THE CHRONOS CHRONICLE:
The Newsletter of the Aging Institute
Summer 2023

Message From the Director

For those who have visited the Aging Institute, you know that we are situated along the Monongahela River, adjacent to a structure known as the Hot Metal Bridge. In the past, that bridge was utilized by the Jones and Laughlin Steel Corporation to carry crucibles of molten iron from the blast furnaces on our side of the river to the open-hearth furnaces on the opposite bank. During World War II, it is estimated that 180 tons of steel crossed this bridge every hour, equating to approximately 15% of the total U.S. steel production during that period.

Looking out my window, there are now fields of green grass and hiking trails where once blast furnaces produced and open railroad ladle cars carried molten metal. While one is hard-pressed to find any trace of that former industrial footprint, the legacy of that era remains omnipresent. This is due, in large part, to the enduring philanthropic efforts of Pittsburgh's leading families. In its industrial heyday, Pittsburgh was noted for such titans as Carnegie, Mellon, and Frick.

Walking around Pittsburgh today, one can still peruse the latest bestseller available at a branch of the Carnegie Library or see the newest touring art exhibition at the Frick Art Museum. Being a native of Washington, D.C., I also remember many a weekend walking through the Mellon Collection of Art that catalyzed the formation of the now famous National Gallery of Art.

While the leading industrialists of the last two centuries devoted their philanthropic efforts towards cultural institutions, many now choose to direct their giving towards medical research. In an era of shrinking NIH budgets, these philanthropic gifts are becoming increasingly important, if not essential, to maintain a robust scientific enterprise. Fortunately, we, at the Aging Institute, have been the beneficiaries of local, national, and international gifts. Early on, we obtained critical support from the Beckwith Institute and the Jewish Healthcare Foundation, two local Pittsburgh institutions.

As you can see in the following pages, recently our faculty have received major gifts from several new and generous donors. These include the Pittsburgh-based Richard King Mellon Foundation, the California-based WoodNext Foundation and the international Hevolution Fund. These gifts act as catalysts for the work we do and allow our faculty to pursue new and often outside-of-the box ideas.

We are incredibly grateful for these past gifts and our most recent support. We like to believe that with these funds, we can build on Pittsburgh's industrial past and, like the Hot Metal Bridge we see every day, create a base of knowledge that is both strong and enduring!

Toren Finkel, MD, PhD
Director, Aging Institute,
University of Pittsburgh/UPMC
Professor of Medicine,
Division of Cardiology
G. Nicholas Beckwith III and
Dorothy B. Beckwith Chair
in Translational Medicine

Faculty Spotlight: Aditi Gurkar, PhD

by Kritika Chaddha, PhD, Postdoctoral Associate in the Gurkar Lab

How would you explain what you do to someone unfamiliar with your work and field?

As we age, we are at a higher risk for developing chronic diseases that affect our quality of life. These diseases and risks include cardiovascular diseases, diabetes, osteoporosis, and neurodegeneration — such as in Alzheimer's disease and dementia. My lab is interested in understanding the biology of aging to help delay the incidence of such debilitating diseases and to ensure everyone can live healthier, more productive lives.

What draws you to your work?

Growing up, I had a very close relationship with my grandparents. They lived with our family and were a huge part of my life. As they got older, my grandmother struggled with memory and mobility issues and was confined to a wheelchair while my grandfather remained active and was rarely sick until the end of his life. Consequently, from a very young age, I was haunted by the question: *why do people age differently?* And this is the question that inspires me every day. I believe if we can understand the rate of aging, we can also intervene and have fuller, healthier lives.

As an Assistant Professor at the University of Pittsburgh School of Medicine, my lab in the Aging Institute strives to answer these fundamental questions. We study how we can best assess biological age and what we can do to promote healthy aging and improve quality of life for all. Our most recent work includes studies of the underlying biology of major weight loss during aging, the role of lipid metabolism in senescence and aging, and deeper understanding of the causal metabolites that drive rapid aging.

How does this research impact society?

As you may know, our older population is growing at an unprecedented and fast pace. One in five individuals will be over the age of 65 by 2030. That is a staggering number! Most individuals over the age of 65 have at least one chronic disease, and a substantial number are affected by comorbidities, which they counter with polypharmacy — by using multiple medications to treat different health conditions.

This reality not only affects the individuals but also impacts their families and burdens our healthcare system. What we are hoping to achieve with our research is a 'precision biological age predictor' in combination with tailored intervention so that we can address these age-related diseases and improve the overall health of individuals, their families, and our health care system.

What do you like most about working at the Aging Institute?

Healthy aging for all is truly what I am passionate about. The Aging Institute has incredible and collegial scientists who believe in a common vision and make my science better! I am also lucky to be part of the Division of Geriatric Medicine and to work with and alongside so many passionate geriatricians who want to improve the lives of our elderly. I am truly inspired by all of them. In addition to my work and my colleagues, I am motivated by living in a community where these needs are present. Pennsylvania is ranked fifth amongst states in the U.S. in terms of its total population over the age 65 — where better to be inspired and serve the community?

Is there any helpful advice you have received in your career?

A friend and colleague of mine told me once that pursuing research in academia is a marathon, not a sprint. To really do impactful science, it may take time, a lot of challenges, and possibly some frustration. But pacing oneself and keeping an eye on the finish line helps!

What is the most enjoyable part of your job?

The people in my lab give me so much joy. Of course, I love the science we do, but it cannot be fun and innovative unless you have the right team. I have been lucky enough to have some of the most dedicated, motivated, and fun-loving people in the lab. And maybe equally enjoyable is any time I get to see new data. It is still so exciting!

Which authors or books have influenced you the most?

The book that influenced me the most was *Time, Love and Memory* by Jonathan Weiner. I remember feeling so connected in some intangible way with what I thought a researcher's life would be. It was truly life changing; I decided to become a geneticist due to Weiner's portrayal of Dr. Seymour Benzer. I was blessed to have a supportive family who cheered me on when I moved to the U.S. at the age of 17 to pursue this dream, and they remain my biggest supporters.

Outside of the lab, do you have any hobbies?

Yes, many! My family is what I call a 'foodie fam.' I am always trying new food and recipes created by my husband and son. My daughter and I love all art forms — we dance, paint, and do origami. I also love science communication — and although I am an introvert — you will see me talking to anyone and everyone about aging!

Finally, if you weren't doing this, what would you be doing?

I do love what I am doing every day. It is not an easy career to pursue, but then I remember my grandparents. I believe if I can help our elderly in some way — today or years from now — I will have made an impact. But, if I must think of something else that I enjoy equally, it would be dancing. I am a classically trained dancer in the artform of Bharatanatyam and got my master's degree at the age of 10. Even today when I dance, I feel like I am 10! I hope to always hold onto that feeling and remain forever youngish.

Trainee Spotlight: Shohini Ghosh-Choudhary

Shohini Ghosh-Choudhary is a trainee under the supervision of Toren Finkel, MD, PhD, director of the Aging Institute and distinguished professor at the University of Pittsburgh School of Medicine. Ghosh-Choudhary is a medical student in the University of Pittsburgh's physician-scientist Medical Scientist Training Program, which combines medical and research training to equip students to both treat patients and undertake cutting-edge research. She joined Dr. Finkel's lab in 2020 as his first PhD student, and her work in the lab focuses on CRISPR-based screening to identify targets for senolytic therapies.

The accumulation of senescent cells over time is implicated in a wide range of age-related diseases. There is a need to develop senolytic drugs to deplete senescent cells without harming healthy cells. In her work, Ghosh-Choudhary uses an unbiased CRISPR Synthetic Lethality Screen to look for mechanisms that will clear cells that have undergone telomere-induced senescence. To date, she and the Finkel Lab have found that endoplasmic reticulum (ER) stress pathways are genetic vulnerabilities for senescent cells. Ghosh-Choudhary and the Finkel Lab are also studying the functional implications of telomere-induced senescence in the cardiomyocytes of mice.

Ghosh-Choudhary is originally from the East Brunswick area of New Jersey and completed her bachelor's degree in biomedical engineering at Georgia Tech. After graduation, she spent two years working at the National Institutes of Health in the Sickle Cell Branch of the National Heart, Lung, and Blood Institute, where she researched sickle cell disease, its outcomes, and potential therapies. Ghosh-Choudhary showed that red blood cells in patients with sickle cell disease abnormally retain mitochondria, which triggers the formation of neutrophil extracellular traps and proinflammatory DAMP signaling. Ghosh-Choudhary is also passionate about optimizing physician-scientist training and studies how best to reduce attrition and bolster funding trajectories for trainees.

Join the Aging Institute – We are hiring!

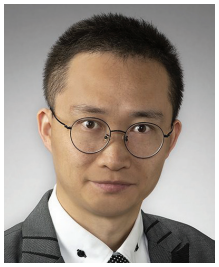
Post Doctoral Associate Positions

- **Dr. Aditi Gurkar Lab**
- **Dr. Shihui Liu Lab**
- **Drs. Shiori & Yusuke Sekine Lab**
- **Dr. Matt Steinhauser Lab**
- **Dr. Jay Tan Lab**
- **Dr. Bokai Zhu Lab**

Staff Positions

- **Laboratory Manager -** Gurkar Lab
- **Research Scientist -** Yuan Liu Lab
- **Research Scientist -** Parkhitko Lab
- **Laboratory Research Specialist -** Parkhitko Lab
- **Laboratory Research Technician -** Li Lab

Faculty Update: Xiaojun (Jay) Tan, PhD



Xiaojun (Jay) Tan, PhD, faculty member of the Aging Institute of the University of Pittsburgh/UPMC, was appointed as assistant professor in the Department of Cell Biology. Dr. Tan joined the Aging Institute in April 2019 as a research faculty member working with Toren Finkel, MD, PhD, director of the Aging Institute. Dr. Tan has since built a robust research program, studying lysosomal quality control in aging and age-related disease, which became the foundation for the establishment of his independent laboratory at the Aging Institute in July 2022. Dr. Tan is also a faculty member of the Cell Biology and Molecular Physiology Graduate Program within the Interdisciplinary Biomedical Graduate Program.

Dr. Tan has had a long-term research interest in lysosomes since he was a graduate student. Lysosomes are essential to cell physiology due to their control of fundamental pathways involved in nutrient sensing and cellular growth. Lysosomes are also important to aging and age-related pathologies, as they play critical roles in cellular responses to stress, clearance of pathogens, and clearance of damaged macromolecules like protein aggregates. Dr. Tan's lab is leveraging expertise in biochemistry, cell and molecular biology, disease models, and large-scale screening methods to discover novel mechanisms that maintain lysosomal integrity and activity. The ultimate aim is to benefit human health through targeted improvement of lysosomal function.

One area that Dr. Tan will focus on in his lab is to further investigate a novel pathway of rapid lysosomal repair that he discovered during his work with Dr. Finkel. The phosphoinositide-initiated membrane tethering and lipid transport (PITT) pathway was initially discovered with an unbiased, proximity-labeling approach involving isolation and characterization of the lysosomal surface proteome before and after lysosomal membrane damage, a hallmark of age-related disease. Dr. Tan elucidated a molecular mechanism in which the PITT pathway drives formation of extensive, new ER-lysosome membrane contacts and subsequent ER-to-lysosome lipid transfer through multiple stages.

The PITT pathway represents a major advance in part due to its many links to conditions and diseases of aging. The PITT pathway is activated by various chemicals that trigger lysosomal membrane permeabilization or osmotic rupture, by silica which causes a long-term lung disease called silicosis, by genetic models of lysosomal storage diseases, or by an effector protein ORF3A of SARS-CoV-2 that is known to induce lysosomal membrane damage.

The PITT pathway may be particularly important to age-related pathologies involving neurons and cardiomyocytes, because these cell-types are particularly reliant on lysosomal activity for homeostasis. Indeed, loss of the PITT pathway exacerbates tau fibril spreading, a key step in the progression of Alzheimer's disease. Loss of the PITT pathway also increases cellular accumulation of lipofuscin — a pathological finding in old lysosomes and a known hallmark of aging and age-related disease.

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In mouse models and human patients, loss of the first enzyme in the PITT pathway causes lysosomal dysfunction, severe neurodegeneration, and premature aging. Dr. Tan's work reveals the potential molecular underpinnings of these genetic conditions, providing his new lab with strong rationale to explore the broader relationship between lysosomal function and pathological processes in aging biology and relevant models of disease.

Dr. Tan's work has been supported by his Aging Institute start-up funds as well as a UPMC Competitive Medical Research Fund award and a K01 Research Scientist Development Award from the National Institute on Aging .

University of Pittsburgh and UPMC

AGING INSTITUTE RESEARCH SEMINAR SERIES

Visiting Speakers, 2023 - 2024

2nd Thursday of the Month | 4:00 pm - 5:00 pm
Bridgeside Point 1 | 100 Technology Drive | 5th Floor Conference Room
Zoom link: https://pitt.zoom.us/j/99688230409

Shin-ichiro Imai, MD, PhD

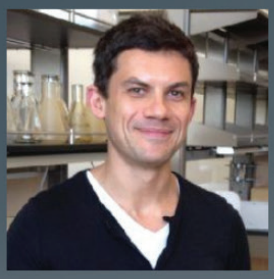
Professor, Developmental Biology and Medicine
Washington University School of Medicine

Vittorio Sebastiano, PhD

Associate Professor (Research)
Obstetrics/Gynecology
Director, Transgenic Knockout and Tumor Model Service (TKTC)
Stanford University

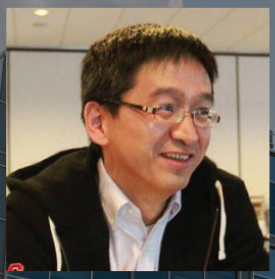
Roberta Diaz Brinton, PhD

Professor, Department of Pharmacology ad Neurology
Professor Psychology and Evelyn F. McKnight Brain Institute
Director, Center for Innovation in Brain Science
University of Arizona



Roberto Zoncu, PhD

Associate Professor, Biochemistry, Biophysics and Structural Biology
University of California, Berkeley



Ling Qi, PhD

Chair, University of Virginia Department of Molecular Physiology/Biological Physics
University of Virginia

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UPMC LIFE CHANGING MEDICINE

Aging Institute Research Seminar Series: February 2023

Richard Youle, PhD: "Mitochondrial Quality Control Mechanisms"

by Shiori Sekine, PhD

In a seminar at the Aging Institute earlier this year, Richard J. Youle, PhD, senior investigator at the National Institutes of Health, presented a recent advance in understanding the mechanisms by which dysfunctional mitochondria lead to Parkinson's disease (PD). Parkinson's disease is an age-related progressive neurodegenerative disease that causes unintended or uncontrollable movements due to a loss of Dopaminergic neurons in the central nervous system. Currently, about 500,000 Americans carry a PD diagnosis.

Based on previous observations that mitochondrial toxins cause parkinsonism in humans — characterized by stiffness, tremors, and slowness of movement — it has been suggested that PD is attributable to mitochondrial dysfunction. Dr. Youle's lab discovered that the products of two genes that are mutated in inherited PD — PINK1 and Parkin — mediate autophagic removal of defective mitochondria. This discovery suggests that one cause of PD is impairment of mitochondrial quality control.

Dr. Youle's lab also revealed and was one of the first to show mitochondrial Parkin recruitment in response to mitochondrial damage for autophagic removal of dysfunctional mitochondria (Narendra et al., *J. Cell Biol.*, 2008). This work has now been cited more than 3,500 times and is a landmark study in the fields of both mitochondrial biology and Parkinson's disease.

Increasing evidence also suggests that impaired mitochondrial quality control promotes mitochondrial damage-associated inflammation, which may ultimately contribute to the progression of PD. A recent murine study published by Dr. Youle's lab (Sliter et al., *Nature*, 2018) revealed that impairment of PINK1/Parkin-mediated mitophagy results in release of mitochondrial DNA (mtDNA), thereby triggering inflammation through the cGAS-STING pathway and resulting in the death of Dopaminergic neurons.

The cGAS-STING pathway is an innate immune pathway that mediates expression of inflammatory cytokines in response to double-strand DNA in the cytosol. Cytosolic DNA usually comes from invaded pathogens. However, in mice with defective mitophagy, the cGAS-STING pathway was activated by mtDNA that leaked from dysfunctional mitochondria, leading to inflammation. Chronic inflammation causes DNA damage, cellular senescence, cancer, cognitive decline, and age-related diseases.

At the seminar, Dr. Youle also highlighted a recent human cohort study (Borsche et al., *Brain*, 2020) that showed a relationship between increased circulating cell-free mtDNA and elevated inflammatory cytokines in serum in PD patients with biallelic *PINK1/PRKN* mutations. This series of studies further demonstrates the impact of inflammation — caused by impaired mitophagy and subsequent mtDNA release — on the pathogenesis of *PINK1/PRKN*-linked PD. Promoting the autophagic removal of damaged mitochondria and/or preventing mitochondrial damage-induced inflammation is a promising therapeutic strategy to prevent PD progression.

Dr. Youle has published more than 180 peer-reviewed research articles and has received several awards, including three NIH Director's awards. In 2021, he was awarded the Breakthrough Prize in Life Sciences for his discovery of the PINK1/Parkin-mediated mitophagy and its implication in PD pathogenesis. His curiosity-driven and inspiring scientific journey never stops.

In addition to detailed analysis of the PINK1/Parkin-mediated mitophagy pathway, his lab is also investigating the biology of the STING pathway itself, in particular the recently identified unique role of STING in LC3B lipidation onto specific membrane structure within the cells (Fisher et al., *J. Cell Biol.*, 2020). With this promising new direction, we anticipate many groundbreaking new discoveries in the coming years from Dr. Youle.

Dr. Youle has published more than **180 peer-reviewed research articles** and has received several awards, including **three NIH Director's awards**.

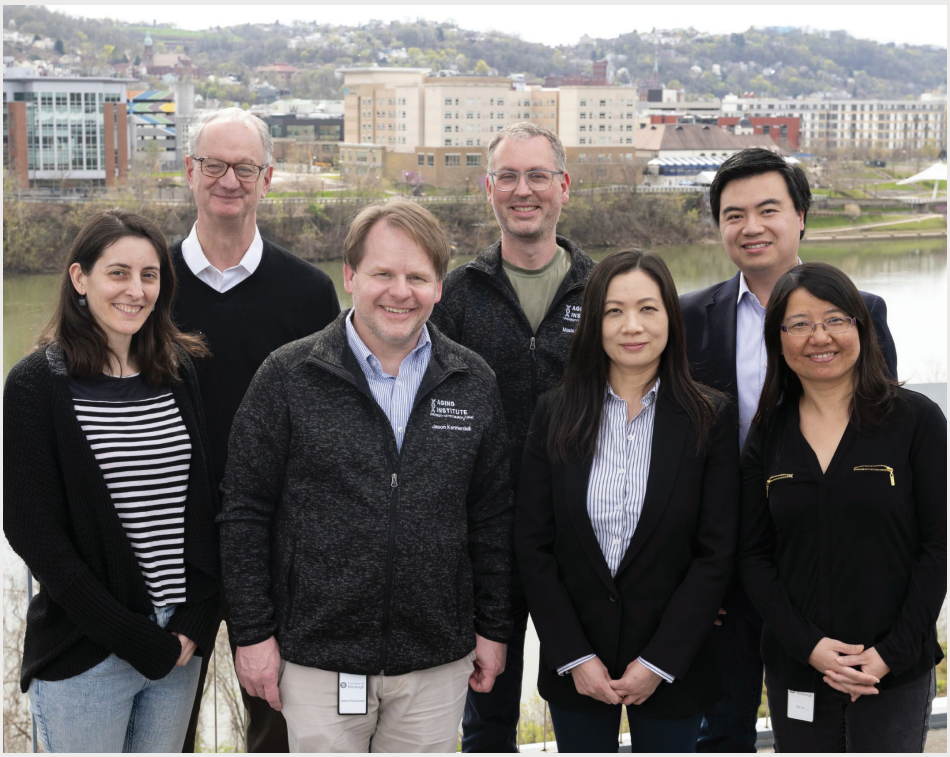
Highlighted Grants at the Aging Institute

Toren Finkel, MD, PhD, and Drug Discovery Team Awarded WoodNext Foundation Grant (Projects 1 & 2)

The WoodNext Foundation is providing generous support to the Aging Institute to develop new drugs for Alzheimer's disease and dementia. This project is led by **Drs. Toren Finkel**, **Yuan Liu**, and **Beibei (Bill) Chen** of the Aging Institute at the University of Pittsburgh/UPMC. Over the last several years, these investigators along with their colleagues, Drs. Irene Alfaras, Bo Lin, Mads Larsen, and Jason Kennerdell, have been pioneering ways to target 'undruggable' proteins. It is estimated that of the 30,000 or so proteins in the human body, more than 85% cannot currently be drugged. This shortcoming limits treatment of common debilitating diseases of old age, even when we know the underlying cause.

Over the last few years, the drug discovery team at the Aging Institute has sought to expand the universe of druggable targets. With the help of the WoodNext Foundation, those efforts will be further expanded and supported. One new focus of the team is to target inflammation in patients with Alzheimer's disease. Many experts believe this neuro-inflammation is a key driver of dementia. As such, developing drugs to combat this problem might be useful for Alzheimer's disease and a host of other related conditions.

One key driver of inflammation in the brain is a protein called TNF-α. Currently, biological therapies that inhibit TNF-α are widely used to treat patients with inflammatory diseases, such as inflammatory bowel disease and arthritis. Epidemiological studies hint that patients taking TNF-α lowering therapies may also have a reduced risk of cognitive decline with age.



Aging Institute Drug Discovery Group:

Front Row (left to right): Irene Alfaras Cardenal, PhD; Jason Kennerdell, PhD; Yuan Liu, PhD; and Bo Lin, PhD

Back Row (left to right): Toren Finkel, MD, PhD; Mads Larsen, PhD; and Beibei (Bill) Chen, PhD

However, these therapies were not designed to work in the brain, and the ability of these medicines to penetrate affected areas of the brain varies considerably from person to person. This research suggests that if a therapy could be designed to get past what is known as the blood-brain barrier and effectively inhibit TNF-α in the brain, it might be effective for treatment of dementia.

Over the next few years, the drug discovery team at the Aging Institute will be launching a major new effort to address this problem. In particular, they are developing a small molecule drug to block the action of TNF-α by inhibiting its receptor, known as TNFR1. Up until now, TNFR1 has been considered undruggable. However, the Aging Institute has applied their sophisticated new high throughput methodology to tackle this important challenge. Initial results are encouraging, although many challenges remain. With generous support from WoodNext, the Aging Institute drug discovery team hopes there will be one less undruggable protein — and a new therapy for dementia.

Highlighted Grants *(continued)*

Anne B. Newman, MD, MPH, is leading Project 3, "Targeting Inflamation: A Hallmark of Aging," of the WoodNext Foundation Grant

With generous support from the WoodNext Foundation, the Aging Institute is launching the "Reducing Inflammation for Greater Health Trial (RIGHT)." In this novel clinical trial, **Anne B. Newman, MD, MPH**, director for the Center for Aging and Population Health, distinguished professor of Epidemiology, professor of Medicine and Clinical and Translational Science, and clinical director at the Aging Institute of the University of Pittsburgh/UPMC, will test whether clazakizumab, a novel inhibitor of interleukin-6 (IL-6), can improve physical and cognitive function in older men and women.

Modest elevations of IL-6 and other cytokines are common in older adults, leading to a slowing in physical and cognitive function, as well as several chronic diseases. The WoodNext Foundation supports the development of new targets to address inflammation in Projects 1 and 2, while Project 3 — the RIGHT study — leverages potential benefits from an existing anti-inflammatory drug to improve aging outcomes, including brain and vascular health.

Evidence from the cardiovascular field supports the approach of Dr. Newman's research. Canakinamab, a monoclonal antibody to interleukin 1 beta (IL1beta), has been shown to reduce recurrent cardiovascular events in patients with cardiovascular disease, and this effect seems to be due to a reduction in IL-6 signaling. Monoclonal antibodies to cytokines and their receptors have been widely used in rheumatological diseases, such as rheumatoid and psoriatic arthritis. The drug being used in RIGHT is clazakizumab, which targets IL-6 directly. Clazakizumab has already been tested in rheumatological disease and other conditions of excess inflammation and is being provided to the RIGHT study at no cost by CSL Behring.

RIGHT will test the effects of clazakizumab on endurance and fatigue, vascular stiffness, endothelial function, and markers of brain health, including cognitive tests, biomarkers of neurodegeneration, and functional brain MRI to assess neurovascular coupling. Additional assessments will include changes in immune cell frequencies, response to stimuli, and changes in multiple pro- and anti-inflammatory cytokines.

A repository of serum, plasma and peripheral blood mononuclear cells will be established to allow additional tests of biomarkers in the future.

Recruitment began in June 2023 and has used mailing lists to reach older adults in the community. Volunteers will be screened in the Health Studies Research Center located in the Bellefield Professional Building in North Oakland. Eligible candidates must be at least age 70, have IL-6 levels averaging 2.5 pg/ml on two occasions about two weeks apart, and have no serious medical problems. The recruitment period is anticipated to continue over a one-year period, with a six-month treatment period for each participant.

People ages 70 and older interested in joining the study can email **RIGHT-Study@pitt.edu** or leave a voicemail at **800-872-3653**.

The RIGHT trial requires complex coordination between multiple units, including:

- Investigational Drug Pharmacy and Clinical and Translational Research Center at UPMC Montefiore.
- Ultrasound Research Laboratory, Epidemiology Data Center, and Health Studies Research Center in the Department of Epidemiology at the University of Pittsburgh.
- Immunological Monitoring and Cellular Therapeutics Laboratory and the Bruno Immunology Laboratory in UPMC Hillman Cancer Center.
- Magnetic Resonance Research Center in UPMC Presbyterian.
- University of Pittsburgh Alzheimer's Disease Research Center.

Highlighted Grants *(continued)*

Aditi Gurkar, PhD, received two new Richard King Mellon Foundation Grants

As we age, our bodies accumulate sick, senescent cells. These cells, often called zombie cells, promote inflammation, damage neighboring cells, and cannot divide, thus driving disease and aging. While the immune system typically helps the body rid itself of these senescent cells, this process slows down with age and leads to an accumulation of senescent cells. This accumulation of senescent cells is associated with age-related diseases.

Aditi Gurkar, PhD, assistant professor of Medicine at the Aging Institute, along with her lab and others in the aging research field have previously shown that elimination of such zombie cells using *senotherapeutics* — drugs which can help the body eliminate these senescent cells — improves health and delays the onset of chronic conditions associated with aging in animal models. Research is now underway to identify and develop senotherapeutics, however some significant challenges still exist. Currently, there is no satisfactory tool to measure senescence load in humans. Without understanding senescent cell load and accumulation in tissues, it will be challenging to translate senotherapeutics into the clinic.

Two recent grants awarded to Dr. Gurkar from the Richard King Mellon Foundation aim to address this fundamental shortcoming. With one of these grants, Dr. Gurkar is working in collaboration with physicist and expert in nanomagnetic materials, Rama Bala, PhD. This project will explore nanomedicine and magnetic force microscopy to quantify changes in senescent cells.

This award not only provides funding for exciting science across disciplines, but also provides unique experiential training opportunities for the next generation of scientists to drive bold research in new directions. Through dedicated mentorship and partnership with STEMnetX — a non-profit STEM empowerment program implemented by the Chance to Change Lives Foundation — this project will allow recruitment of a diverse group of trainees to further scientific discovery in aging research.

The second Richard King Mellon Foundation grant is in partnership with Patricia Opresko, PhD, the Dr. Bernard F. Fisher, Chair for Breast Cancer Discovery Science, co-leader of UPMC Hillman Cancer Center's Genome Stability Program, and professor of Environmental and Occupational Health in the School of Public Health and in the Department of Pharmacology and Chemical Biology in the School of Medicine at the University of Pittsburgh. This project will explore telomeres — protein-DNA structures that cap chromosome ends and shorten with every cell division — as markers of senescence and aging. Shortened telomeres are known to trigger replicative senescence, or senescence that is associated with cells that frequently divide.

However, most cells in the human body rarely divide. It is now known that cells that do not actively divide — like neurons and cardiomyocytes — also exhibit features of senescence, and that oxidative damage — caused by reactive oxygen species — to telomeres can trigger senescence without shortening. This project will examine whether telomere damage independent of shortening can be used as a detector of senescence in non-dividing cells.

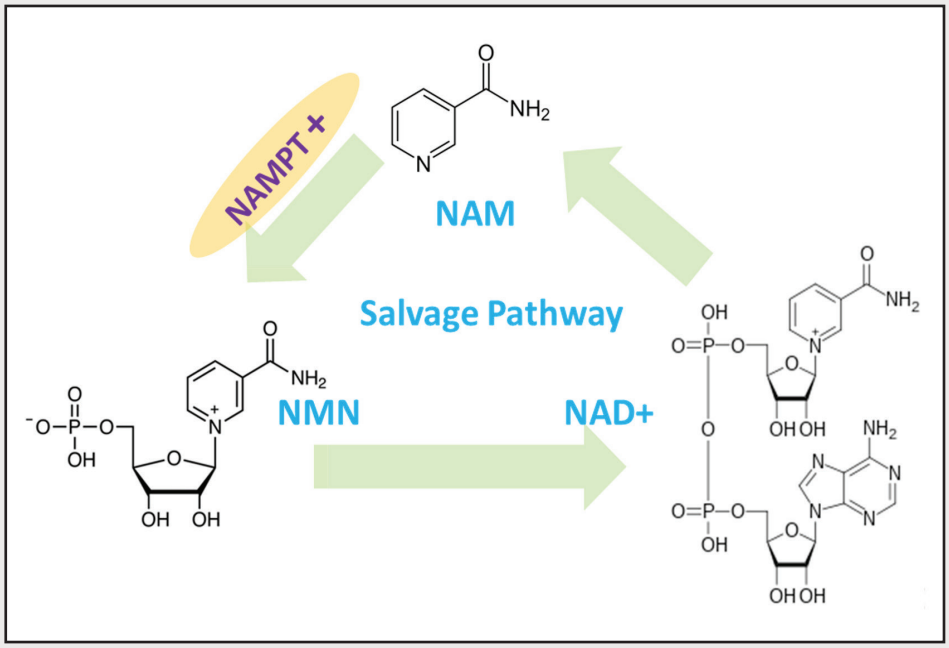
Detection of biological age is a formidable challenge and requires a cross-disciplinary approach. Dr. Gurkar says she is truly honored and excited for this support from the Richard King Mellon Foundation (RKMF), which will allow her to explore novel and innovative ideas in the field of aging research. She envisions that the RKMF grants will support the development of innovative tools to advance aging research and make these discoveries accessible to both the research community and the public.

To learn more about Dr. Gurkar's ongoing work and other exciting new research in aging at our Institute, please join us for the annual Aging Institute Open House on August 30 from 3-5 p.m.!

Highlighted Grants *(continued)*

Yuan Liu, PhD, received a grant from the Richard King Mellon Foundation for her project, "Boosting NAD+ to treat Alzheimer's."

Yuan Liu, PhD, assistant professor at the Aging Institute and in the Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine at the University of Pittsburgh School of Medicine, was recently awarded a Richard King Mellon Foundation grant for her project titled, "Boosting NAD+ to treat Alzheimer's." With this new grant, Dr. Liu aims to develop an oral NAD+ booster to treat Alzheimer's disease and hopes to establish new opportunities to promote healthy aging and reduce complications of a broad spectrum of age-related diseases.



Nicotinamide adenine dinucleotide (NAD) is a critical signaling molecule for numerous biological functions, including energy metabolism and stress responses. The human body has a limited supply of NAD+, and that level steadily declines with age, resulting in increased disease susceptibility. At the same time, restoration of NAD+ levels in old or diseased animals has been shown to promote health and extend lifespan. In particular, decreased brain NAD+ levels in elderly human patients and animal models are associated with cognitive decline, while restoring NAD+ can improve cognitive function. Thus, NAD-boosting molecules could potentially increase resilience to many age-related diseases, including late-onset Alzheimer's disease, and thereby improve health and longevity.

To date, NAD+ boosting strategies have largely relied on high-dose nutraceutical NAD precursor molecules, such as nicotinamide mononucleotide (NAM) or nicotinamide riboside (NR). These molecules have unclear cellular uptake and metabolism, extremely short serum half-lives, and overall poor potency. It is well established that nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme of NAD biosynthesis via the salvage pathway. NAMPT-controlled NAD+ production is compromised in elderly humans and mice. The aim of Dr. Liu's project is to develop a novel brain penetrant, direct NAMPT activator to treat late-onset Alzheimer's disease. This drug may also be useful for a broad range of age-related conditions driven by NAD+ decline.

Thanks to the generous support from the Richard King Mellon Foundation, Dr. Liu and her lab have already started their research project to study NAD+ levels. Leveraging the high throughput screen platform at the Aging Institute, the Liu Lab screened a library of 100,000 small molecule compounds and identified three classes of NAMPT protein binders. The results confirmed that these small molecules directly bond to NAMPT and increase cellular NAD+ levels in human iPSC-derived neurons. One class of "hit" compounds displayed excellent oral bioavailability and good brain penetration. Dr. Liu plans to further optimize the compounds for better potency and safety. The successful lead compound will be orally administered to an Alzheimer's mouse model to test its efficacy to elevate NAD+ levels in the brain, which, if effective, could be a breakthrough therapy to slow cognitive decline and promote healthy aging.

Highlighted Grants *(continued)*

Bokai Zhu, PhD, was awarded a grant from the Richard King Mellon Foundation to study whether rejuvenating nuclear speckles will delay aging.

Bokai Zhu, PhD, assistant professor of Endocrinology and Metabolism at the Aging Institute, received a new grant from the Richard King Mellon Foundation to study whether genetic or pharmacological rejuvenation of nuclear speckles can be used to delay aging and treat aging-related diseases.

One of the major hallmarks of aging and a critical risk factor for the development of many aging-related diseases is the progressive decline of proteostasis — also called protein homeostasis — leading to the accumulation of damaged proteins and reduced cell viability. In pre-clinical models, boosting endoplasmic reticulum (ER) proteostasis reduces protein aggregation, protects against age-related tissue dysfunction, and extends both lifespan and health span.

The unfolded protein response (UPR) signaling pathway controls ER proteostasis by detecting and mitigating unfolded/misfolded proteins. While activation of UPR was traditionally viewed solely as an adaptive response to ER stress, previous work by Dr. Zhu and his lab showed that under normal physiological conditions, the UPR is manifested as a cell-autonomous 12-hour ultradian rhythm regulated by the transcriptional factor X-box binding protein 1 (XBP1).

By studying this 12h-oscillator, Dr. Zhu's team uncovered a novel role for nuclear speckle fluid dynamics in regulating UPR transcriptional capacity. Nuclear speckles are droplet-like subnuclear organelles important for mRNA processing and transcriptional regulation. Higher expression of the nuclear speckle scaffolding protein, SON, generates diffuse and fluid nuclear speckles, increases their interactions with chromatin, transcriptionally amplifies the UPR, reduces protein aggregates, and protects against proteome stress.

An analogy would be the water level fluctuations in a lake: Nuclear speckles with higher SON level are like a lake filled with free-flowing water and fast currents, while nuclear speckles with reduced SON expression resemble a nearly dried-out lake with a few disconnected pools of stagnant water (Figure 1). Reduced SON gene expression and defective UPR activation are strongly associated with aging and observed in many aging-related diseases. Dr. Zhu hypothesizes that by 'rejuvenating' nuclear speckles via increasing their fluidity genetically or pharmacologically, it may be possible to restore ER proteostasis and thus delay, or even reverse, aging and extend health span — much like restoring the water level and fluidity of an "aging" dried-out lake.

With the philanthropic support of the Richard King Mellon Foundation, Dr. Zhu's lab will test SON overexpression as a genetic approach to rejuvenate nuclear speckles. Dr. Zhu's team will perform high-throughput drug screening to search for small molecules capable of increasing nuclear speckle fluidity and diffuseness. There is promise that these approaches will robustly activate protein quality control pathways, improve overall proteostasis, and delay aging and aging-related diseases, such as tauopathy, Alzheimer's disease, and metabolic syndromes.

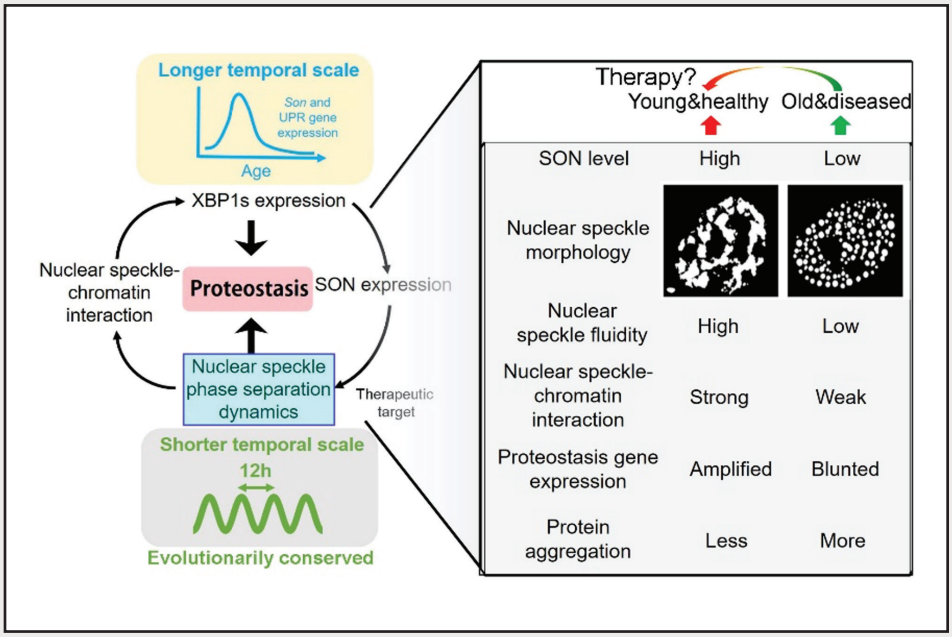


Figure 1

Highlighted Grants *(continued)*

Andrey Parkhitko, PhD, received a new Richard King Mellon Foundation grant to investigate therapeutic synergies to slow aging.

Andrey Parkhitko, PhD, assistant professor of Endocrinology and Metabolism at the Aging Institute, received a new grant from the Richard King Mellon Foundation to investigate how combinations of anti-aging therapies — including targeting nutrient sensing, epigenetics, and cellular senescence — administered in late life can extend healthspan.

Methionine metabolism is critical for cellular growth, protein translation, and various metabolic pathways. Ongoing studies by Dr. Parkhitko and his lab in aged mice are evaluating potential benefits of methionine restriction on aging by measuring frailty, epigenetic clocks, and molecular and functional metrics in critical organs. The Parkhitko Lab discovered that dietary methionine restriction administered for six months in 18-month-old mice (or the equivalent of about 60-year-old humans) improved health, delayed motor function impairments, and promoted resilience to frailty.

Previous studies have targeted the nutrient-sensing signaling pathway with dietary restriction or with rapamycin — an immunosuppressant often used to prevent organ rejection in transplantation and which can treat certain types of cancer. Another focus of considerable effort entails targeting the burden of senescent cells with senolytic drugs — a combination of dasatinib and quercetin. Yet, no single intervention against these central processes has been sufficiently effective to delay aging.

With the generous support of the Richard King Mellon Foundation, Dr. Parkhitko and his lab will conduct a new study in aged mice to test therapeutic efficacy of methionine restriction, rapamycin, senolytics, and their pairwise combinations using a comprehensive framework for assessing overall aging and age-dependent organ deterioration. Dr. Parkhitko is optimistic that targeting different drivers of aging in late adulthood will have additive, or even synergistic, aging benefits.

Additional **New Grants** at the Aging Institute

Principal Investigator	Grant Title	Grant Type	Sponsor
Liu, Shihui	Mechanisms of anthrax lethal toxin-induced mortality and the novel biological-based targeted therapies	R01	National Institutes of Health
Sekine, Shiori and Sekine, Yusuke	Deciphering a Novel Mechanism for Iron-sensing at Mitochondria and Its Role in Erythropoiesis	R01	National Institutes of Health

Highlighted Manuscript at the Aging Institute

Shiori Sekine, PhD, Yusuke Sekine, PhD, Ryan Houston, and Team – Published Manuscript in *Molecular Cell*

A new study published in *Molecular Cell* in 2023 by **Shiori Sekine, PhD**, assistant professor of Cardiology at the Aging Institute and University of Pittsburgh School of Medicine, and **Yusuke Sekine PhD**, assistant professor of Endocrinology and Metabolism at the Aging Institute and University of Pittsburgh School of Medicine, as well as Sekine Lab member, **Ryan Houston**, and their team, identified a novel iron monitoring system in mitochondria that protects erythroid cells against intracellular iron deficiency.

Iron plays a crucial role in our body as a biological catalytic center of various proteins and regulates a wide range of biological reactions. For example, in mammals, approximately 70% of total body iron exists as heme — the majority of which is found in erythrocytes in the form of hemoglobin that carries oxygen from the lung to the whole body. Insufficient iron supply is serious, as it causes so-called iron deficiency anemia, the most common form of anemia worldwide.

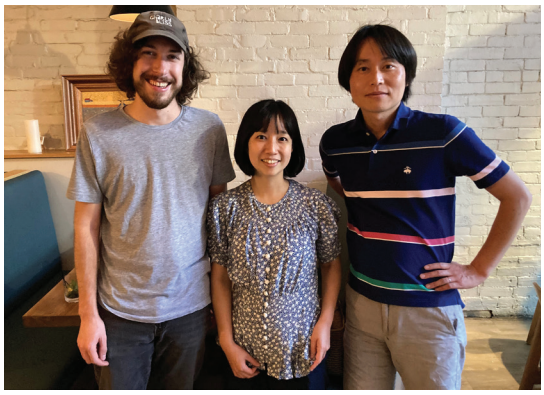
On the other hand, excessive iron is also toxic for our body, as iron is a highly reactive molecule that can mediate the production of reactive oxygen species — a harmful molecular species that would otherwise damage our cells. Therefore, the amount of intracellular iron must be precisely monitored, and appropriate cellular responses should be induced depending on the intracellular iron availability. This mechanism is particularly important for erythroid cells that incorporate large amounts of iron for hemoglobin synthesis as described above.

Mitochondria are one of the crucial organelles of the cells that produce cellular energy, adenosine triphosphate (ATP). Mitochondria harbor their own DNA but the majority of mitochondrial localized proteins are encoded by nuclear DNA, and these proteins are transported into the mitochondria through mitochondrial protein transporters. Recent studies have shown that the transport of certain mitochondrial localized proteins is tightly regulated in response to various external and internal factors surrounding mitochondria. More importantly, such regulation of mitochondrial protein transport has also been found to serve as a communication tool between mitochondria and other cellular compartments and can evoke appropriate cellular responses.

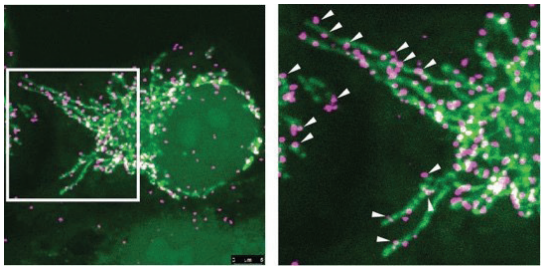
The Sekine Lab found that mitochondrial import regulation is also utilized as a novel tool to monitor intracellular iron availability and activate intracellular stress signaling pathways under iron deficient conditions. The lack of this pathway sensitized erythroid cells against iron deficiency, suggesting a novel role of mitochondria as a signaling platform to activate cell survival signal upon iron deficiency in the iron-demanding cell lineage.

Besides the aforementioned ATP production, mitochondria are also known to play an important role in intracellular iron metabolism, as these organelles harbor the biosynthetic pathways for two major iron-containing cofactors: heme and iron-sulfur cluster. The Sekine Lab is currently investigating whether the identified mitochondrial iron monitoring system is also involved in the intracellular iron and/or iron- cofactor metabolism.

This enhanced understanding of the relationship between mitochondria and iron could help in future efforts to balance and obtain the optimal amount of iron in the body, as well as reduce side effects of iron deficiency, including risk of heart and lung problems, fatigue, developmental delays, and complications in pregnancy.



(From left to right) Sekine Lab member, Ryan Houston, Shiori Sekine, PhD, and Yusuke Sekine, PhD



Iron-dependent mitochondrial import regulation of DELE1 activates cell survival signal through the HRI-ISR pathway in the erythroid cells upon iron deficiency.

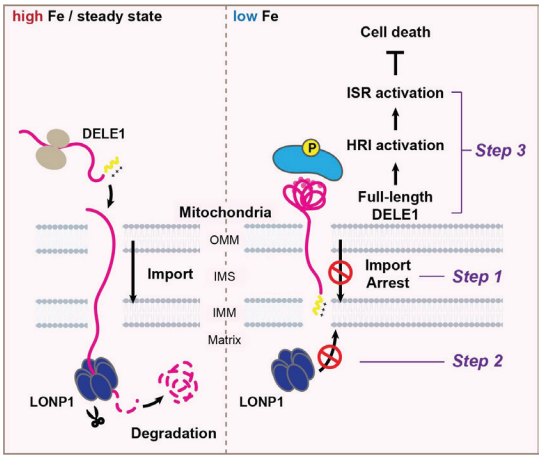


Image of the activation of stress signaling pathway (mazenda spots) at mitochondria (green) under iron deficiency. The Sekine Lab recently identified a novel mitochonria-resident iron-responsive protein, which was recently published in Molecular Cell.

Sekine Y*, Houston R*, Eckl EM, Fessler E, Narendra DP, Jae LT, Sekine S. A mitochondrial iron-responsive pathway regulated by DELE1. *Mol Cell*. 2023 Jun 15;83(12):2059-2076.e6. doi: 10.1016/j.molcel.2023.05.031

TriState SenNet

As highlighted in a previous edition of *The Chronos Chronicle*, **Toren Finkel, MD, PhD**, director of the Aging Institute and distinguished professor at the University of Pittsburgh School of Medicine, is the contact principal investigator of a U54 grant from the National Institutes of Health Common Fund called the TriState SenNet Tissue Mapping Center (TMC).

The TriState SenNet TMC comprises four universities: the University of Pittsburgh, The Ohio State University, University of Rochester Medical Center, and Carnegie Mellon University — and draws upon the expertise of researchers across its four universities to study senescent cells in human heart and lung tissue.

The TriState SenNet TMC held its annual hybrid conference at The Ohio State University in Columbus, Ohio on April 10 to 11, 2023. The meeting was attended by members of all four TriState SenNet universities, Internal and External Advisory Boards, and National Institutes of Health personnel. At the meeting, the TriState SenNet team had the opportunity to collaborate in person, evaluate current study progress, share data and techniques, and discuss strategic plans.

The conference included presentations related to current research in aging and cellular senescence by several members of the SenNet Consortium, including Dr. James Kirkland of the Mayo Clinic, Dr. Finkel, Dr. Ljiljana Pasa-Tolic of the Pacific Northwest National Laboratory, and Dr. Vera Gorbunova of the University of Rochester Medical Center, as well as data and poster presentations by postdoctoral fellows, students, and trainees of the TriState SenNet TMC.

The next hybrid conference for the TriState SenNet TMC is scheduled for November 2023 at the University of Rochester in Rochester, New York.

Trainees Receive Research Awards

Ankit Sharma, an MD/PhD student pursuing graduate training in the Aging Institute's Steinhauser Lab, recently received a one-year American Heart Association (AHA) Predoctoral Fellowship award for the following research proposal, **"Defining the role of IGF2 in regulating adipogenesis and promoting hyperplastic, metabolically healthy adiposity."**

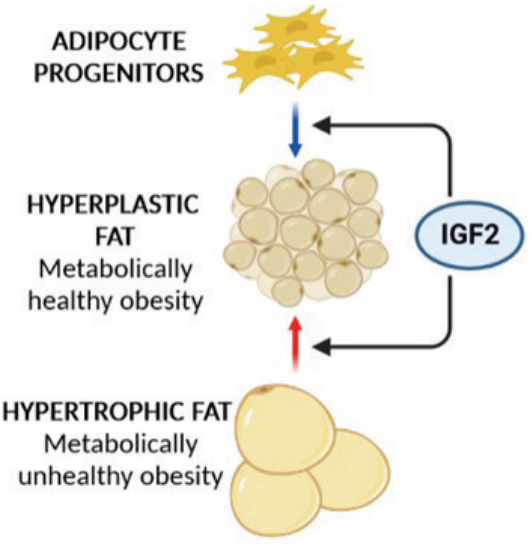
Results of this proposal could lead to a better understanding of how fat expansion is regulated as well as inform novel therapeutic strategies for treatment of aging- and obesity-related metabolic diseases by promoting metabolically healthy fat expansion.

After completing his first two years of medical school at the University of Pittsburgh School of Medicine, in July 2022, Sharma transitioned into his PhD training under the mentorship of Matthew Steinhauser, MD, an associate professor of Medicine in the University of Pittsburgh School of Medicine and deputy director of the Aging Institute of the University of Pittsburgh/UPMC. Sharma was interested and motivated to join the Steinhauser Lab in large part due to his several years in metabolism research prior to medical school at the University of Texas Southwestern Medical Center, where he was involved in studies assessing the role of abnormal sphingolipid metabolism in linking obesity to the development of insulin resistance.

This work was driven by the simple question: *Why is obesity unhealthy?* The Steinhauser Lab, with its focus on studying the intersection between adipose biology and systemic disease, gave Sharma the unique opportunity to build on his research background by looking not only at the underlying causes of obesity-related diseases, but also at the complimentary questions: *Can obesity be healthy? And if so, is it possible to transform* ***unhealthy*** *obesity into* ***healthy*** *obesity?*

Adipose tissue and overall metabolic function are inextricably linked. This link is perhaps most important in the context of adipose tissue expansion, particularly during weight gain or aging. In other words, how fat is stored in adipose tissue during obesity or aging plays a key role in determining the extent to which these processes are metabolically unhealthy. During obesity and aging, hypertrophic fat expansion — defined by the growth in the size of pre-existing fat cells, or adipocytes — predominates.

Adipocyte hypertrophy involves storing lipids in old, overburdened fat cells that are susceptible to inflammation, fibrosis, and metabolic dysfunction. As a result, excess calories are no longer stored as lipids in the organ designed for their storage and are instead deposited into tissues that cannot properly handle them, such as heart, liver, and skeletal muscle. This is the process that makes obesity "metabolically unhealthy," linking obesity to the development of diabetes, cardiovascular disease, and fatty liver disease.



continued >

On the other hand, a growing body of data has shown that hyperplastic fat expansion, which predominates during early development and declines precipitously with age, is associated with "metabolically healthy obesity." Driven by adipogenesis – the process by which adipocyte progenitor cells mature into functional, lipid-storing adipocytes – hyperplasia generates new adipocytes that are more capable of handling lipid storage than their hypertrophic counterparts. This stark contrast has provided rationale for the identification of novel approaches to reprogram "old" hypertrophic adipose tissue to a "younger" hyperplastic state. The Steinhauser Lab's approach has focused on better understanding the molecular basis for a discrete "hyperplastic-to-hypertrophic" growth switch that occurs in the adipose tissue of both mice and humans.

Sharma's early work in the Steinhauser Lab identified a stark, near complete loss in the expression of insulin-like growth factor 2 (IGF2) – a well characterized promoter of cellular proliferation and early developmental organ growth – in the adipose tissue of mice between 5 days and 20 days old, the period of postnatal development within which the "hyperplastic-to-hypertrophic" adipose growth switch occurs. These preliminary findings indicate that the loss of IGF2 may play a key role in the loss of adipogenesis, and conversely, that the targeted "re-introduction" of IGF2 signaling to adipocytes and their progenitors can restore adipogenesis, thereby unlocking hyperplastic, metabolically healthy fat expansion that decouples obesity from its associated co-morbidities.

This AHA Predoctoral Fellowship award provides support for Sharma's further studies that will delineate the role of IGF2 signaling in regulating adipose tissue growth and adaption to stressors, such as obesity and aging. Ultimately, the results of this proposal could lead to a better understanding of how fat expansion is regulated as well as inform novel therapeutic strategies for treatment of aging- and obesity-related metabolic diseases by promoting metabolically healthy fat expansion.

Dion hypothesizes that this research will illuminate new connections between disruption of 12-hour rhythms, cellular senescence, and age-related diseases.

William Dion, MS, third-year PhD candidate in Integrative Systems Biology (ISB) at the University of Pittsburgh School of Medicine and member of Dr. Bokai Zhu's laboratory at the Aging Institute, was awarded the Ruth L. Kirschstein National Research Service Award Individual Predoctoral Fellowship (Parent F31) from the National Institute on Aging to pursue his research project, "Nuclear speckle liquid-liquid phase separation dynamics in senescence and aging." Dion previously received the Diana Jacobs Kalman/AFAR Scholarship for Research in the Biology of Aging from the American Federation for Aging Research — highlighted in the prior edition of *The Chronos Chronicle* — for his research project on the same topic and now plans to expand his ongoing work with this new award.

Dion earned his bachelor of science degree from Michigan State University and his master of science degree at Michigan Technological University, where he studied the evolution of animal color pattern development. In 2020, he joined the Zhu Lab. Dr. Zhu is an assistant professor in the Division of Endocrinology and Metabolism at the University of Pittsburgh School of Medicine and the Aging Institute. His lab conducts research on biological clocks and physiological rhythms — a scientific field called chronobiology.

Previous research by the Zhu Lab suggests that the 12-hour biological clock deteriorates with age and can lead to cellular senescence and a decline of protein homeostasis — or proteostasis — disrupting the equilibrium of proteins in the body. Many age-related diseases, such as Alzheimer's disease, Parkinson's disease, and other neurodegenerative disorders, are associated with cellular senescence and deterioration of proteostasis.

With this research project, Dion will build upon his previous work in the Zhu Lab, which demonstrated that rhythms of a biomolecular condensate, the nuclear speckle, and its shape are essential to maintenance of proteostasis. Dion will further investigate how the nuclear speckle is linked to a decline in proteostasis. He is also investigating how the rhythms of nuclear speckle shape change with cellular senescence. Dion hypothesizes that this research will illuminate new connections between disruption of 12-hour rhythms, cellular senescence, and age-related diseases.

SAVE THE DATE

AGING INSTITUTE RESEARCH DAY

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Thank You from the Aging Institute Team

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