PROGRESS REPORT TO
THE H. L. SNYDER MEDICAL FOUNDATION
JULY 2022

Nearly 270,000 American men will be diagnosed with prostate cancer in 2022, making it the most common cancer diagnosis among men. Almost all prostate cancers are adenocarcinomas that start in the glands that line the prostate, the walnut-sized organ that is part of the male reproductive system. Prostate cancer is rare in men under 50 years of age; however, the chance of developing the disease increases with age.

At Dana-Farber Cancer Institute, the Lank Center for Genitourinary Oncology has been instrumental in changing the treatment landscape for prostate cancer and other genitourinary malignancies, leading research and clinical efforts to improve and extend the lives of patients. World-class investigators at the Lank Center, including **William Hahn, MD, PhD,** and **Matthew Freedman, MD,** continue to make critical progress in the field of prostate cancer research and care, thanks in no small part to the philanthropic support of the H. L. Snyder Medical Foundation. Thank you for your continued commitment to their work.

William Hahn, MD, PhD
Executive Vice President and Chief Operating Officer, Dana-Farber Cancer Institute
William Rosenberg Professor of Medicine, Harvard Medical School
Institute Member, Broad Institute of MIT and Harvard

In 2019, Hahn and his colleagues identified the overexpression of the CREB5 protein as a driver of resistance to standard hormone therapy in men with metastatic castration-resistant prostate cancer (CRPC), a late-stage, aggressive form of the disease. They subsequently found that CREB5 interacts with FOXA1, which helps the androgen receptor (AR) bind to DNA and promotes prostate cancer development and resistance to AR-targeted therapies. AR is a master transcription factor that regulates prostate cancer development and progression.

In a study published in the May 2022 *eLife*, Hahn and his colleagues used a mass spectrometry approach called RIME (Rapid Immunoprecipitation of Endogenous Protein) to define how CREB5 mediates resistance to treatment. The team, which included Freedman and scientists from multiple institutions, found that the dynamic binding properties of CREB5 facilitate the assembly of essential AR and FOXA1 factors to promote treatment resistance. At the molecular level, the findings suggest a complex model of therapy resistance that occurs in the nucleus of cells that activates oncogenic signaling pathways.

Among these pathways is Wnt, which is inappropriately activated in many cancers, and epithelial-mesenchymal transition, an important step in metastasis. Hahn's findings may lead to new ways to intervene in this resistance process, as well as precision strategies to treat patients with CREB5-dependent prostate cancer and other malignancies.

Matthew Freedman, MD
Associate Physician, Medical Oncology Service, Dana-Farber Cancer Institute
Professor of Medicine, Harvard Medical School
Associate Member, Broad Institute of MIT and Harvard

Since the discovery of the androgen-sensitive nature of prostate cancer, suppression of systemic testosterone has remained the most effective initial therapy for advanced disease, although progression almost inevitably occurs. Recent preclinical studies have shown that activation of AR with supraphysiological (greater than normally found in the body) levels of testosterone can paradoxically inhibit late-stage CRPC.

In the May 2022 *JCI Insight*, Freedman and a team of scientists from Dana-Farber, Washington state, and Canada, reported for the first time that tumor models that respond to supraphysiological levels of testosterone (SPT) exhibit a distinct AR cistrome compared to non-responding tumors. The cistrome is a collection of regulatory elements for a set of genes; in this particular case, it is the set of AR binding sites. The findings provide an opportunity to identify a subset of patients with CRPC who may benefit from SPT therapy.

Treatment with SPT has demonstrated anti-tumor efficacy in a subset of patients with CRPC; therefore, identifying signatures that predict SPT response would have important implications for choosing which patients should receive this therapy and which should avoid it. In patient-derived models, Freedman and his colleagues demonstrated that the different AR cistromes of SPT responders and non-responders can be clearly stratified in preclinical settings. The researchers are now validating this signature in patients treated with SPT to further support its use in the clinic.

Conclusion

Your vision and generosity enable Hahn and Freedman to uncover new insights into the biology and genetics of prostate cancer. The advances described in this report continue to help pave the way for new, more effective therapies for patients. We are grateful for your support of Dana-Farber's lifesaving mission.

William Hahn, MD, PhD, 2021-2022 Publications

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Stanford | MEDICINE

MEDICAL CENTER DEVELOPMENT

2021 – 2022 Activity and Progress Report

Laboratory of Seung Kim MD, PhD

Prepared for H.L. Snyder Medical Foundation

Thank you. Your continued, generous support of **Dr. Seung Kim** and his colleagues has allowed the team to make exciting progress in the development of novel diagnostic and therapeutic strategies for pancreatic disease.

With support of the H.L. Snyder Medical Foundation, Dr. Kim and colleagues have developed a novel transplant protocol that cures diabetes in a mouse model -- a breakthrough that that shows real promise as a novel treatment for people with diabetes, and this has fostered Stanford Health Care (SHC) support for the **Stanford Pancreatic Islet Replacement and Immune Tolerance (SPIRIT) program**.

Below we detail the major achievements of Dr. Kim over the past year – all made possible thanks to the vision of the H.L. Snyder Medical Foundation, and the foundation's support of collaborative research throughout Stanford University, and beyond.

1. New approaches for achieving islet replacement and immune tolerance to cure diabetes

Thanks to ongoing collaboration with Stanford Immunology colleagues, including long-time recipient of H.L. Snyder Medical Foundation support **Dr. Judith Shizuru**, Dr. Kim has developed antibody-based 'conditioning' (as the basis of 'reduced intensity conditioning') to achieve successful **simultaneous hematopoietic stem cell transplantation with engraftment of matching islets** in a mouse model.

In these **first-in-kind studies** Dr. Kim and team demonstrate that their protocol for combining bone marrow and islet transplantation post-antibody-conditioning can cure diabetes in preclinical animal models (Bhagchandani et al 2022, Chang et al 2022) – an essential step towards FDA-approved clinical trials for people with diabetes. Dr. Kim and his colleagues are currently developing these preclinical findings into a proposal for a clinical trial with members of the Stanford Pancreatic Islet Replacement and Immune Tolerance (SPIRIT) program, to the California Institute of Regenerative Medicine (CIRM).

Dr. Kim will be happy to present and discuss data from these promising studies at the 2022 H.L. Snyder Medical Foundation Annual Meeting.

2. NCI support for a pancreas cancer program at Stanford

Together with 6 other faculty at Stanford, Dr. Kim directs a P01-supported center focused on diabetes and pancreatic cancer. We are excited to report that funding from the National Cancer Institute (NCI) began in 2021-22 and will extend for 5 years. In turn, this achievement was instrumental in helping the Stanford Cancer Institute (SCI) renew its NCI-designated cancer center award in 2022.

3. Renewal of NIH support for the Stanford Diabetes Research Center

Thanks to the vision of the H.L. Snyder Medical Foundation, and based on a vigorous effort in the past decade, Dr. Kim and his Stanford colleagues have developed a world-class research base for diabetes investigations. This culminated in Dr. Kim founding of the Stanford Diabetes Research Center (SDRC) in 2016, and we are thrilled to report that in 2022 the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) renewed the SDRC funding for the next 5 years. Through leadership by Dr. Kim and other members of the SDRC, Stanford Health Care (SHC) has also initiated funding to launch the new Stanford Pancreatic Islet Replacement and Immune Tolerance (SPIRIT) program.

4. Seung Kim named K.M. Mulberry Professor of Developmental Biology

Thanks to the generosity of an anonymous donor, Dr. Kim was honored to be named the inaugural holder of the KM Mulberry Endowed Professorship at Stanford University. He was also honored to be appointed a Professor in Medicine. He also continues to hold a courtesy appointment in Pediatrics.

5. Kim Lab alumna awarded Assistant Professorship

Dr. Romina Bevacqua, an instructor in the Kim group, accepted a position as Assistant Professor at the Mt. Sinai School of Medicine in New York, New York. She will continue her groundbreaking studies of human islet genetics and signaling there, using CRISPR- and genomics-based approaches.

6. Summary

Dr. Kim had team have continued to grow Stanford research programs focused on islet replacement and protection, with goal of generating cellular therapeutics for type-1 diabetes (T1D). Dr. Kim's productivity and success is reflected in his selection to **lead four Stanford groups devoted to basic and translational diabetes research**; the SDRC, the Northern California JDRF Center of Excellence at Stanford, Stanford

Pancreatic Islet Replacement and Immune Tolerance (SPIRIT) and a National Cancer Institute (NCI) center focused on understanding diabetes links to pancreatic cancer.

Dr. Kim's progress would not have been possible without the support of the H.L. Snyder Medical Foundation –Thank you again.

Citations

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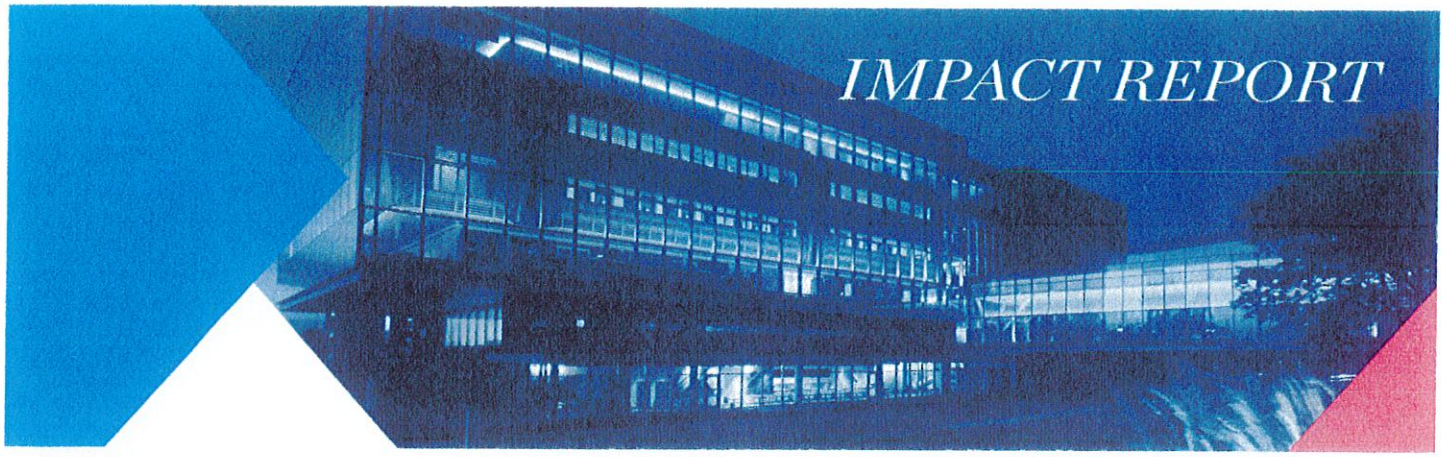

H.L. Snyder Medical Foundation

Prepared September 2022

When it comes to funding research, seed money is crucial — and private grants can be transformative. Since 2019, the H.L. Snyder Medical Foundation's investment in Alzheimer's disease research led by Dr. Russell Swerdlow at the University of Kansas Medical Center has led to significant results. Through the **H.L. Snyder Medical Foundation Fellowship Fund** at KU Endowment, Dr. Swerdlow and his team of investigators have been able to advance research and pioneer innovation. Below are stories of how the Foundation's generosity has made a difference at KU and beyond.

Progress Highlights

Since our September 2021 reporting, Dr. Swerdlow continued his research into Alzheimer's disease (AD) mitochondrial dysfunction. Studies focused on genes that underly mitochondrial function including mitochondrial DNA (mtDNA) and the APOE gene; mitochondrial connections to the classic AD pathologies including amyloid plaques and tau tangles; and how to manipulate mitochondria to treat AD patients. Efforts advanced Dr. Swerdlow's "Mitochondrial Cascade Hypothesis," which uniquely proposes AD arises as a primary consequence of mitochondrial stress. According to this novel paradigm, mitochondria initiate AD, drive its progression and pathologies including plaques and tangles, and provide a roadmap for developing effective therapies. Figure 1 illustrates the program's overall organization:

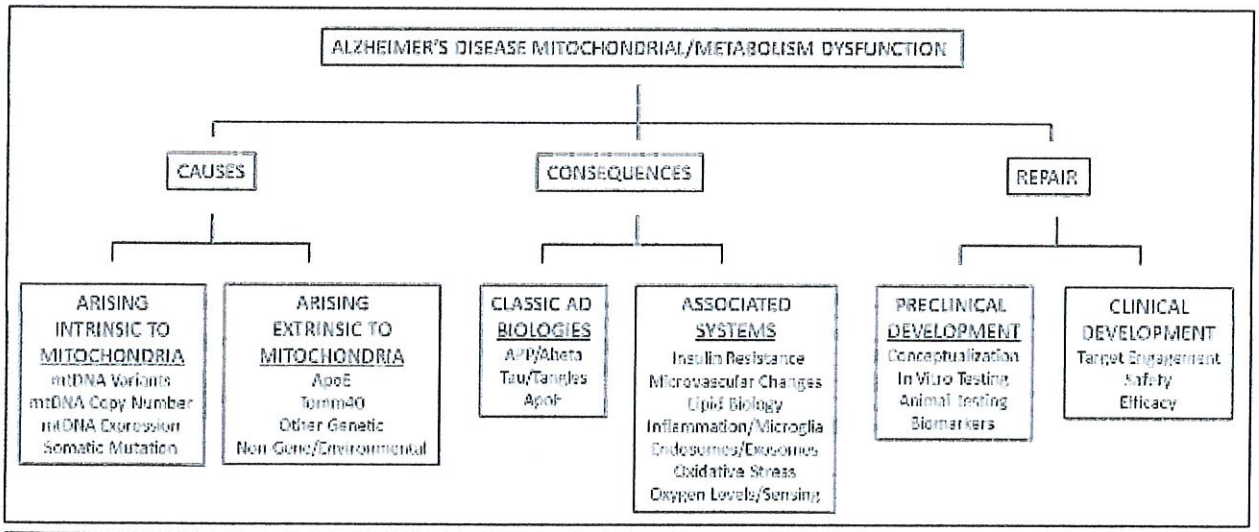

Figure 1. Research program overall organization.

Of note, in June 2022 Dr. Swerdlow received an international award, the Oskar Fischer Prize. The awarding committee recognized the Mitochondrial Cascade Hypothesis as one of the world's most promising attempts to conceptualize AD. In the past year Dr. Swerdlow delivered invited Mitochondrial Cascade Hypothesis national and international presentations to these forums:

- The Target Enablement to Accelerate Therapy Development for Alzheimer's Disease Consortium (TREAT-AD)
- The Annual Conference of the American Society for Neural Therapy and Repair
- The national Alzheimer's Disease Research Center network
- The Oskar Fischer Prize Awards symposium
- The Accelerating Medicine Program for Alzheimer's Disease (AMP-AD) Mitochondrial Interest Group

Relevant publications since the previous progress report include:

[1] Morris JK,McCoin CS, Fuller KN, John CS, Wilkins HM, Green ZD, Wang X, Palash S,Burns JM, Vidoni ED, Koseva BS, Mahnken JD, Grundberg E, Shankar K, **Swerdlow RH**, Thyfault JP. Mild cognitive impairment and donepezil impact mitochondrial respiratory capacity in skeletal muscle. Function 2021;2:zqab045.

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[12] Ding WX, **Swerdlow RH**. Editorial of special column on Drug targets and drug development for Alzheimer's disease. Acta Pharm Sin B 2022;12:1686-1687.

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Specific major accomplishments over the past year, and directions for the coming year, are discussed below.

Genetic Studies

We reported mitochondrial DNA (mtDNA) copy number was lower in brains of persons who died with AD than it was in the brains of persons who did not. We further demonstrated inverse associations between mtDNA copy number and AD histology changes (plaques and tangles) and the magnitude of cognitive impairment within an individual. Essentially, brains containing less mtDNA contained more plaque and tangle pathology, and persons with less mtDNA had more cognitive impairment.

Some investigators will assume reduced mtDNA copy number is a consequence and not a cause of AD. We suspect it is more likely a cause and data we generated support this view. We found a specific heritable mtDNA sequence, haplogroup J, associates with higher mtDNA copy number and increased plaque/tangle pathology. Haplogroup J carriers also show evidence of reduced late-life memory capacity, which manifests prior to AD symptoms. In a related study, we observed aging mice normally upregulate their brain mtDNA copy number. Given this perspective, we propose that to maintain normal function neurons containing haplogroup J mtDNA must maintain a higher baseline mtDNA copy number, and as those neurons age their capacity to mount an age-determined compensatory increase in their mtDNA is consequently diminished.

The APOE gene that encodes the apolipoprotein E protein associates with AD for unclear reasons. We developed a novel hypothesis to explain this. The APOE gene sits adjacent to the Translocase of the Outer Mitochondrial Membrane 40 kilodalton (TOMM40) gene, which expresses a critical mitochondrial protein. Our data suggest TOMM40 affects APOE expression through an enhancer-promoter relationship. Our data suggest mitochondrial dysfunction, including dysfunction induced by low mtDNA copy numbers, reduces TOMM40 expression, and reduced TOMM40 expression promotes increased APOE expression. This is important because neurons normally do not express APOE. We subsequently examined AD autopsy brains to look for evidence of neuron APOE expression and found APOE-laden neurons (Figure 2). This is remarkable because APOE is a secreted protein, so in addition to aberrantly expressing APOE the neurons appear unable to properly secrete it.

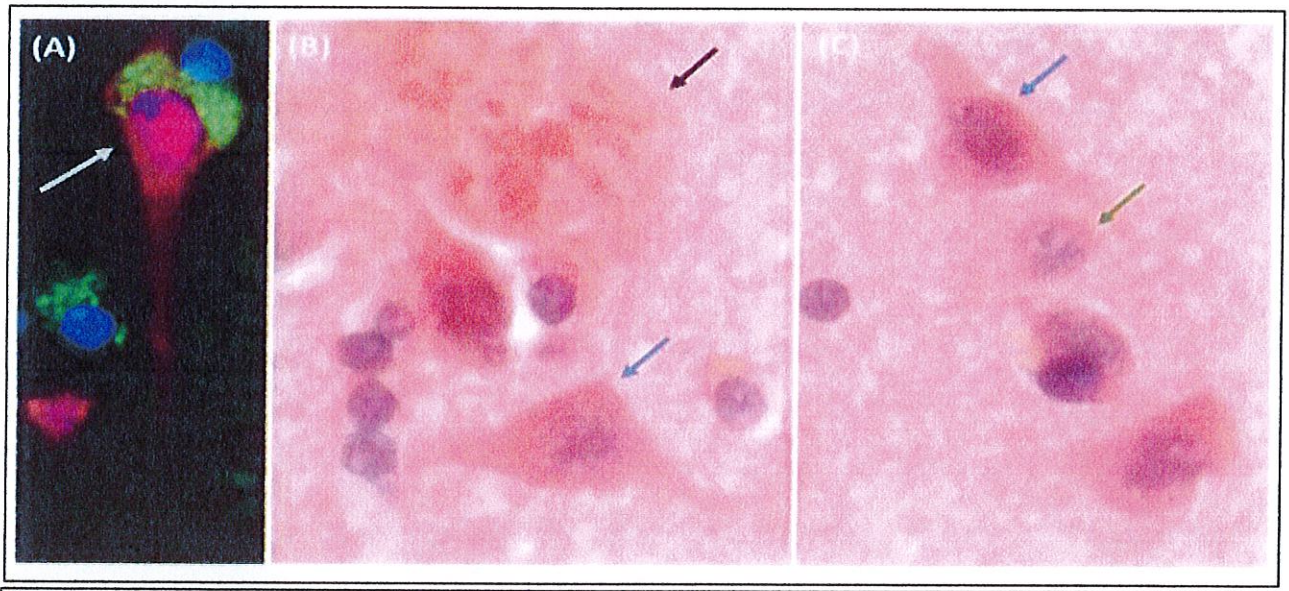

Figure 2. Neurons in a Human Brain Contain ApoE. (A) Immunofluorescence merged image with DAPI, a primary antibody to NeuN (red), and a primary antibody to ApoE (green). The cell indicated by the arrow is a neuron, and we suspect the green signal above the nucleus is ApoE. (B) In this immunohistochemistry picture obtained using a primary antibody to ApoE and AEC as the chromogen, the blue arrow shows a reasonably healthy cell with a large nucleus. This is likely an ApoE-laden neuron. The black arrow reveals a plaque decorated by ApoE. (C) Similar to (B), the blue arrow shows an ApoE-laden neuron next to a neuron in which we do not clearly detect ApoE (indicated by the green arrow).

Per our working model, an age-related decline in mitochondrial function turns on neuron APOE expression; the neurons cannot secrete the apolipoprotein E protein they produce; and the retained apolipoprotein E further perturbs neuron function and integrity. Figure 3 summarizes our conceptual model. Over the past year we also obtained insight into the mechanisms through which mitochondrial dysfunction triggers neuron APOE expression. The

mechanism involves two stress signaling pathways, ERK and JNK, and the Nrf2 transcription factor. We are currently preparing a manuscript that summarizes these discoveries and conducting further experiments to define additional components of the proposed TOMM40-APOE regulatory circuit.

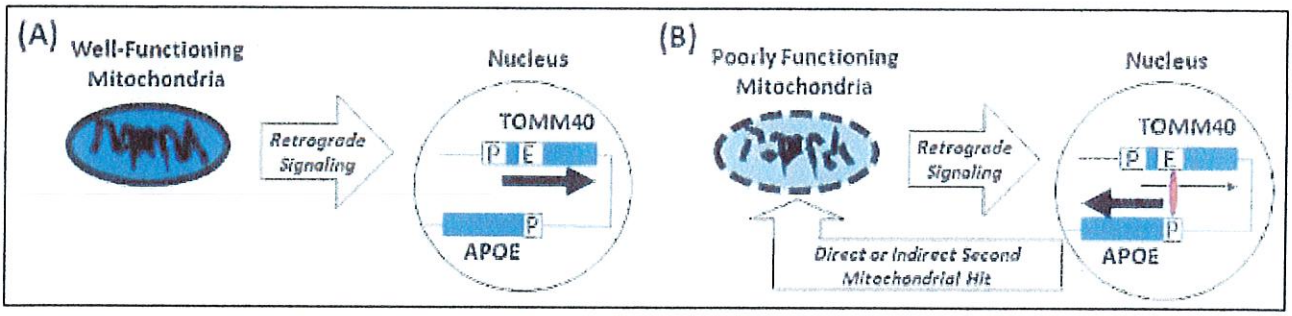

Figure 3. Mitochondrial function influences APOE transcription. (A) With normal mitochondria, TOMM40 expression is robust which prevents the TOMM40 enhancer from engaging the APOE promoter; APOE expression is low. (B) With perturbed mitochondria TOMM40 expression declines, the TOMM40 enhancer-APOE promoter engage, and APOE expression turns on; this directly or indirectly further damages the cell. E=enhancer, P=promoter.

Linking Mitochondria to AD Pathologies

Our recently published data show when neuronal cells express the amyloid precursor protein (APP), only a small amount localizes to mitochondria. Most APP distributes to the plasma membrane. Neurons process APP to beta amyloid (Aβ), which they secrete. We found that in settings of mitochondrial dysfunction, APP redirects to mitochondria, less reaches the plasma membrane, and less Aβ secretion occurs. We believe this can at least partly explain why cerebrospinal (CSF) Aβ levels are reduced in AD patients.

We in fact believe CSF levels can serve as a biomarker of brain mitochondrial function. To address this hypothesis, we recently began obtaining CSF from comatose patients hospitalized in The University of Kansas Hospital intensive care unit with traumatic brain injuries or subarachnoid hemorrhage. Brain mitochondria in comatose patients are relatively inactive, but activity returns as the coma resolves. We are testing for associations between level of consciousness and Aβ in these samples. We are also measuring the tangle-forming protein tau, as well as mitochondrial components including mtDNA. Through this we hope to develop CSF assays that will inform the status of a living person's brain mitochondria without having to perform an actual brain biopsy.

As discussed above, this past year we continued to link mitochondrial and APOE biology. We are interested in how neuronal apolipoprotein E impacts overall neuron integrity, and directly or indirectly affects mitochondrial function. We therefore created neuronal cell lines that uniquely express the different APOE2, 3, and 4 isoforms. We predicted APOE4, the isoform that associates with increased AD risk, would disproportionally impair mitochondria but surprisingly discovered constitutive expression of all APOE isoforms induces equivalent states of mitochondrial stress. In concordance with our data that show TOMM40 and APOE constitute part of a regulated expression unit, we hypothesize APOE4's ability to increase AD risk arises because the proposed TOMM40-APOE enhancer-promoter relationship is more efficient when APOE4 is the allele. In other words, a state of primary mitochondrial dysfunction is more likely to trigger aberrant neuron APOE expression when APOE4 is present. We are currently testing this using two advanced technologies, Nanostring and Hi-C chromatin capture.

The apolipoprotein E protein plays a recognized role in lipid biology, which is broadly altered in AD patients. Accordingly, we initiated a "lipidomics" study to determine how mitochondrial dysfunction alters neuronal cell lipidomes. Finally, we are concluding a project to more completely inform how a state of primary mitochondrial dysfunction alters the expression of the APP gene, as well as its processing to Aβ. Our data reveal reducing the mtDNA copy number increases APP RNA and protein levels (Figure 4).

Mitochondrial and Metabolism-Targeted AD Therapeutics

We recently enrolled our 30th participant into a randomized trial that is evaluating how a ketogenic diet affects cognitive abilities in AD patients. Our goal is to is matriculate 80 participants. The rationale is that inducing ketone body production through a ketogenic diet will partly compensate for the state of reduced glucose utilization we know exists in AD. We believe this state reflects a consequence of mitochondrial dysfunction, and that adding the ketone fuel will enhance the brain's ability to generate energy and signals that will promote mitochondrial maintenance. As part of this study, we perform extensive assays using blood cells from our trial participants. This includes applying a powerful omics technique, RNASeq, to determine how a ketogenic diet effects overall gene transcription in the trial participants.

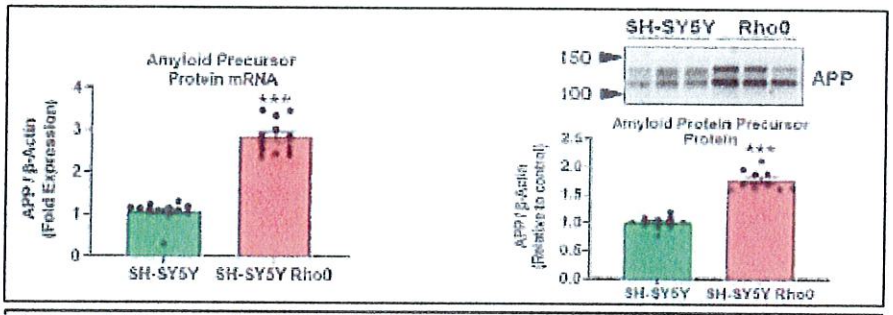

Figure 4. Neuronal mtDNA depletion increases APP RNA and protein.

This past year we also completed a study assessing the impact of a diabetes drug, dapagliflozin, on AD patients. Dapaglifozin, an SGLT2 inhibitor, blocks the local re-uptake of renally excreted glucose. This leads to a mild reduction in blood glucose levels and a mild state of ketosis. We are currently analyzing the dapagliflozin trial data.

In 2020 we reported that administering adequate doses of the metabolism intermediate oxaloacetate (OAA), a dicarboxylic acid, can promote brain bioenergetic fluxes in human AD patients. Because OAA is unstable in solution, we created OAA prodrugs in which OAA's carboxyl ends are esterified to a ketone body, β-hydroxybutyrate, or a lactate precursor, propylene glycol. The former molecule was designed to deliver OAA as part of a ketogenic diet mimetic intervention, and the latter to deliver OAA as part of an exercise mimetic intervention. We are currently determining oral absorption, tissue distribution, and pharmacokinetics in the preclinical setting. These studies, which required us to generate isotope-labeled versions of the compounds, are intended to move us towards investigational new drug application (IND) filings with the FDA.

Our long-term plan is to treat AD by attacking the disease at two points: (1) reversing aging-associated reductions in bioenergetic fluxes, and (2) blocking the pathway through which mitochondrial stress initiates neuron APOE expression (steps 1 and 2 as shown in Figure 5).

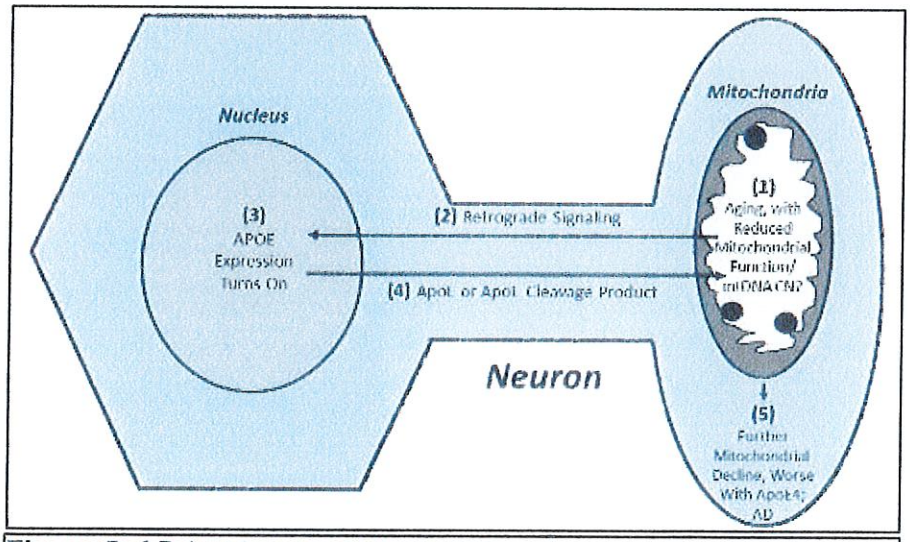

Figure 5. AD treatment strategy. Efforts focus on enhancing mitochondrial flux (step 1) and inhibiting the neuron APOE expression (step 2).

You Can Continue to Make a Difference

Support from the H.L. Snyder Medical Foundation has helped to dramatically accelerate the pace and elevate the quality of Dr. Swerdlow's research program at KU. In addition to the publications generated and international recognition received over the past year, the University of Kansas doubled Dr. Swerdlow's laboratory space from 1,200 to 2,400 square feet. This has allowed Dr. Swerdlow to hire additional personnel and commit to training additional graduate students. Hiring quality postdoctoral fellows and research associates is costly, and Dr. Swerdlow is required to pay a stipend to and tuition costs for the graduate students he trains. Procuring equipment, lab reagents, and sophisticated instrumentation is also a major investment. Critically, the advanced omics applications (RNASeq, Hi-C chromatin conformation capture, Nanostring RNA and protein measurements, lipidomic analyses) are incredibly expensive. It could take years to procure NIH grant funds that would allow Dr. Swerdlow to take advantage of these advanced techniques. For these reasons, the H.L. Snyder Medical Foundation's support has been instrumental to our recent successes and is sincerely appreciated. **Thank you!**

Russell H. Swerdlow, MD
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KU Alzheimer's researcher wins international prize for his work with mitochondria

Russell Swerdlow, M.D., professor of neurology, earns the Oskar Fischer Prize for innovative Alzheimer's disease research

June 15, 2022 | Leilana McKindra

Russell Swerdlow, M.D., director of the University of Kansas Alzheimer's Disease Research Center, has chiseled out a rock-solid reputation as an unconventional thinker. Being recognized as one of 10 Oskar Fischer Prize winners only adds to that legacy.

Russell Swerdlow, M.D., director of the University of Kansas Alzheimer's Disease Research Center, was recognized as one of 10 Oskar Fischer Prize winners. The prize, designed to expand society's understanding of the causes of Alzheimer's disease, is the world's largest of its kind, with $4 million awarded across three categories.

The prize, aimed at expanding society's understanding of the causes of Alzheimer's disease, is the world's largest of its kind, with $4 million awarded across three categories. As an Oskar Fischer Prize recipient, Swerdlow will receive $300,000.

An expert in brain energy metabolism, Swerdlow's research focuses on the Mitochondrial Cascade Hypothesis, which represents his effort to tie together a field of work that argues mitochondria play a critical role in the development and progression of Alzheimer's disease.

For Swerdlow, this hypothesis represents the evolution of research he initiated as a medical student at New York University in the 1980s, continued as a neurology resident at the University of Virginia, pursued as a postdoctoral fellow and faculty member at the University of Virginia, and brought with him to the University of Kansas Medical Center in 2007. The hypothesis asserts that deficient brain energy and malfunctioning mitochondria initiate late-onset Alzheimer's disease. The theory, which has slowly gained traction over the last 15-plus years, runs counter to the longstanding hypothesis that the disease is caused by a buildup of beta-amyloid, a protein that forms plaques, and tangles of a protein called tau, which are always present with Alzheimer's.

"It is difficult to find words that capture my appreciation and gratitude to the Oskar Fischer Prize Committee for this recognition," Swerdlow said. "It means so much to me that the committee sees the hypothesis that has guided my research efforts over several decades, the Mitochondrial Cascade Hypothesis, as a serious hypothesis that warrants serious consideration by the broader field."

In addition to serving as the Gene and Marge Sweeney Professor of Neurology at KU Medical Center, Swerdlow also holds professorships in the departments of Molecular and Integrative Physiology and Biochemistry and Molecular Biology. He directs the KU ADRC, one of 31 Alzheimer's Disease Research Centers designated by the National Institute on Aging and a world leader in the study of the role of mitochondria in Alzheimer's disease. Swerdlow previously received the 1997 S. Weir Mitchell Award, a scientific research award from the American Academy of Neurology, and he was the 2014 recipient of the University of Kansas Chancellor's Club Research Award.

"I also want to thank the mentors, mentees, collaborators, colleagues, and supporters that helped me to conceive, develop and advance the Mitochondrial Cascade Hypothesis," he said. "I hope it will continue to lead to a better understanding of what drives Alzheimer's disease, and ultimately to more effective treatments."

The Oskar Fischer Prize was established in 2019 with a philanthropic gift to the University of Texas at San Antonio from Texas businessman James Truchard, Ph.D. As part of the rigorous competition, entrants were challenged to boil down the breadth of Alzheimer's research to date into a single explanation for the cause of the disease. The winning entries include unique theories that highlight key aspects of Alzheimer's and provide new frameworks for potential causes of the disease.