

ROUND 2:
3/24/23



What's going on in the
brains and bodies of
people with Alzheimer's?



THE GRAYEST OF GRAY MATTERS

WHAT DO WE REALLY KNOW ABOUT ALZHEIMER'S, AND WHAT ARE THE MOST PROMISING WAYS TO APPROACH THE DISEASE? PITT IS BUILDING A CRITICAL MASS OF RESEARCHERS IN THE SEARCH FOR ANSWERS.

BY ANITA SRIKAMESWARAN

“**H**elp wanted.” The post-pandemic plea, now commonplace on the doors of restaurants and retail shops, could also be displayed in the homes of the estimated 6 million Americans now living with Alzheimer’s disease. That’s 6 million people who are likely to lose the ability to remember whether they had breakfast. Six million who are flummoxed by the simplest problems. Who are disoriented, anxious and may become distraught enough to lash out toward caregivers. Their diagnosis is typically made with cognitive and other neurological testing, family discussions and brain scans—well after symptoms have started and the brain changes may be, tragically, irreversible.

Suspicion has fallen on aluminum pans, artificial sweeteners and flu shots, but despite decades of research, the underlying causes of Alzheimer's have remained elusive. For the most part, the hunt has centered on the beta-amyloid plaques in the brain that are found during autopsy of patients who had the disease.

Last November, findings from the double-blind, multicenter Clarity AD trial, funded by the pharmaceutical and biotech companies Eisai and Biogen, were published in the *New England Journal of Medicine*. Among the nearly 900 of 1,795 study participants with early Alzheimer's given the amyloid-targeting monoclonal antibody lecanemab, cognitive decline slowed by an average of 27 percent compared to those given placebo after 18 months of treatment.

"This sets the bar for future studies. Everything else that is coming has to be the same or better," says Oscar Lopez, director of the Alzheimer's Disease Research Center (ADRC), Levidow-Pittsburgh Foundation Professor in

caused by having three rather than two copies of chromosome 21, have a substantially higher risk of developing the dementia—and chromosome 21 contains the amyloid precursor protein gene. Other genes associated with elevated risks of developing Alzheimer's play roles in amyloid metabolism.

But prior to lecanemab, which works with the body's immune system to clear amyloid, trial after trial of amyloid-targeting drugs failed to make patients better even if they reduced amyloid burden, and amyloid brain plaques also can be found during autopsies of people who didn't have Alzheimer's.

That indicates to Lopez that "it's not the whole thing. Amyloid has something to do [with it], but there are some other things going on."

Research teams at Pitt and around the world are closely examining tau, another protein that gets misfolded into clumps called neurofibrillary tangles, in the brains of people with Alzheimer's.

cast or distracting set dressing? Are there protective mechanisms at work in people who do not develop Alzheimer's? And how will scientists raise the curtain to see what's really unfolding on the stage?

The number of people with Alzheimer's is predicted to rise to nearly 13 million by 2050. Pitt is building a critical mass of researchers with approaches and insight, as well as opinions, that stem from a range of perspectives. All of them are determined to quell the coming storm.

WINDOWS TO THE BRAIN

Pitt made an indelible notch in the timeline of Alzheimer's research in 2002.

At an international meeting that year, William Klunk, an MD, PhD, who recently retired as a Distinguished Professor of Psychiatry and Neurology, and Chester Mathis, a PhD who's now a Distinguished Professor of Radiology and

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Alzheimer's Disease and Related Dementias and an MD professor of neurology at Pitt. Alzheimer's symptoms of those who received lecanemab progressed more slowly than those in the placebo group, he explains, so "it's a step forward."

He acknowledges the clinical impact, while measurable, is small, far from the giant leap needed to cure the forgetfulness, confusion and other cognitive issues that patients—and their families—face. The study suggests there may be merit to the "amyloid hypothesis," the notion that amyloid aggregation triggers a biochemical cascade that leads to dementia-inducing neuronal death, and therefore treating or eliminating the plaques will treat or eliminate Alzheimer's symptoms.

Amyloid abnormalities have been a suspect in the genesis of Alzheimer's since scientists saw during autopsies that the brains of people who had the disease were riddled with clumps of the protein. Also, people with Down syndrome,

It's thought that amyloid aberrations lead to changes in tau, which is needed to maintain the internal, microtubular structure of neurons.

Yet, studies of tau-reducing drugs in patients have also failed to improve Alzheimer's symptoms.

The relationship between amyloid plaques and tau tangles in the Alzheimer's brain remains a mystery, says Julia Kofler, an MD associate professor of pathology and an ADRC codirector. Tau abnormalities can be found in many other neurodegenerative diseases.

Also, "some tau pathology is almost a part of normal aging," she notes. "If you get a brain without any tau pathology, it would be the exception, not the rule." As the leader of Pitt's Brain Bank, Kofler would certainly know. She preserves postmortem brain tissue from patients who died with neurodegenerative diseases.

The science of Alzheimer's appears to be the grayest of gray matter. If beta-amyloid and tau are factors, are they leading actors, supporting

UPMC Professor of PET Research, gave a presentation on their development of Pittsburgh Compound B, or PiB, a radiopharmaceutical. PiB was able to, for the first time, reveal amyloid deposition in the brains of living patients. The feat changed the research landscape, making it possible to use brain scans to track how experimental drugs affect the presence of amyloid.

Pitt scientists are now creating new tools to monitor what's happening inside the aging brain.

Just last fall, a team led by Ann Cohen (a PhD who trained with Klunk), Howard Aizenstein, an MD, PhD, and Victor Villemagne, an MD, all of psychiatry, as well as Milos Ikonovic, an MD in neurology, was awarded a \$33.5 million National Institute on Aging grant renewal to examine neuroinflammatory processes in subclinical Alzheimer's using a novel imaging agent. The agent tracks astrocytes, or astroglia, star-shaped cells that play key roles in neuronal metabolism.

"When amyloid and tau proteins begin to deposit in the brain, astrocytes react in ways that

**KLUNK AND MATHIS
IMAGE T/K HERE
WITH CAPTION**

may either benefit or harm surrounding nerve cells,” says Villemagne. That process is known as reactive astrogliosis, and Villemagne’s team has developed a method to measure it in living persons. Preliminary data indicate that astrogliosis becomes abnormal early on in Alzheimer’s, even before changes associated with amyloid and tau. Further, it seems that persistent astrogliosis might lead to increased vulnerability to Alzheimer’s disease pathology (meaning amyloid and tau aggregation).

The investigators will use the project grant to examine the roles played by astrogliosis in the brain. For instance, how does the process work in people with known risk factors to Alzheimer’s, notably cardiovascular disease? Does it contribute to cognitive impairment or alter behavior? What does it do in a routinely sleep-addled brain?

Other biomarker stars include Tharick Pascoal, an MD, PhD, and Thomas Karikari, a PhD, who both recently joined Pitt as assistant professors of psychiatry, and are part of the astrogliosis program project grant.

In October 2021, Pascoal and his team were awarded a five-year, \$40 million NIH grant to compare two tau tracers in studies across eight medical centers. Karikari, came to Pitt from Sweden’s University of Gothenburg, to adapt a new blood biomarker for tau, which he described in the December 2022 issue of the journal *Brain*.

About 30 percent of people with Down syndrome develop Alzheimer’s in their 50s; that goes up to 90 percent when they get to their 60s. The NIH awarded in 2020 a five-year, \$109 million grant to a multicenter effort led by Benjamin Handen, PhD professor of psychiatry, who is

looking for Alzheimer’s biomarkers in this population.

Building on the revolution of PiB, “we have continued to grow our place in biomarker research, and we think we’ve assembled the world’s best team in Alzheimer’s imaging,” says psychiatry department chair David Lewis, Distinguished Professor of Psychiatry and Neuroscience.

NEW STARTING POINTS

In 2017, several years after Klunk and Mathis first revealed PiB, Peter Strick stared at Klunk’s PowerPoint slide of PET scans at an Alzheimer’s-themed Brain Day symposium. The slide revealed neon red, yellow and green PiB-highlighted splotches of beta-amyloid in the brains of cousins who carry a gene known to cause early onset Alzheimer’s disease.

The basal ganglia are chock-full of plaques, but these people have no symptoms, noted a perplexed Strick, scientific director of Pitt’s Brain Institute, PhD chair of Pitt’s Department of Neurobiology and the Thomas Detre Professor. What the self-professed “basal ganglia guy,” was seeing made no sense. The basal ganglia play a role in how we move, learn, process emotions and many other tasks. *If amyloid accumulation causes dementia, why weren’t these 30-somethings ill already?*

Klunk and Mathis had shown the world that people with the early onset Alzheimer’s gene or Down syndrome somehow show up with plaques in the basal ganglia without apparent symptoms—unlike late-onset cases where basal

ganglia aggregation becomes disabling.

Inspired by Klunk’s presentation and the conundrum presented in that slide, Strick launched a basic neuroscience research effort that, in October 2022, received a five-year, \$32.5 million grant from the National Institute on Aging to create what could be a gamechanger—a model of late-onset Alzheimer’s disease in marmosets. Unlike rodents, these squirrel-sized primates have sensory and motor systems as well as neural networks that are similar to humans. If successful, the model will be the first of its kind in the world.

In the project, nicknamed Marmo-AD, researchers will breed animals that are born with genes known to be associated with late-onset forms of Alzheimer’s. The marmosets will be closely followed throughout their lifespans with blood tests, behavioral assessments, brain scans and sampling of skin cells called fibroblasts, which can be made into neurons for further studies in the lab.

“Each marmoset will be monitored in clinically relevant ways comparable to what is and can be done in humans,” but with the bonus of gathering information from birth and not just at symptom onset, says coprincipal investigator Afonso Silva, Endowed Professor in Translational Neuroimaging and professor of neurobiology.

“If we are successful, we could learn how Alzheimer’s begins and what might be done to stop it,” he notes.

And there are many more promising efforts underway at Pitt.

Amantha Thathiah, like many first-time visitors to Pittsburgh, was happily shocked to discover it was not the dark, smoky Steel City of legend. She was looking for a place to start her own lab after postdoctoral training and a faculty appointment in Leuven, Belgium, and her mentor, world-renowned Alzheimer’s expert Bart De Strooper, saw that Pitt, the birthplace of PiB, was recruiting.

Her interest in Alzheimer’s arose because, as she puts it, she “followed the science.” For her doctorate, Thathiah studied proteases, enzymes that break down proteins, in a cancer biology lab. Wanting to expand her knowledge of protease biology in her postdoctoral training, she focused on Alzheimer’s, because the disease involves three classes of these enzymes, thrilling her scientific sensibilities.

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What role, if any, do amyloid plaques play in Alzheimer's? (Plaques are shown here in pink.)



Thathiah arrived in 2016 as a PhD assistant professor of neurobiology—among the first in a wave of recruits focused on the basic science of Alzheimer's. She's had some success with interrupting what seem to be disease mechanisms of the disease.

In one of her projects, Thathiah and her team focus on G protein-coupled receptors, or GPCRs, a group of proteins that are involved in numerous biological processes. Forty percent of current-day medications, including drugs for hypertension, asthma, motion sickness and schizophrenia, work by targeting GPCRs. Thathiah had previously found that a subset of Alzheimer's disease patients has elevated levels of the GPR3 protein. Deleting the *Gpr3* gene (which makes the GPR3 protein) in a mouse model reduced the amyloid plaque burden in the brain but elevated anxiety levels. In an October 2022 paper published in the Proceedings of the National Academy of Sciences, Thathiah's team showed in an Alzheimer's mouse model that modifying the *Gpr3* gene to block binding of a cellular-signaling protein led to reduced amyloid plaque and cognitive difficulties—without causing anxiety and other significant side effects that occurred with gene deletion. (That's a half-vote for amyloid burden contributing to the disease, if you are keeping track.)

The findings suggest drugs that lead to appropriately biased GPCR signaling could inspire

new approaches to treating Alzheimer's.

In another project, she's examining neurons made from skin fibroblasts of patients who've died with Alzheimer's. That project could help reveal relationships between aging and dementia. Her energies are focused on both alleviating suffering and understanding the disease.

She finds it inspirational to meet people who are caring for Alzheimer's patients. "It gives me purpose. I'm going to investigate disease mechanisms, but I'm always thinking of therapeutically targeting that mechanism to benefit the patients," says Thathiah.

TOWARD CRITICAL MASS

While the psychiatry department has been building an elite team of biomarker investigators, Pitt's ranks of neurobiologists with an interest in Alzheimer's disease, like Thathiah, have continued to grow. That's been due in part to Strick's curiosity about the images he saw in 2017 and the interests of Arthur S. Levine, former dean of the medical school and senior vice chancellor for the health sciences, who became executive director of the Brain Institute in 2020.

Shortly before the pandemic, Pitt recruited two young PhD neuroscientists from MIT: Hansruedi Mathys, who is using his expertise in

single-cell RNA sequencing to uncover how and, hopefully, why Alzheimer's-affected brain cells differ from those taken from people who don't have dementia; and Or Shemesh, who is studying the possible influence of viruses, bacteria and other infectious agents as an Alzheimer's trigger.

Established PhD investigators are also making their professional homes here. Afonso Silva brought his expertise in brain imaging—and his marmosets—from the National Institutes of Health. Stacey Rizzo, renowned expert on the use of rodents in behavioral studies, came from the Bar Harbor, Maine-based Jackson Laboratory to apply her knowledge to nonhuman primate research models. Karl Herrup, who thought to leave Hong Kong University and return to Pittsburgh to retire, was persuaded to instead set up a new lab and continue his search for an answer to Alzheimer's.

Likewise, a cadre of investigators from a number of disciplines throughout Pitt are probing a range of possible culprits and antagonizers.

For instance, M. Ilyas Kamboh, a PhD, is well-known for his work in Alzheimer's genetics and pathologies; he was studying the APOE gene before it was identified in 1992 as the most significant risk factor for Alzheimer's. Likewise, Rada Koldamova, an MD, PhD, focuses on the variant APOE4, which increases the risk of developing Alzheimer's to those who carry one (four times) or two (10 times) copies of it. Both are based in

the School of Public Health. (By the way, brain protein APOE is mainly produced by astrocytes and is used to transport lipids from astrocytes to neurons.)

Stephen Chan, an MD, PhD cardiologist who is Pitt's Vitalant Professor of Vascular Medicine, and Toren Finkel, an MD, PhD Distinguished Professor of Medicine and director of the Aging Institute, are studying how inflammation leads to both cardiovascular disease and neurodegeneration. Anne Newman, MD, MPH Distinguished Professor of Epidemiology and clinical director of the Aging Institute, will oversee a clinical trial evaluating whether a monoclonal antibody that reduces inflammation can lead to improved cardiovascular and cognitive function. This multi-tiered approach is supported by \$14.3 million from the WoodNext Foundation.

Those projects are complemented by ongoing work of psychiatry faculty members such as Rebecca Thurston, a PhD and the Pittsburgh Foundation Professor in Women's Health and Dementia, who is examining menopause and dementia; Mary Ganguli, MD, MPH professor, whose focus is the epidemiology of cognitive impairment and dementia; Meryl Butters, a PhD who studies depression and dementia; and many others.

Simply put, "We're all looking for clues," Silva says.

FRESH PERSPECTIVES

Globally, "there is no question that the tenor of the investigations has changed." And that's good, says Karl Herrup, PhD professor of neurobiology and author of "How Not to Study a Disease: The Story of Alzheimer's" (MIT Press, 2021).

Herrup, an outspoken critic of the amyloid hypothesis, says "a lot of basic scientists have certainly realized that [targeting amyloid] is not a productive way of approaching the disease, and pharmaceutical companies are coming to the same realization."

After faculty appointments at Yale, Case Western Reserve University and University Hospitals at Cleveland (where he directed its Alzheimer's Center) and the Hong Kong University of Science and Technology, Herrup returned to his hometown in 2019. Pitt has one of the nation's best Alzheimer's Disease Research Centers (led by Lopez), and Herrup had already collaborated with Kofler for several years.

Much has been learned about amyloid biology, notes Herrup. Yet: "It's as if we spent all this time figuring out why hair turns gray because people with gray hair are at higher risk for Alzheimer's. Both of those statements are true. It's interesting to learn about hair follicles, pigment cells and so on," but it doesn't cause the disease, he says.

He sometimes dismays his colleagues with his frank views on the failure to move the needle on Alzheimer's, but he takes that in stride. "Science moves faster if everyone is questioning everyone else's thinking. It forces you to sharpen your argument," Herrup says.

In his own lab, Herrup has been examining the role of DNA damage and noncoding genetic variants in Alzheimer's, as well as the cellular response to DNA damage and neuroinflammation.

Yet he wonders if Alzheimer's could be what he calls an "emergent property" of the aging brain, like a hurricane forming from water and wind.

"If so, that would be really troubling because it doesn't lend itself to a biotech approach of the disease" Herrup says, because "no one element is responsible for the emerging property. You can't predict a hurricane just by knowing the physical and chemical characteristics of water."

From his perspective, an Alzheimer's-research do-over should go back to the clinical symptoms and work to fix those. He's also closely following work at Pitt and elsewhere that probes contributions of the Alzheimer's-associated APOE4 gene and its protein's role in cholesterol transport, vascular abnormalities, myelin changes, synaptic decay and viruses.

"We need to reach beyond the usual suspects. We need to be talking to people who aren't now working in Alzheimer's," he says.

Prior to 2018, when Silva came to Pitt, he was focused on stroke, not Alzheimer's.

He decided to leave the NIH in part because the need for answers for Alzheimer's is urgent, he says. Strick encouraged him to work with Gregg Homanics, PhD professor of anesthesiology and perioperative medicine, to breed marmosets with abnormalities in the presenilin-1 (or PSEN1) gene that causes an inherited version of Alzheimer's. It's the same gene carried by the cousins who had amyloid plaques in the PiB brain scans Strick had seen a year earlier in the Brain Day talk.

During the last few years, Silva, Homanics

and their colleagues have quietly developed an early onset AD marmoset model. The new Marmo-AD (late-onset) award builds on this early onset modeling effort. "The primary goals for the first five years of this grant will be to start characterizing the early onset animals we have generated and to use genetic alignment information between mice, marmosets and humans to identify which risk genes for late-onset Alzheimer's we should focus on," Silva says.

Late-onset Alzheimer's is not an inherited disease in the conventional sense. The vast majority—95%—of Alzheimer's cases are considered sporadic, but some mutations (like those mentioned earlier) increase risk. Introducing such genes into marmosets and closely following gene expression patterns, brain scans and behavior could shed some much-needed light on what the underlying problems might be. And also accelerate the pipeline to clinical trials.

This year, results are expected from other big clinical trials of antibodies targeting amyloid. Lopez says it will be encouraging if more drugs are able to slow disease progression, as lecanemab did, but he doesn't expect them to be "magic." He recalls the painstaking process of vetting statin drugs to combat high cholesterol; those first came out in the 1990s.

"The first ones were not that good, and with time we improved them. It took so long to get really good statins"—and that was "in a disease where we know exactly the physiopathology," he says. But, as Lopez notes, the physiopathology, meaning the underlying disease-causing biological aberrations, are not yet known in Alzheimer's.

As a site for multiple national and international clinical trials, Pitt's Alzheimer's Disease Research Center has long been at the forefront of Alzheimer's research. For instance, the center contributed to the finding that careful blood pressure control in midlife reduces the risk of developing Alzheimer's.

Herrup, who is planning to write a second book on Alzheimer's research, is encouraged by "bright spots," like Lopez's work with blood pressure.

The wait for answers is hard, both for families living with Alzheimer's and for scientists conducting the research.

"We need a hit so badly," says Herrup. " ■