

WHEN REPLICATION RUNS AWRY

Tuberculosis kills more than a million people each year. The World Health Organization says it's the leading cause of death from a single infectious agent — more deadly than HIV/AIDS.

One reason TB is so dangerous is that, out of the 10 million or so infections that arise each year, roughly 500,000 are from drug-resistant *Mycobacterium tuberculosis* — bacteria immune to physicians' first, and sometimes second, choice of antibiotics.

M. tuberculosis' ability to mutate and evolve drug resistance is poorly understood. It's a problem that naturally interests Dr. Eric Josephs, whose research centers on mutation.

Most organisms use an ancient molecular proofreading mechanism called DNA mismatch repair. As strands of DNA are replicated in the process of cell generation, mismatch repair reads each new string and, if it finds mistakes, fixes them.

"Almost every organism on the planet has the same proofreading mechanism, except for bacteria related to tuberculosis," says Josephs, an assistant professor at the Joint School of Nanoscience and Nanoengineering. "They appear to have a weird, independently-evolved proofreading mechanism suggestive of mismatch repair. But people don't know what proteins are involved or how it works."

Understanding that mechanism, and what turns it off and allows drug resistance to evolve, could one day unlock new treatments for TB and other diseases.

As a postdoc, Josephs used nanoscale techniques to study mismatch repair and mutation. A year after arriving at UNCG, he received a \$291,000 grant from the National Institute of Allergy and Infectious Diseases to apply those same techniques to TB bacteria.

The R21 grant supports early stage research with potential to create transformative breakthroughs. Josephs' mutation research has implications not only for drug-resistant TB, but also for other diseases.

"Proteins involved in mismatch repair can malfunction and cause cancers," Josephs says. "They can also influence the onset of Huntington's disease and a number of neuromuscular diseases."

This year, Josephs also won a \$1.7 million NIH R35 Maximizing Investigators' Research Award, a grant designed to support outstanding researchers early in their careers.

The funding will support a wide-ranging exploration of the mechanisms of mutation and mutation avoidance. Josephs hopes his work will shed further light on how diseases work and point the way to new potential treatments.

"New understanding about genetic mutation can be applied broadly to a number of different disease systems," he says.

The work could also have other applications in biotechnology and agriculture, where the ability to engineer changes in an organism's genetic makeup is critical.

By Mark Toszczak • Learn more at go.uncg.edu/josephs

Josephs' work on strategies to prevent accidental CRISPR mutations in the wrong genes was recently published in leading international journal "Nature Biotechnology." CRISPR is a molecular tool that enables scientists to introduce "targeted" mutations into specific genes in living organisms; many researchers think it holds the key to new disease treatments. "Preventing unwanted mutations," he explains, "is critical for future therapeutic applications."

JOGGING YOUR MEMORY

DR. JENNIFER ETNIER recently gave her 79-year-old mother a smartphone. Her mother's memory is almost impeccable, and Etnier knew she would quickly adapt to the technology. Her father, on the other hand, is in the early stages of late-onset dementia.

One difference between the two? Exercise. Throughout her life, Etnier's mom has maintained high levels of physical activity. By contrast, her dad was active as a younger man, but let his exercise decline in his later years.

"I started wondering if their differing exercise patterns contributed to this phenomenon," says Etnier, now the Julia Taylor Morton Distinguished Professor in the Department of Kinesiology at UNC Greensboro.

It's that curiosity that has guided Etnier's research since she arrived on campus in 2004, put her ahead of the curve in her field, and led to her latest \$3.4 million National Institutes of Health study.

The funding will support Etnier and her fellow researchers as they work to determine what effect exercise might have on middle-aged and older adults with a genetic risk of Alzheimer's disease — a leading cause of death in the U.S. They hypothesize that those with a family history of dementia or Alzheimer's disease can cognitively benefit from exercise.

"Dementia is a term used to describe cognitive declines serious enough to interfere with daily living," says Etnier, who is a fellow of the National Academy of Kinesiology and the American College of Sports Medicine. "Alzheimer's disease is the most common cause of dementia and is characterized by the death of neurons in the brain."



It's a fear woven deep in the fabric of humanity, and especially prevalent as we age: the fear of suddenly losing your ability to think and process – to remember. And the fear is valid, as the prevalence of Alzheimer's disease – a specific form of dementia – is on the rise, with no known cure or pharmacological interventions to prevent the disease.

Etnier gets that, and her work is proving that we can do something about it. She's already discovered that long-term physical activity is beneficial for older adults with a family history of Alzheimer's, regardless of their genetic risk for the disease.

In 2013, Etnier and team conducted their first Physical Activity and Alzheimer's Disease research study, or PAAD, with \$394,625 from the NIH.

In the original PAAD study, Etnier and her fellow researchers observed that exercise correlated with improvements in memory, with effect sizes ranging from small to large.

"If you think of a test where the average grade is 70 and the standard deviation is a 10, essentially we found that exercise increases your performance on that test by between 2.7 and 10.5 points," Etnier said. "If it increased it by the largest amount, that would be like increasing your 'grade' by a full letter grade."

The initial study was small – with 54 subjects – and lacked a control group for comparison, thus limiting the researchers' ability to definitively say that changes in memory were in response to the exercise.

This time around, in PAAD2, Etnier is including a control group and also wants to find out whether or not there are genetic variables that identify people who might benefit more from exercise than others. She is looking at the apolipoprotein E, or APOE, genotype as a potential variable to determine how much one can benefit from exercise. The APOE genotype is a predictor of Alzheimer's.

After the age of 30, cognitive decline begins even in healthy adults. But people with a genetic predisposition or family history of Alzheimer's may be experiencing these declines at a faster rate.

"We know the brain is malleable across the lifespan. Although the brain is more plastic – able to change in response to experiences – at young ages, it is still plastic even in older adults," Etnier says. "Physical activity is important at all ages – but the benefits may be even more critical as you get older."

IMPROVED COGNITIVE PERFORMANCE IN 8 MONTHS

Etnier's team has found that participating in a regular exercise program for as few as eight months is associated with improved memory performance in older adults. Among those at genetic risk for Alzheimer's, the researchers also observed alterations in brain function.

Graduate students and postdoctoral fellows, like Jarod Vance (far left) and Dr. Kyoungshin Park (third from left), run participants through various cognitive tests at the beginning, middle, and end of the year-long study. Below, Vance and Park administer one such test to a staff member (not a project participant).

ON THE MOVE

Etnier and her team are now working to recruit 240 people from Guilford and surrounding counties over the next three years. Unlike other studies looking at physical activity for prevention of Alzheimer's disease, PAAD2 focuses on a younger age group – 40- to 65-year-olds – and will utilize group exercise instead of solo exercise. These factors help distinguish the study from any other in the country.

"If an individual is cognitively normal when they are 60-80 and have both a family history of Alzheimer's and a genetic risk for Alzheimer's disease, then they may have some other genes in their make-up that are giving them protection against Alzheimer's," Etnier says. "By targeting a younger cognitively normal group, we hope to bypass that issue." The researchers also hope using a group exercise program will offer participants social support.

Participants undergo testing at the beginning, middle, and end of a year-long period, to measure thinking abilities, brain structure and function, and biomarkers related to Alzheimer's disease.

Half of study participants commit to an hour-long exercise program of walking and training with resistance bands three times a week for a full year, while the other half continue living their normal lives without regular exercise. Importantly, at the end of the study year, participants in the control group receive a short-term YMCA membership to encourage them to get active as well.

Executive Director David Heggie of Greensboro's Bryan Family YMCA helped Etnier secure partnerships with YMCAs in Alamance, Davidson, Forsyth, Guilford, Randolph, and Rockingham counties for the project's exercise programs.

Participating YMCAs provide exercise instruction and help collect participant data, such as heart rate and ratings of perceived exertions. UNCG Associate Professor and exercise physiologist William Karper provides regular oversight at the YMCA locations to ensure uniformity across the programs. Below, PAAD2 participants work out at the Kathleen Price Bryan Family YMCA, with guidance from graduate student Delaney Thibodeau.

THE BENEFITS OF A SINGLE WORKOUT
Etnier's team has found that a single 20-minute session of moderate-intensity exercise produced significant benefits for both short-term and long-term memory.





ALL IN THE BRAIN

In her undergraduate and graduate studies in clinical exercise physiology, Dr. Alexis Ganesh (right) developed a passion for understanding the aging brain. She chose UNCG for her doctoral studies specifically to work with Etnier, pictured above working at the UNCG Gateway MRI Center.

"From the first day of my doctoral program, I was excited to use the MRI scanner," says Ganesh. She was soon designing studies around the technology and learning to analyze brain images. Now, as a postdoctoral fellow, she's responsible for neuroimaging on the PAAD2 project.

She values the opportunities and training she has received here, she says – not just on the MRI but also on effectively communicating her results to scientific peers and the community.

BENEATH THE SURFACE

"What's intriguing about the measures we're taking is that the behavioral measures are not likely to be the most sensitive ones we have," says Etnier. "The ability to perform a task may or may not change over the course of a year, but brain structure and blood biomarkers are very sensitive."

Etnier works with Dr. Laurie Wideman, Safrit-Ennis Distinguished Professor in Kinesiology, to take blood samples at pre- and post-test appointments. They are looking at blood-based biological markers relevant to Alzheimer's and exercise.

Etnier is also using the University's Gateway MRI Center to assess brain structure and function.

"When you compare yourself to someone else, there are a lot of factors – like lifestyle, environment, and genetics – that might affect your brain structure and how it functions," says Dr. Alexis Ganesh, the postdoctoral researcher responsible for the project's neuroimaging. "We suggest that physical activity can provide benefits, but the degree of benefit may depend on where you're starting."

High-resolution MRIs of participants' brains show structural volume of brain regions, while functional imaging allows the team to see neural activation during tasks and investigate how functionally connected the brain is.



CHANGING LIVES

"Physical activity may give you the protection you need to stall the onset of clinical impairment or to prevent it altogether," Etnier says. "If we can delay the onset of Alzheimer's by five years, that's five more years with better quality of life – that's five more years with your children and grandchildren."

It was a radio ad asking for PAAD2 study participants that got Ginny Ebert's attention.

"I was excited right away," she says. "It touched home."

Ebert's grandmother and father have Alzheimer's, and at 50, she's pretty sure she's on the way. Her mind feels like a pinball machine, she says. Things that once came easily are now more difficult, and she has trouble processing and making connections. Then she forgets what she's trying to figure out in the first place.

Ebert graduated from UNCG in 1990 with a degree in nutrition, and throughout most of her life she was very active – swimming, running, and weightlifting. But then she took a long hiatus.

She began the PAAD2 exercise program in August, and so far, she has felt a lot better, both mentally and physically. But the thing she values most is the community she has built with the other participants.

"Getting to meet other people in a similar situation has created a supportive and positive environment," Ebert says.

Community is just a small piece of the enormous impact Etnier's work could have on both individuals and the future of Alzheimer's research.

"I hope that once we finish the PAAD2 study, we can give people at risk the tools they need to potentially delay or even prevent the onset of this disease,"

Etnier says. "If our expectations are realized, this could give hope to the millions of individuals who have seen a family member suffer the heart-wrenching effects of Alzheimer's disease and who may fear the same fate because of their family history."

By Elizabeth L. Harrison

Learn more about PAAD2 and how you and your loved ones can participate at go.uncg.edu/PAAD2.

The project is a great training ground, says Etnier. "Dr. Nate Berry, a UNCG doctoral graduate who worked on the project as a postdoctoral researcher, was recently hired by Under Armour as a lead research scientist."

Shonda Mobley (top photo, right), who received her master's in public health education at UNCG, serves as the PAAD2 project coordinator. Her responsibilities include screening and scheduling participants. Graduate students like Towfiqul Alam (bottom left) and Sam Kibildis (bottom right) evaluate participants' fitness by measuring respiration and perceived exertion during exercise.

