Ray Smith, 70 (left), and Carol Turner, 68 (right), with their mother Mattie Smith, 90, who has Alzheimer's disease. Ray Smith inherited two copies of the Alzheimer's risk gene *APOE4*; Turner has one copy.

PHOTO: BILL TIERNAN

THE BURDEN OF A GENE

A variant called *APOE4* is notorious for its link to Alzheimer's. Can new insights into its function help stave off disease? *By* **Jocelyn Kaiser**

Carol Turner and her brother Ray Smith have seen up close what it's like to live with Alzheimer's disease. In 2020, their father died of it at age 93 in a dementia ward, after months of seeing his family through a window because of the COVID-19 pandemic. Not long after, their mother showed the signs—she had trouble sleeping, she couldn't recognize some faces, and her beloved brownie recipe didn't come out quite right. Brain scans revealed abundant beta amyloid, the sticky protein that is a hallmark of Alzheimer's.

Given this family history, their mother's geriatrician at Eastern Virginia Medical School in nearby Norfolk knew the siblings could be at high risk of Alzheimer's. He pointed them to a clinical trial with a site at the medical college seeking participants to test a preventive treatment. There, DNA testing revealed more troubling news. Smith, now 70, has two copies of the most common—and perhaps the most scientifically beguiling—of the genetic variants strongly linked to Alzheimer's late in life: *APOE4*. Turner, 68, carries one copy.

The variant has loomed large since the finding that it dramatically increases Alzheimer's risk electrified the field more than 30 years ago. The estimated 164 million people globally who carry two copies of *APOE4*, one of three variants of the *APOE* gene, can have a risk of late-onset Alzheimer's from eight to 25 times higher than those who carry only the gene's most common version, *APOE3*. About 1.6 billion people have a single copy of the variant—this writer among them. For us the risk of the disease can also be increased, threefold or more, depending on one's ancestry.

The 1993 discovery of *APOE4*'s role in Alzheimer's seemed to promise key insights into the roots of the mystifying disease, and perhaps a new target for treatment. But progress has been slow. The protein that *APOE* encodes, apolipoprotein E (ApoE), has many roles in the brain, making it hard to tease apart which are relevant to the development of disease and why certain *APOE* variants affect risk. But scientists may finally be gaining traction. New insights into *APOE*'s effects, as well as a wave of studies on rare protective versions, are fueling interest in potential therapies that would counter *APOE4*'s harms.

Adding new urgency to such efforts is a study published by Spanish researchers in May in *Nature Medicine*, which bolstered the case that *APOE4* should be considered not just a risk factor for the disease, but a direct

cause. It found that of the 2% of people of European ancestry with two copies of *APOE4*, 75% will show amyloid accumulation in brain scans (though not necessarily dementia) by age 65. The variant's role in disease has "become clearer and clearer," says *APOE4* expert Yadong Huang of the Gladstone Institutes, who wrote an accompanying commentary.

Such findings have energized the small sliver of Alzheimer's researchers who have devoted their careers to *APOE4*. "*APOE* plays a big role not only in risk for Alzheimer's, but also likely the progression of disease," says neurologist and neuroscientist David Holtzman of Washington University in St. Louis. "It's really fantastic that now people are looking into ways to target it."

After being told she and her brother had "the Alzheimer's gene" and already had brain amyloid, Turner was worried. "[The doctor] said, 'You and your brother will likely get it down the road if you don't do something about it.'" In late 2021 Turner became the first African American to join the trial, in which people with significant levels of amyloid but no Alzheimer's symptoms get either a placebo or biweekly or monthly infusions of an amyloid-removing antibody called lecanemab to perhaps stave off the disease. Smith joined the trial soon after. More than 2 years into the 4-year treatment, both say they haven't noticed any decline in their memory.

***APOE* WAS ORIGINALLY** known for its role in regulating blood cholesterol, which contributes to cardiovascular disease. (Most people with the gene variant *APOE2* have low cholesterol and are protected from heart disease, whereas *APOE4* carriers are at highest risk.) But in the early 1990s, researchers at Duke University identified a DNA region linked to higher risk of late onset Alzheimer's, which begins after age 65 and is the most common form of the disorder. The Duke team and others also turned up another clue: The ApoE protein, whose gene lay in the suspect region, was present in the amyloid plaques suspected of causing neurological damage in Alzheimer's.

Then in 1993, Duke geneticist Margaret Pericak-Vance's group pinpointed the *APOE4* risk variant by comparing the genes of people who developed late onset Alzheimer's with those of their unaffected relatives—234 people from 42 families in all.

The finding, published in *Science*, was heralded as a major breakthrough for the field. Neurologist Allen Roses, who led the overall team investigating *APOE* at Duke, predicted in *The New York Times* that "in 10 to 15 years, we'll have a safe and effective medication that a 50-year-old could take every day to prevent Alzheimer's disease."

Larger studies soon revealed that *APOE4* carriers accumulated more amyloid earlier in life than noncarriers, long before developing Alzheimer's symptoms. The Duke group and others made the equally compelling discovery that people with one or two copies of a different variant, *APOE2*, developed little amyloid and were protected from the disease. Intriguing differences based on ancestry also emerged: Risks for African Americans with *APOE4* are somewhat lower than for people with European ancestors, whereas in East Asians the risk is much higher, up to 25-fold for people with two copies, compared with those with two *APOE3* genes.

And yet despite nearly 7000 papers published on *APOE4* and Alzheimer's, Roses's prediction about safe, effective treatment targeting the protein remains a distant hope. That's partly because ApoE has multiple sources in the brain and diverse roles. The protein is made mainly by astrocytes, cells that help nourish and maintain neurons, as well as by microglia, the brain's immune cells. Under stress, neurons also produce it.

"*APOE* plays a big role not only in risk for Alzheimer's, but also likely the progression of disease."

David Holtzman
Washington University in St. Louis

Work starting in the 1980s revealed that ApoE's main job in the brain is to assist in transporting and processing lipids, vital to cells' ability to repair damage, transmit signals, and more. The protein binds to cholesterol and other lipids to form particles that are delivered to brain cells, which then break them down into forms the cells can use.

Studies also showed ApoE helps clear away amyloid—a job that ApoE4, which differs from ApoE2 and ApoE3 at just two amino acids, does less efficiently. The brains of *APOE4* carriers not only develop more amyloid than noncarriers, but are less able to break up plaques if they do form. They are also more likely to produce misfolded tangles of a protein called tau that is thought to contribute to the disease. But those aren't the only possible routes by which *APOE4* could drive Alzheimer's: Researchers have also found that the variant's protein impairs cell powerhouses called mitochondria, makes the blood-brain barrier more permeable to toxins, hinders lipid transport and metabolism, and revs up inflammation, which harms neurons.

Holtzman suspects that of these many effects, "there's just probably a few that are truly having a major impact on disease." But the challenge of identifying the relevant ones and untangling their mechanisms deterred would-be drug developers. It didn't help that researchers were unsure what a treatment should do. If ApoE4 was causing harm because it was too wimpy to remove amyloid, an ideal therapy should ramp up its levels in the brain. If it was intrinsically harmful, on the other hand, a treatment should curb it.

Researchers say interest in *APOE4* also lagged because the Alzheimer's field was fixated on amyloid as the disease's main cause. "We were in competition with the amyloid hypothesis," particularly the notion that directly removing plaques could treat the disease, says Pericak-Vance, now at the University of Miami. Billions of public and private research dollars went into attempting to show that amyloid-stripping antibodies could slow Alzheimer's.

That work led to the 2023 approval in the United States of lecanemab, and of a second drug, donanemab, in July, for people with early Alzheimer's disease. But the benefits of these antiamyloid therapies are modest, and they can cause serious side effects, including brain swelling and bleeding. *APOE4* carriers are especially prone to them, possibly because they have more amyloid-laden brain blood vessels that become weaker when the plaques are removed.

Yet *APOE4* studies were never fully eclipsed by amyloid research. Starting in 2018, for example, Massachusetts Institute of Technology neuroscientist Li-Huei Tsai and collaborators showed how impaired lipid processing in specific cell types harboring *APOE4* alters them in ways that could cause disease. Neurons become hyperactive, astrocytes and microglia become stuffed with lipids and less effective, and cells called oligodendrocytes fail to maintain the sheathing around nerve fibers.

Studies of protective *APOE* variants have also invigorated the field. In 2019, Harvard Medical School researchers described a Colombian woman who, like much of her extended family, carried a rare mutated gene that almost inevitably causes Alzheimer's around age 40. Yet unlike many of her relatives, she did not develop cognitive problems until her 70s, even though she had abundant amyloid.

The apparent explanation was another unusual mutation, this one in both of her two copies of *APOE3*. Known as Christchurch for its discovery in a New Zealander with a lipid disease in 1987, the mutation makes ApoE less efficient at binding to heparan sulfate proteoglycans, molecules that coat brain cells—an effect the researchers thought might explain the puzzlingly low levels of tau found in the woman's brain. In June, the same team reported in *The New England Journal of Medicine* that 27 people with the Colombian family's Alzheimer's gene and

just one copy of the Christchurch mutation stayed cognitively healthy 5 years longer than those who lacked the mutation. The work suggests future drugs might stave off disease by mimicking this genetic change.

Researchers also seem to have finally reached some consensus on the long-running debate over whether a therapy should ramp up or block ApoE4. Last year, National Institute on Aging Director Richard Hodes and former National Institutes of Health (NIH) Director Francis Collins asked a working group of *APOE4* researchers to review the latest evidence.

One clue came from large studies of Alzheimer's in African Americans led by Jeffery Vance of the University of Miami, who is Pericak-Vance's husband. His team found that people who inherited *APOE4* from African ancestors have differences in surrounding DNA that lower the gene's expression in brain cells compared with those who got it from European ancestors. That could explain why African American *APOE4* carriers don't face as high a risk of Alzheimer's as white carriers. Another study out this year led by neurologist Michael Greicius at Stanford University found two non-Hispanic white individuals with one copy of *APOE4* who remained dementia and amyloid free at ages 76 and 90, apparently because of mutations that disabled the variant—more evidence that reducing ApoE4 may be helpful.

After reviewing these and other studies, the NIH working group published in January what Collins calls "a very through paper" in the *Annals of Neurology*. It concludes, he says, that, at least in people of African and European ancestry, ApoE4 "is clearly a toxin."

THAT CONSENSUS and the wealth of new findings are propelling work on a range of strategies for targeting *APOE4* to treat or prevent Alzheimer's. Some teams hope to use a strand of RNA to bind and partially shut off *APOE4*. (Some ApoE may be necessary to maintain brain function.) Such antisense oligonucleotide drugs, injected into spinal fluid, have been approved to treat two rare neurodegenerative diseases. Holtzman's team has reported that an antisense drug aimed at *APOE* could curb production of tau tangles and brain damage in mice with the *APOE4* variant. But it blocked amyloid only if first given to mice as newborns.

Chemical biologist Anastasia Khvorova thinks that's because the mice still made too much ApoE protein. Her team at the University of Massachusetts Chan Medical School reported earlier this year that in adult mice prone to Alzheimer's-like pathology, short double strands of genetic material called small interfering RNA, which mark a gene's messenger RNA for destruction, can "silence *APOE* in the brain in a very potent fashion" without lowering needed levels outside the brain, Khvorova says. The approach curbed the formation of amyloid plaques. Both RNA drug approaches are now under development by companies.

Such drugs would be injected into the spinal fluid at least every few months. But some researchers are exploring what would be a one-time treatment that edits a gene in brain cells. As a proof of concept, in a *Nature Neuroscience* study last fall, Huang's lab showed that newborn mice engineered to carry the Christchurch mutation in one or both of their copies of *APOE4* were partially or fully protected from developing Alzheimer's-like pathology as adults. A group led by physiologist Lance Johnson of the University of Kentucky genetically engineered a mouse so that a drug could switch *APOE4* into *APOE2* in specific brain cell types. At a meeting last year, he reported that making the switch in midlife in astrocytes alone reversed amyloid accumulation in the animals.

One gene-modifying approach has reached the clinic. In 2019, a group led by gene therapy researcher Ron Crystal of Weill Cornell Medicine began to infuse a virus carrying *APOE2* into the upper spinal column of 15 patients who carry two copies of *APOE4* and have mild cognitive impairment or Alzheimer's symptoms. Cerebral fluid samples from the first few patients suggested their brains were making *APOE2* and producing less amyloid and tau, according to meeting reports from Crystal's team, which plans to share full results this fall. The trial's sponsor, the biotech Lexeo Therapeutics, is also working on introducing an *APOE2* gene with the Christchurch mutation, and on another therapy that would both add *APOE2* and silence *APOE4*.

More precise viral vectors in the works that home in on the brain could make it possible to inject a treatment into a person's blood instead of their spinal column. Still, genetically modifying the brain could be a costly, extreme step for *APOE4* carriers such as Turner and Smith who are cognitively normal but at elevated risk for Alzheimer's.

Holtzman and his group have developed a less drastic intervention: an antibody that binds to and removes an especially harmful form of the ApoE4 protein. They've shown it can prevent Alzheimer's-like pathology in mice. Tsai's group is exploring whether a cocktail of already approved drugs or supplements could thwart some of the *APOE4* damage her group has observed. The team has reported, for example, that the nutrient choline can correct lipid processing in cultured human astrocytes with *APOE4*. In a small trial in Texas, Tsai's collaborators are testing the effects of choline supplements on lipid profiles in spinal fluid of cognitively normal people with at least one copy of *APOE4*.

It's "a small step toward fulfilling our idea of an affordable, accessible, safe way to move the needle for carriers," Tsai says.

Carol Turner (left) moved into the home of her mother, Mattie Smith, who has Alzheimer's disease, to care for her.

PHOTO: BILL TIERNAN

ALL THIS PROGRESS raises hopes for people who know they carry the *APOE4* variant and are eager to improve their odds. Their ranks are growing fast. Many, including me, learn of their status from direct-to-consumer DNA testing such as 23andMe. Several physician-researchers interviewed for this story said *APOE4* carriers are turning up at their offices, concerned about their risk.

A treatment someone like me could take to ward off Alzheimer's now seems a possibility—though it could take years. What do we *APOE4*s do in the meantime? As a carrier of one copy of *APOE3* and one *APOE4*, I have triple the risk of a white person with two *APOE3* copies—a 30% chance, or more by some estimates, of developing Alzheimer's by age 85, which is 26 years from now. I inherited *APOE4* from my father, who had dementia and barely remembered me when he died.

University of Southern California (USC) Alzheimer's researcher Hussein Yassine points out that carrying *APOE4* isn't inherently a defect. The variant may actually be the original form of the gene—the one our early human ancestors had—and could in

fact offer advantages. "There is a reason why so many people have it," he says. Some studies have proposed *APOE4* helps carriers fight off childhood pathogens. Other research even suggests young adults with *APOE4* have better spatial memory and other subtle cognitive advantages.

But there's also evidence that the sedentary lifestyle and heavy consumption of refined sugars common among modern humans could exacerbate the disadvantages of the gene, Yassine says. "Modern day life does not work for certain individuals with *APOE4*."

Scientists' advice for *APOE4* carriers: Eat right, exercise, get enough sleep, control cholesterol and blood pressure, and stimulate one's mind. All are habits that seem to lower the risk of Alzheimer's for everyone. At the USC Center for Personalized Brain Health, launched in 2023 expressly to recruit *APOE4* carriers for prevention and drug discovery studies, Yassine is planning trials testing some of those lifestyle strategies. He is also wrapping up a study of high doses of omega-3s, a form of fat that some evidence suggests protects *APOE4* carriers from neuroinflammation.

One way Turner has stayed active is by helping recruit Black Americans to the prevention trial, in part through visits to local churches. Smith keeps his mind sharp as an umpire for softball games, which have "a lot of rules," he says. The two find comfort in the fact that if they show signs of cognitive impairment, possibly while receiving a placebo, they can choose to receive lecanemab. "That's the biggie to me, that you will have the choice of getting the drug," Smith says.

As for me, I signed up for the same trial but learned that I didn't have enough amyloid to enroll. So like many other *APOE4*s—there's an online forum (APOE4.info) where they gather—I now scour the web for new research about the variant, dabble in taking supplements like choline and omega-3s, and ponder tracking my scores on online cognitive tests. I hope any decline, if it happens, is slow enough for me to help my two daughters, who 23andMe says don't have *APOE4*, make plans for me.

And I try to remember what experts have told me: that many people with one *APOE4* gene will not develop cognitive problems, and even some with two copies are dementia free in their 90s. "Understand that [being a carrier] is not diagnostic," says Alzheimer's researcher Goldie Smith Byrd of Wake Forest University School of Medicine, who founded a community health research center that supports Black Americans with Alzheimer's in their families. Her words resonate with me: "Get your business in order, try to be as healthy as you can. And try not to stress over it so much." ■

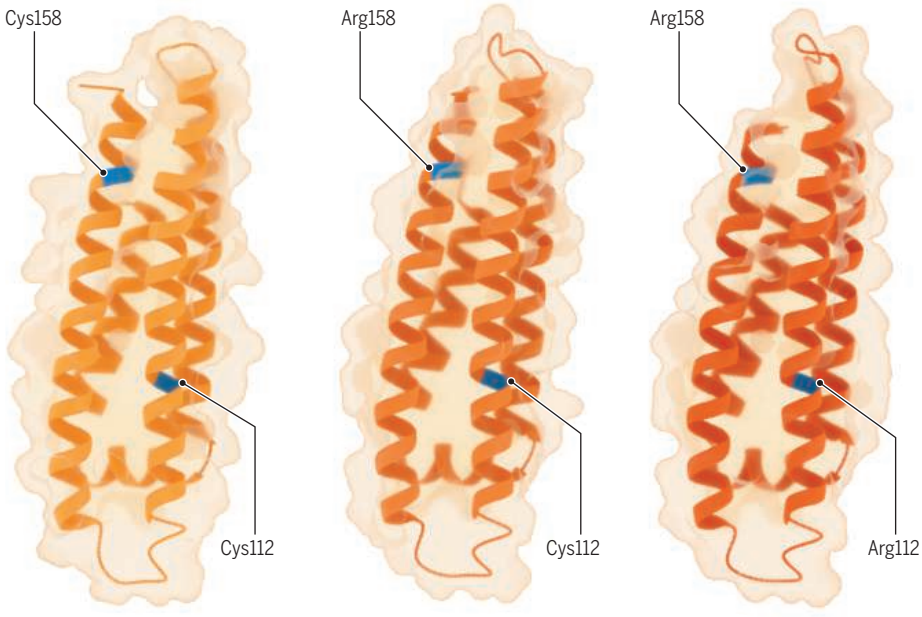
Small differences, big effects

The three protein forms encoded by the genetic variants *APOE2*, *APOE3*, and *APOE4* differ by just one or two amino acids (blue, below), yet lead to dramatic differences in risk for Alzheimer's disease.

ApoE2
Carried by about 5% to 10% of people worldwide, this protein **protects** against Alzheimer's—although two copies of its gene can lead to a rare lipid disease.

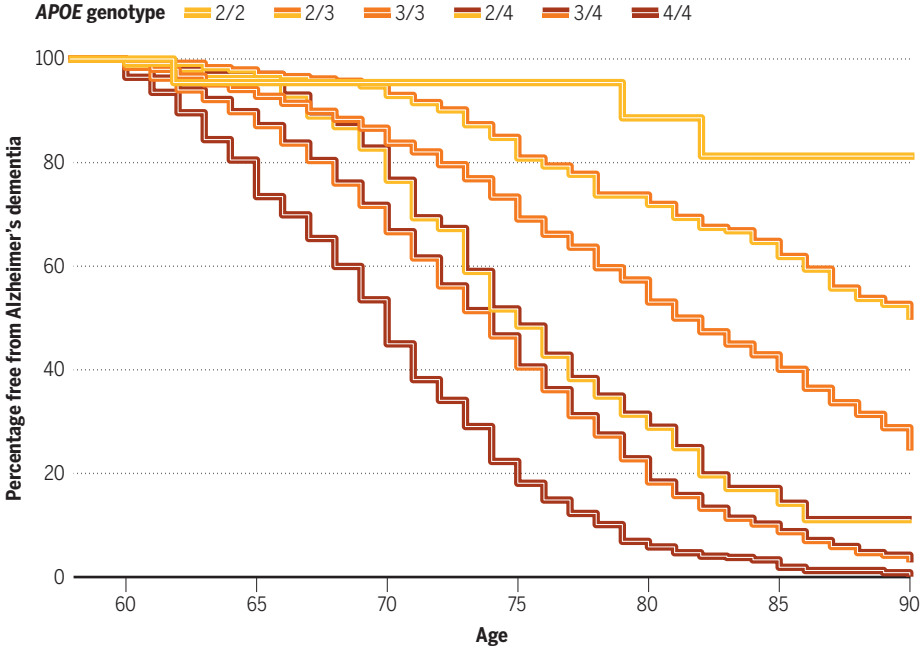
ApoE3
This form of the protein is present in about 70% to 80% of the global population. It is considered to confer **average risk** of Alzheimer's.

ApoE4
Roughly one-fifth of the global population has one copy of this protein's **risk-raising** gene variant and 2% have two copies. About half of Alzheimer's patients carry it.



Luck of the draw

Various pairings of the three *APOE* gene variants—*APOE2*, *APOE3*, and *APOE4*—raise or lower risk for Alzheimer's disease late in life. These curves are from a 2020 study of 5000 deceased non-Hispanic white people with and without Alzheimer's. Such retrospective studies may exaggerate risks; in studies that followed cognitively normal people over time, at least 40% of *APOE4/4* carriers were still dementia free at 85.



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