now serve as the basis for a future comprehensive drug discovery and translational research program to devise potential treatment strategies for AD. The investigators were able to produce in high-quality all six recombinant human tau isoforms that are competent to form aggregates and mature filaments. They discovered that human tau 3 repeat isoforms have significantly faster aggregation than those of the corresponding 4 repeat isoforms. Using a molecular biology approach, the researchers have identified that the second repeating segment is a key contributor to the formation of toxic aggregations of human tau isoforms. The conditions for growing each of the six human tau isoform filaments have been developed, and multiple detailed biochemical and biophysical characterizations of selected tau aggregation inhibitors (TAIs) have been identified. Several strong TAIs in cell-based assays have been validated for their neuroprotective effects. This project laid a strong foundation that allows the collaborators to perform *in vivo* efficacy tests of selected compounds in a human tau AD mouse model and discover potential new treatment strategies. Their results serve as the basis for high-resolution structure studies of the filaments of human tau isoforms and the design of future structure-based inhibitors.

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**VCU** **Shijun Zhang, PhD***

***Development of NLRP3 Inflammation Inhibitors for AD***

Inflammasomes are important protein complexes that regulate innate immunity. The Nucleotide-binding Oligomerization Domain-like receptor family pyrin domain-containing-3 (NLRP3) inflammasome plays a role in inflammatory disease and is the one that has been most often studied. NLRP3 inflammasome activation leads to the production of interleukin (IL)-1β and promotes inflammatory cell death. Notably, recent studies have indicated a critical role for NLRP3 inflammasome and IL-1β in the pathogenesis of AD. Therefore, development of novel NLRP3 inflammasome inhibitors (NLRP3Is) may represent novel effective disease modifying agents for AD. Previously, the investigators developed small molecule NLRP3Is and identified one lead compound with *in vivo* efficacy to reduce AD pathology and improve memory functions. The goal of this funded project was to develop more potent NLRP3Is analogs based on the lead compound and evaluate them *in vitro* for their inhibitory potencies. The research team successfully synthesized and structurally characterized a series of analogs with modifications on two domains of the lead structure, and completed biological characterization to evaluate the synthesized analogs. The studies established that structural modifications on the sulfonamide domain can only be limited to certain substituents. Modifications on the phenyl ring, in general, improved inhibitory potencies, although potency was decreased if the substituent was beyond a certain length. This could be due to limited cell permeability, so future work will further characterize a new lead compound.

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**UVA Zhiyi Zuo, MD, PhD***

***Empathic Transfer of Postoperative Cognitive Dysfunction***

Caregiving spouses of patients with dementia have an increased chance of suffering from dementia later. Although the mechanism for this phenomenon is not clear, increased stress due to caregiving to the patients and similar living environments are thought to contribute to it. Postoperative cognitive dysfunction (POCD) is a relatively new but well-documented clinical entity that affects patients after heart and non-heart surgeries. POCD not only affects patients' daily activity but also predicts high mortality. Recent studies from the investigator's laboratory (and others) have indicated that inflammation in the brain (i.e., neuroinflammation), an abnormal process for many chronic brain diseases including AD, may be involved in POCD. The researcher's preliminary data showed that mice living in the same cage with mice that have surgery (cage-mates) also develop neuroinflammation and POCD. Results from this funded project showed that cage-mates had increased levels of inflammatory mediators in the blood. Mice that could see, but did not live in the same cage with, mice that had surgery also had increased inflammatory mediators in the brain. Cage-mates also presented with anxious behavior. Finally, this study showed that the ventral posterior nucleus of the thalamus, which relays somatosensory information to the cerebral cortex, is activated in the cage-mates of mice with surgery. These results suggest the transfer of pathological process through sight and have significant implications for bystander health. These findings may help us understand how caregiving spouses of patients with dementia may develop dementia later in life**.**

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