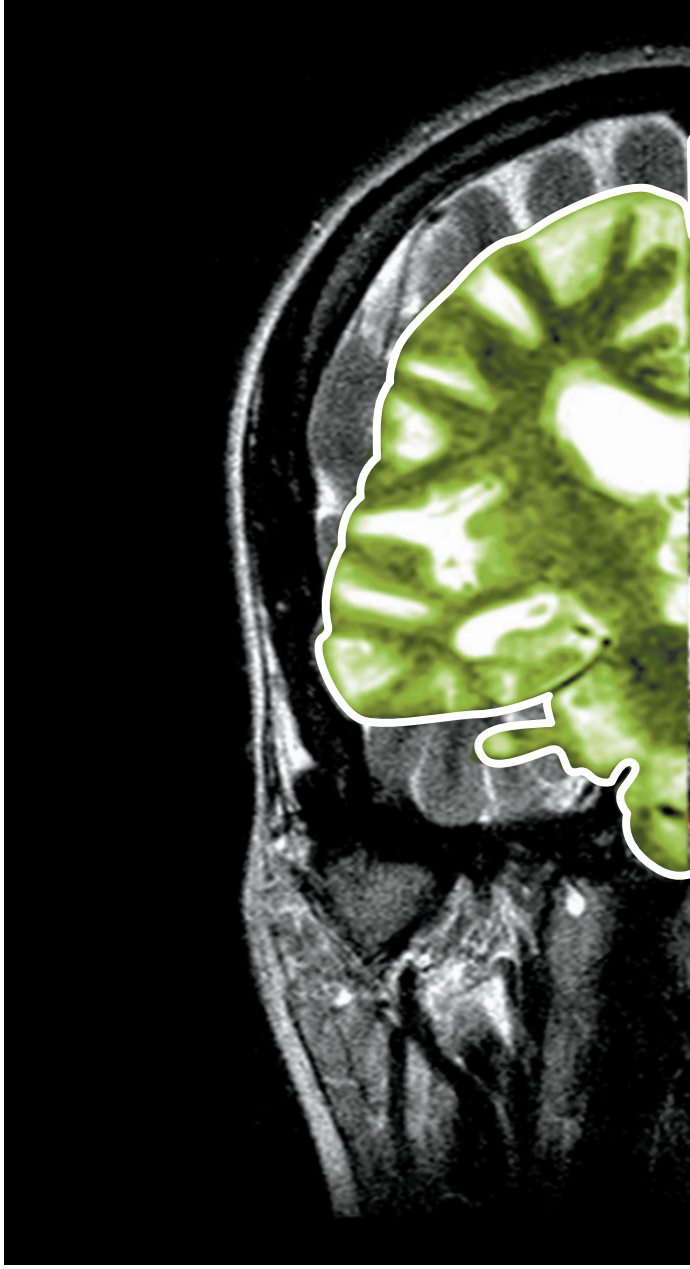
The forgetting GENE



For decades, most researchers ignored the leading genetic risk factor for Alzheimer's disease. That is set to change.

BY LAURA SPINNEY

MEDICAL BODY SCANS/JESSICA WILSON/SPL

One day in 1991, neurologist Warren Strittmatter asked his boss to look at some bewildering data. Strittmatter was studying amyloid-β, the main component of the molecular clumps found in the brains of people with Alzheimer's disease. He was hunting for amyloid-binding proteins in the fluid that buffers the brain and spinal cord, and had fished out one called apolipoprotein E (ApoE), which had no obvious connection with the disease.

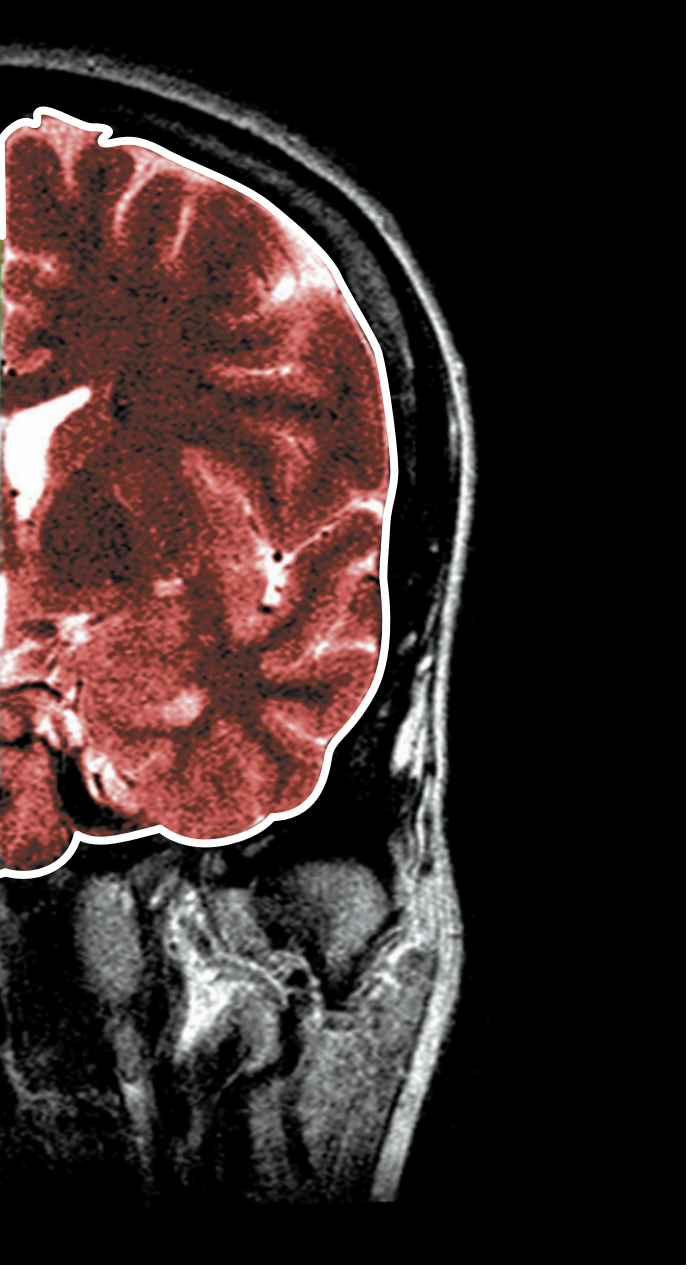
Strittmatter's boss, geneticist Allen Roses of Duke University in Durham, North Carolina, immediately realized that his colleague had stumbled across something exciting. Two years earlier, the group had identified a genetic association between Alzheimer's and a region of chromosome 19. Roses knew that the gene encoding ApoE was also on chromosome 19. "It was like a lightning bolt," he says. "It changed my life."

In humans, there are three common variants, or alleles, of the *APOE* gene, numbered 2, 3 and 4. The obvious step, Roses realized, was to find out whether individual *APOE* alleles influence the risk of developing Alzheimer's disease. The variants can be distinguished from one another using a technique called the polymerase chain reaction (PCR). But Roses had little experience with PCR, so he asked the postdocs in his team to test samples from people with the disease and healthy controls. The postdocs refused: they were busy hunting for genes underlying Alzheimer's, and *APOE* seemed an unlikely candidate. The feeling in the lab, recalls Roses, was that "the chief was off on one of his crazy ideas".

Roses then talked to his wife, Ann Saunders, a mouse geneticist who was skilled at PCR. She had just given birth to their daughter and was on maternity leave, so they struck a deal. "She did the experiments while I held the baby," he says. Within three weeks, they had collected the data that would fuel a series of landmark papers showing that the *APOE4* allele is associated with a greatly increased risk of Alzheimer's disease[1].

Twenty years on, *APOE4* remains the leading genetic risk factor for Alzheimer's, the most common form of dementia (see 'Risky inheritance'). Inheriting one copy of *APOE4* raises a person's risk of developing the disease fourfold. With two copies, the risk increases 12-fold. Yet Roses' data were largely criticized or ignored. Within a couple of years, interest in ApoE had dwindled as researchers flocked to study amyloid-β. The handful of labs that continued to pursue ApoE did so in the face of indifference from funding agencies and the neuroscience community, and without the resources needed to validate experimental findings with larger studies.

Today, the function of the ApoE protein in the brain remains mostly unknown. This neglect of such a strong



Over time, Alzheimer's disease drastically shrinks the brain.

lead has puzzled some outside the Alzheimer's field. At a forum on brain diseases in Frankfurt, Germany, Thomas Bourgeron, an autism researcher at the Pasteur Institute in Paris, voiced his confusion. "If I had a risk factor like that, I'd be hot on its trail."

But interest in the lipoprotein is picking up, in part because attempts to target amyloid-β have repeatedly disappointed in major clinical trials. Pharmaceutical companies are pulling back from amyloid-based approaches and some academics have begun to question the focus on the molecule. For the first time, researchers are developing drugs aimed at the ApoE4 protein and drawing attention from industry.

"The amyloid hypothesis became such a strong scientific orthodoxy that it began to be accepted on the basis of faith rather than evidence," says Zaven Khachaturian, president of the nonprofit campaign Prevent Alzheimer's Disease 2020, and former coordinator of Alzheimer's-related activities at the US National Institutes of Health. Until recently, he says, "no one has stepped back to ask the fundamental question of whether our basic premise about the disease is the correct one".

STIFF COMPETITION

Opinions differ as to why Roses' finding was neglected, but many agree that bad timing played a part. In 1991, John Hardy and David Allsop had proposed the 'amyloid cascade hypothesis'. This posits that Alzheimer's disease results from the abnormal build-up of amyloid-β clusters, or plaques, in the brain[2]. Others rallied around the idea and it has won most of the funding available to the field ever since.

But Roses did not subscribe to that theory. "Amyloid is one of many substances that builds up in plaques as a result of dying cells and atrophy in the brain," he says. "I never did think it was the cause." In saying so, he may have deterred others from investigating a possible ApoE–amyloid link, and inadvertently set up a competition between the two hypotheses for funding. He never got another grant to work on ApoE.

But there were also technical obstacles to ApoE research. The protein is found throughout the body, making it difficult to target the molecule specifically in the brain. And ApoE is bound to fat, so it tends to stick to other molecules in biochemical assays, says Menelas Pangalos, who leads research on small-molecule discovery at AstraZeneca in Macclesfield, UK, and has long had an interest in ApoE.

Working with such proteins requires an intimate understanding of lipid biochemistry. "If you want to study ApoE biology, you really need to devote a laboratory to understanding the techniques," says neurologist David Holtzman of Washington University in St. Louis, Missouri. Holtzman did just that, establishing a separate lab dedicated to developing techniques for handling lipoproteins in the central nervous system.

Amyloid was the easier target. Two decades of intensive pursuit have yielded a range of drugs that alter the metabolism of amyloid-β, but these have yet to fulfil expectations. Of the six drugs that were in phase II or III clinical trials in 2012, half have since been dropped because of either safety concerns or lack of effectiveness. This comes against a backdrop of ageing populations, overstretched health-care systems and a dearth of medications for Alzheimer's disease. "The large number of major failed trials in Alzheimer's is quite frightening," says Lennart Mucke, director of the Gladstone Institute of Neurological Disease at the University of California, San Francisco. "It has really scared off big pharma."

The three remaining drug candidates that target amyloid-β are currently being tested in people with Alzheimer's, as well as in individuals who have a high risk of developing the disease but who have not yet developed symptoms. Imaging studies have shown that the brains of high-risk individuals look and behave differently from controls decades before the onset of Alzheimer's, and long before they start to accumulate amyloid-β or lose grey matter[3]. The trials will examine whether the drugs prevent or delay the onset of the disease; they are due to wrap up over the next six years. There is a growing sense in the field — among academics and industry representatives alike — that these efforts are the last chance for the amyloid hypothesis. Amid these concerns, the spotlight has swung back to ApoE.

If the prevention trials fail, it will be up to academics to persuade companies back to the table with solid preclinical and early clinical data, says Mucke. He is optimistic that ApoE researchers may soon have that leverage. Despite the obstacles in this area, there is an emerging understanding of how ApoE4 increases risk, which Holtzman's and Mucke's groups have explored through transgenic mice that they have developed to express human forms of ApoE.

The molecule seems to contribute to Alzheimer's through two distinct pathways, one of which is amyloid-dependent. In both animals and humans, ApoE4 strongly promotes amyloid-β deposition in the brain, compared with ApoE3, long considered the 'neutral' form when it comes to Alzheimer's risk. ApoE2, which is considered the protective form, decreases the build-up[4]. "These are compelling data," says Holtzman.

The other mechanism does not involve amyloid. When neurons are under stress, they make ApoE as part of a repair mechanism. The 'bad' ApoE4 form tends to be broken down into toxic fragments that damage the cell's energy factories — the mitochondria — and alter the cell skeleton.

The relative contribution of these two pathways to Alzheimer's risk is not known, says Holtzman, but he and others think that changing a harmful form of ApoE into a less damaging one might prove a promising therapeutic approach. At the Gladstone, cardiovascular scientist Robert Mahley, working with a team including neuroscientist

Yadong Huang, has identified small 'corrector' molecules that modify the structure of ApoE4 protein to one more like that of ApoE3, thereby reducing abnormal fragmentation[5].

In cell culture, low concentrations of these corrector molecules can reduce mitochondrial impairment and neuronal dysfunction[6]. They are now being tested more rigorously in a range of animal models. If the molecules ultimately prove safe and effective in humans, Mucke foresees a day when doctors will prescribe them for people deemed at risk of Alzheimer's, just as statins are offered to those with high cholesterol and an elevated risk of cardiovascular disease.

ABOVE AND BEYOND

Such drugs could also have implications beyond Alzheimer's. "The mitochondrial-impairment hypothesis provides a pretty logical and parsimonious explanation for why ApoE4 does bad things," says Mucke, "not only in the context of Alzheimer's, but maybe also in other diseases." There is evidence that it may be a risk factor in Parkinson's disease and epilepsy. It is also associated with an increased risk of a poor outcome after brain injury, and more rapid progression of untreated HIV infection. Fifteen biotechnology companies are already collaborating with the Gladstone to develop these and similar drugs.

Despite his inability to get grants, Roses never gave up on ApoE. But a few years after his group discovered the link between ApoE and Alzheimer's, he wearied of the constant battles for funding. He left academia and spent ten years in industry — where he continued to work on ApoE, among other things — before returning to Duke in 2008.

In 2009, his group described a stretch of non-coding DNA in a gene called *TOMM40* that sits next to *APOE* on chromosome 19. This stretch of DNA, known by the shorthand 523, varies in length. The length of 523 can determine the extent to which *TOMM40* and *APOE* are expressed[7].

The discovery was important, Roses says, because the protein encoded by *TOMM40*, called Tom40, is crucial to healthy mitochondria. Tom40 forms a channel in the outer mitochondrial membrane that is used to import proteins. Without these proteins, mitochondria cannot divide as they should throughout a cell's life. "It's a big effect that's been known about for a decade," says Roses, "But it's not well-known in the Alzheimer's field."

Roses went on to suggest that 523 could be exploited to develop therapies and improved tests for Alzheimer's risk. Most people will develop Alzheimer's if they live long enough, but only about 25% of people carry an *APOE4* allele. As a result, a prognostic test for *APOE4* will only ever be partially informative. But genotyping both *APOE* and *TOMM40* could provide information about a wider swathe of the population, Roses says. His group has found, for example, that *APOE3* — by far the most common *APOE* allele in humans — is usually inherited either with a short or a very long 523 tract. In those who inherit two *APOE3* alleles, age of onset differs depending on which combination of the two 523 variants they also inherit.

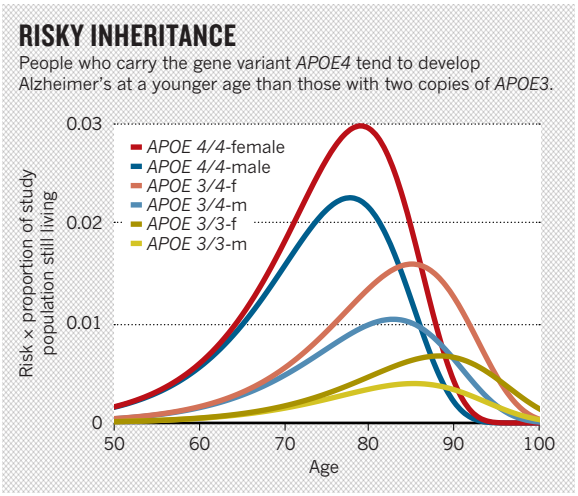
Other labs have found evidence supporting Roses' hypotheses, but some attempts to replicate his *TOMM40* findings have failed. In 2012, Hardy, now at University College London, and a colleague, geneticist Rita Guerreiro, wrote an editorial[8] in which they argued that *TOMM40* did not independently affect Alzheimer's risk.

Roses' faith in his hypothesis has not wavered: he believes he has a sound mechanistic explanation for his findings. And he says that the genome-wide studies that failed to reproduce his results lacked sufficient power to reveal the association between *TOMM40* and Alzheimer's disease. Khachaturian says a proper test of Roses' findings — using Roses' methods in a larger cohort of patients — has not yet been done.

Roses hopes to soon be able to back up his findings with more clinical data and has launched a company called Zinfandel Pharmaceuticals in Durham, fuelled in part by his own funds. Along with the Japanese pharmaceutical company Takeda, based in Osaka, Zinfandel is currently funding a phase III trial, called TOMMORROW, that will put his ideas to the test. TOMMORROW is expected to run for about 5 years and will recruit close to 6,000 healthy, elderly individuals. It will evaluate a risk-assessment algorithm based on age, *APOE* and *TOMM40*.

The trial will also investigate whether a low dose of a drug called pioglitazone — already approved at much higher doses for certain patients with type 2 diabetes — can delay disease onset in those individuals deemed by the algorithm to be at high risk of Alzheimer's. Evidence from animal and small-scale human studies suggests that pioglitazone may prevent or reverse Alzheimer's-related pathology and symptoms[9]. Roses thinks it may do so by stimulating mitochondria to divide.

The ongoing trials could have major consequences even without yielding a cure: research has shown that an intervention that could delay the onset of Alzheimer's by just 2 years would result, 50 years later, in nearly 2 million fewer cases of the disease in the United States than projected otherwise[10]. And the results coming in over the next few years could force researchers to re-evaluate their understanding of dementia. It is time it was recognized for what it is, says Khachaturian: a failure of complex, interacting physiological systems. Looking at any one of these systems — even those involving ApoE4 — in isolation is unlikely to fully explain changes in behaviour. "The field is going to recognize the limitations of current approaches and step back," he says. "And if we're honest with ourselves, we'll start forging new directions." ■



SOURCE: J. RABER *ET AL. NEUROBIOL. AGING* **25,** 641–650 (2004)

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