New Piece in Alzheimer's Puzzle

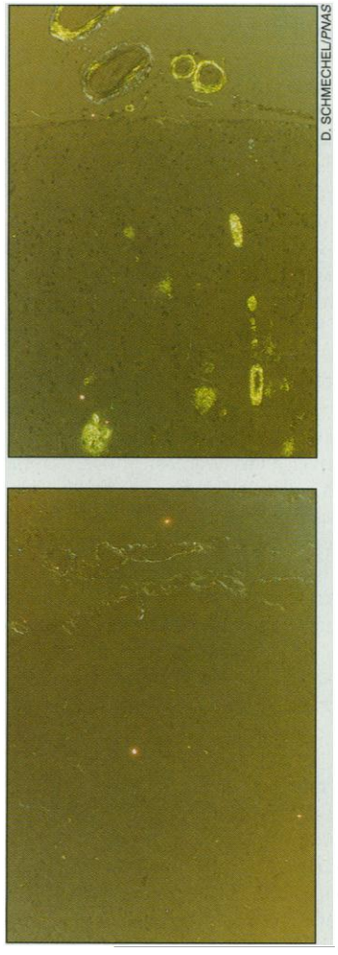
Genetics research convincingly shows that one form of a cholesterol-carrying protein is a major risk factor for late-onset Alzheimer's. Why?

In the winter of 1992, biochemists Warren Strittmatter and Guy Salvesen went fishing for proteins that might be involved in Alzheimer's disease. Working in Allen Roses' lab at Duke University Medical Center, they cast into collected cerebrospinal fluid an interesting hook, a peptide called β-amyloid, which some investigators think plays a key role in the dreaded neurological disorder. Among other proteins that bound to the peptide, the two scientists reeled in apolipoprotein E, a well-studied protein that ferries cholesterol through the blood stream.

It turned out to be a real catch, since ApoE fit neatly and unexpectedly with an ongoing effort at Duke to identify a gene implicated in Alzheimer's. Both the ApoE gene and the suspect region in Alzheimer's were in the same place on chromosome 19. This coincidence triggered an intense biochemical and epidemiological investigation over the past year or so, a search that has now culminated in identification of the first genetic risk factor for late-onset Alzheimer's, the form of the disease that begins after age 65 and makes up more than three-fourths of all cases. That risk factor is the gene for ApoE4, which codes for one of the three major versions of the protein found in humans.

In this issue of *Science*, the Duke group and their collaborators report on a study of ApoE genes in 234 people from 42 families afflicted with late-onset Alzheimer's. By age 80, almost all those in the study who had two copies of the ApoE4 gene developed the disease. Their overall risk was more than eight times greater than that of people with no copies of the gene. The ApoE4 story has electrified the Alzheimer's research community. "It's the most exciting thing that's happened in 6 to 8 years," says Alzheimer's expert Robert Katzman of the University of California, San Diego. Neurologist Richard Mayeux of Columbia University, who has already begun a large-scale prospective study to explore further what risk ApoE4 might confer upon various populations, agrees: "It's the first major breakthrough in approaching regular garden-variety Alzheimer's. It's an epidemiologist's dream."

Indeed, biotech firms and drug companies are already scurrying to visit Roses at Duke and learn whether treatments for the hitherto untreatable illness will arise from the discovery. One reason for the fervor is that before ApoE4, the only genes implicated in Alzheimer's development (the β-amyloid gene on chromosome 21 and another, not yet isolated, on chromosome 14) were linked to relatively rare inherited early-onset versions of the disease, which begin before age 60.



D. SCHMECHEL/*PNAS*

Alzheimer's disease allele. Patients with two ApoE4 alleles *(top)* appear to have more β-amyloid in their brain lesions *(yellow)* than do patients who have two ApoE3 alleles.

Though provocative, the research needs to be confirmed in larger populations, and there's a chance that ApoE4 isn't a cause of Alzheimer's, but merely a marker for a nearby gene that does play a direct role in the disease. Yet even that would be important and could not have been discovered if Roses' lab had not been perfectly positioned to understand what Strittmatter and Salvesen hooked in 1992. A year earlier, Duke genetic epidemiologist Margaret Pericak-Vance and her colleagues had presented a genetic linkage study of Alzheimer's families that tentatively implicated a region on the long arm of chromosome 19, and they were zeroing in even closer. But few outside of Duke had followed the lead, since Pericak-Vance had excluded unaffected family members from her analysis, statistically biasing the study towards the linkage. "We thought it was a flawed study," ruefully recalls University of South Florida geneticist John Hardy, who first identified a β-amyloid mutation on chromosome 21.

But after the binding studies fished out ApoE, the Duke group quickly recognized its importance. The pieces began to fall into place. "We immediately changed the entire focus of the lab," recalls Roses. The first thing they did was to examine brain tissue from Alzheimer's patients, and, confirming earlier results of Japanese researchers, they found that antibodies revealing ApoE's presence bound to the three lesions characteristic of the disease: extracellular senile plaques, vascular amyloid deposits, and tangles found within brain cells.

On the epidemiology front, the Duke team made some surprising observations of the frequency of the ApoE gene. Somewhat like blood-types, the specific composition of ApoE in the body is determined largely by the presence or absence of three major alleles (variants of a gene). The variants are known as E2, E3, and E4 and the apolipoproteins they produce differ only by one or two amino acids. Normally the ApoE3 allele is the foremost type: More than 90% of the general population inherit one copy, and around 60% inherit two copies. In 30 Alzheimer's patients randomly selected from different families prone to the disease, however, Duke geneticist Ann Saunders found a striking pattern. Among those people the E4 allele, not E3, was the common form, appearing in more than half the patients. These provocative results were first presented at a meeting last October and published this March in the *Proceedings of the National Academy of Science (PNAS)*.

A similar skew towards E4 shows up among the "sporadics" (patients with no known family history of Alzheimer's), who make up the largest group of people with the disease. In a paper appearing this week in *Neurology*, authored by Roses' team and researchers from Massachusetts General Hospital (MGH), the University of Toronto, and the State University of New York at Stony Brook, the researchers report on blood samples from nearly 500 individuals who are thought to be sporadic Alzheimer's cases. They find that 64% have at least one copy of the E4 allele, compared with just 31% in various control groups. Word on the scien-

tific grapevine is that the findings in both late-onset families and sporadics have been confirmed. "We and everyone in the world has tested it and it's a remarkably robust finding," says Hardy.

But the most dramatic evidence pointing to a role for E4 in Alzheimer's comes from additional genetic studies of families prone to late-onset Alzheimer's, which appear on page 921. People with two copies of the ApoE4 allele, compared to those who have none or one, are much more likely to get Alzheimer's and appear to develop it much earlier as well, report Pericak-Vance, her postdoc Elizabeth Corder, and Jonathan Haines at MGH's Molecular Neurogenetics Laboratory. Among people in late-onset Alzheimer's families with no copies of E4, just 20% had Alzheimer's disease by age 75. Of those with one copy, more than 45% of the people were affected; of those with two copies, the proportion rose to 90%. Furthermore, in an analysis of all the late-onset family members, average age of Alzheimer's onset went down as the gene dose went up, shifting from 84 to 75 to 68 as the dose went from zero to one to two.

DUKE UNIVERSITY MEDICAL CENTER

Gene hunters. The Duke team *(left to right)*: Warren Strittmatter, Allen Roses, Guy Salvesen, Ann Saunders, John Gilbert, Margaret Pericak-Vance, Mark Alberts, Elizabeth Corder, and Donald Schmechel.

Epidemiology like this is intriguing, but the epidemiologic data do not deal with the crucial issue of how ApoE4 actually brings on Alzheimer's disease—if it brings it on at all. Instead of answers, it prompts several questions. Why, for instance, do slightly different forms of this cholesterol-carrying protein dramatically change an individual's vulnerability to Alzheimer's? Since the protein and β-amyloid bind to each other, will ApoE4 support the controversial hypothesis that β-amyloid causes Alzheimer's by killing brain cells? Almost since the moment investigators discovered that β-amyloid was the major component of the senile plaques and vascular deposits typically found in the brains of Alzheimer's patients, the peptide has been a prime suspect. But could ApoE4 point to some new, as-yet-unknown mechanism?

If the epidemiology can't necessarily pin down a mechanism for the protein to do neurological damage, studies of the interaction between ApoE4 and β-amyloid just might and they're already under way. In a paper already accepted at *PNAS*, Strittmatter, Roses, and Karl Weisgraber, an ApoE expert at the University of California, San Francisco, show that ApoE4 binds much more rapidly and tightly to β-amyloid than does the more common protein, ApoE3. In a second paper also accepted at *PNAS*, Donald Schmechel, another Duke neurologist, finds that Alzheimer's victims who have two copies of the E4 allele have more β-amyloid in the plaques and vascular deposits in their brains than those with two copies of E3.

Exactly what these differences mean depends on what you make of β-amyloid. For those who believe the peptide is the cause of Alzheimer's, it could mean ApoE helps form β-amyloid into the plaques and vascular deposits seen in the disease. Last year, even before the E4 allele came to prominence, Blas Frangione and Thomas Wisniewski of New York University Medical Center had suggested something approaching that hypothesis. According to their theory, which dealt with all amyloids, ApoE could act as a "pathological chaperone" that binds to normally soluble β-amyloid peptide, making it insoluble and thus more likely to form plaques and other lesions.

Soluble β-amyloid has been found attached to another protein, apolipoprotein J. As a result, Frangione and others suggest that ApoJ, which may protect β-amyloid's solubility, and ApoE may be in a delicate balance within the brain. If ApoE levels increase, β-amyloid could be deposited in large amounts, forming the well-known plaques and vascular clumps of amyloid. And if ApoE4 is particularly adept at binding β-amyloid, as it seems to be, that could explain the increase in amyloid deposition and high risk of developing Alzheimer's disease that has been observed in people with two copies of that allele.

Roses, however, has doubts. His data may explain the observed amyloid deposits and their variability from patient to patient, he says, but "there's no solid evidence, to my mind, that β-amyloid is the magic bullet in Alzheimer's disease." There are certainly other fruitful areas to explore, he notes. ApoE also binds to the neurofibrillary tangles found in the brain tissue of patients with Alzheimer's disease, for instance, and the major protein found there is not β-amyloid but another one called tau. Though the notion has fallen out of favor lately, as the β-amyloid movement has gathered steam, many investigators have wondered whether the tangles are the culprits behind the brain cell loss seen in the disease.

Of course, there are still those who have doubts about the ApoE finding itself. "It's premature to say it's ApoE," says Rudolph Tanzi, director of MGH's Laboratory of Genetics and Aging. The real story, he says, could be an Alzheimer's-causing gene lying close enough to the ApoE locus that genetic epidemiology confuses the two; in that case, the ApoE gene would be a marker for the cause of the disease.

But skeptics like Tanzi seem to be in the minority, for now. Labs all over the world are gearing up to explore ApoE4's possible role in the disease. Epidemiologists, for instance, will be tracking thousands of individuals among different populations for years to see how E4 translates into a risk for Alzheimer's. Then there's the question of other Alzheimer's risk factors that might interact with the E4 allele or have been obscured by its influence. Columbia's Mayeux, for instance, hints that in a so-far unpublished pilot study "some very interesting ethnic variations have emerged."

As these studies proceed, and a simple diagnostic test to count the number of E4 alleles becomes available, physicians will face a difficult issue. If there were drugs that halted or slowed the Alzheimer's, knowing your risk could be a blessing. But since there is no effective treatment for Alzheimer's, many wonder whether such a test might do more harm than good, leading to unneeded and pointless anxiety or even discrimination by insurance companies and others. "That's a thorny question. I wish the wisdom of Solomon was granted to me and I could give you an easy answer," admits Zaven Kachaturian, director of Alzheimer's research at the National Institute on Aging (NIA).

Despite these worries, ApoE4 is clearly the biggest fish to be reeled in recently by the Alzheimer's research community. "Clearly this is something we will keep our eyes on and track. Even people who opposed Roses' ideas are now saying he has something. It's becoming a crescendo," says Kachaturian. And that crescendo may be a cue for many encores.

–John Travis