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More than 90% of Alzheimer's cases manifest in people over 65 years of age.

GENETICS

Finding risk factors

Uncovering genes that are linked with Alzheimer's disease can help researchers understand what causes the disease. But it's not easy.

BY MICHAEL EISENSTEIN

Headlines trumpet the discovery of genes associated with Alzheimer's disease so often that one might think the genetic foundations of the disease must surely be mapped out in their entirety. Certainly for those who develop the early onset, or familial, form of the disease in late middle age, the lion's share of the blame can be attributed to three genes: *APP, PSEN1* and *PSEN2*. Each of these genes plays a role in producing amyloid-β, the accumulation of which is widely thought to trigger the disorder's characteristic neurodegeneration.

However, more than 90% of Alzheimer's cases are of the late-onset form, which typically manifests in people older than 65 years and seems to have a separate pool of genetic risk factors. Efforts to identify factors directly involved in the processing and accumulation of amyloid-β have yielded at least a dozen candidate genes implicated in this form of the disease, but their roles are still unclear and their total contribution cannot account for the estimated 60–80% hereditary risk of late-onset disease[1].

One factor — a common variant of the gene encoding apolipoprotein E (ApoE) — has come to dominate the Alzheimer's landscape[2]. Just one copy of this variant, called *APOE4*, increases disease risk fourfold; two copies raise the risk about tenfold. "If you're going to try to predict who's going to get Alzheimer's, *APOE* is probably equivalent to the rest of the genes combined," says Gerard Schellenberg, director of the US-based Alzheimer's Disease Genetics Consortium.

Although *APOE* plays a leading role in the Alzheimer's story, it relies on a large supporting cast. Discovery of these other genetic players gained momentum with the rise of genome-wide association studies (GWAS). In this approach, researchers analyse millions of single nucleotide polymorphisms (SNPs) — variations scattered throughout the genome — in tens of thousands of affected and healthy individuals. By finding genomic changes that correlate with disease, they can uncover candidate genes or harmful mutations.

STATISTICAL POWER

Well over a dozen GWAS studies on Alzheimer's disease have been published, most of them from large consortia in Europe and the United States. Studies of this sort are often criticized for finding false positive associations, which cannot be replicated by other studies, and the early Alzheimer's studies were no exception. But later efforts analysed many more SNPs in the genomes of large populations of people with little overall genetic variability between them, increasing the statistical power and allowing scientists to identify variants in more than ten genes associated with increased risk[3–5].

At a 2009 meeting, for example, Philippe Amouyel, chair of the EU Joint Programming Initiative on Neurodegenerative Diseases, compared data with Cardiff University geneticist Julie Williams, a long-time colleague. "We had found exactly the same genes," Amouyel recalls. "This was really important because it reinforces the fact that these genes were not just appearing through statistical bias."

The results have been further bolstered by validation in independent study groups, as well as by meta-analyses, which collectively examine multiple studies and assess their statistical power. "When people criticize GWAS, the best answer is that when we do a large, completely independent study, we get the same result," says Schellenberg.

The candidate genes also make biological sense, as most are involved with the inflammatory damage and metabolic disruptions that scientists have long associated with the disease (see 'Genetic risk factors for Alzheimer's disease'). "It's an assortment of genes that seem to be associated with lipid metabolism and immune response," says Richard Mayeux, co-director of Columbia University's Taub Institute for Research on Alzheimer's Disease and the Aging Brain in New York. "This was sort of predictable, but we didn't have the data to support it until now." Importantly, many of the genes also interact with the amyloid-β pathway, which is still widely seen as the

initiating trigger for the disease (see 'Little proteins, big clues', page S12).

But these newly discovered genes do not resolve any debates about the origin of the disease — if anything, they potentially provide support for many different models of Alzheimer's pathogenesis. "Those who have been working on amyloid-independent pathways will say that genetics is proving it, while those working on amyloid will say, 'See, it's as we've said'," says Christine Van Broeckhoven, a molecular geneticist affiliated with Belgium's University of Antwerp.

DELIVERY TRUCK

Several of the candidate genes tie into multiple pathways, further complicating the picture. For example, clusterin (encoded by the *CLU* gene), which is one of the new risk factors most strongly associated with Alzheimer's disease, is thought to be involved in both amyloid-β aggregation and clearance. It is also known as apolipoprotein-J, and is best known for helping ApoE facilitate cholesterol trafficking in the central nervous system. Another risk factor, complement receptor 1 (CR1), is an important component of the innate immune response against infection, but is also linked to the clearance of circulating amyloid-β. But variants in genes such as *CLU* and *CR1* make relatively small contributions to the overall risk, increasing it by roughly 15%, so they have much less effect on the risk than *APOE*.

Exactly how ApoE might cause Alzheimer's disease is a matter of debate. As well as being the main transporter of cholesterol and other lipids and lipid-soluble molecules into the central nervous system, it is also thought to help remove amyloid-β from the brain, although the mechanism is not yet clear. There are three major variants of the gene for ApoE. The protein produced by the high-risk *APOE4* variant is the least stable, significantly impairing the movement of cholesterol and amyloid-β within the brain, whereas *APOE2* encodes a protein that is more abundant and actually confers protection against Alzheimer's disease relative to the common *APOE3* allele.

ApoE also modulates the inflammatory response to cellular damage in the brain, points out Thomas Montine, director of neuropathology at the University of Washington in Seattle. This reaction, mediated by the body's innate immune system, could be triggered by amyloid-β-induced cell death, but it might also be a response to other neurological trauma, such as stroke. In either case, a prolonged inflammatory response can result in the gradual build-up of toxic chemical by-products that further accelerate the death of neurons. Similar damage is seen in other neurodegenerative conditions, such as Parkinson's disease. "Almost all of the hypotheses are covered by *APOE4*," says Amouyel.

"We're now talking about seven strongly replicated genetic factors, all associated with lipid homeostasis."

Several researchers are convinced that ApoE's role in cholesterol transport is the key to its importance in Alzheimer's disease. "The brain has 25% of the body's cholesterol content, even though it only makes up 2% of the body weight," says Judes Poirier, a neurobiologist at McGill University in Montreal, Canada. The brain's capacity to rewire itself, a property known as plasticity, depends on the ability to build and stabilize new synaptic connections. This in turn requires cholesterol, and mice that lack ApoE or express the *APOE4* variant exhibit dramatic problems in the repair of synaptic damage. "ApoE is your ultimate delivery truck when you need lipids to maintain or restore neural plasticity," Poirier says.

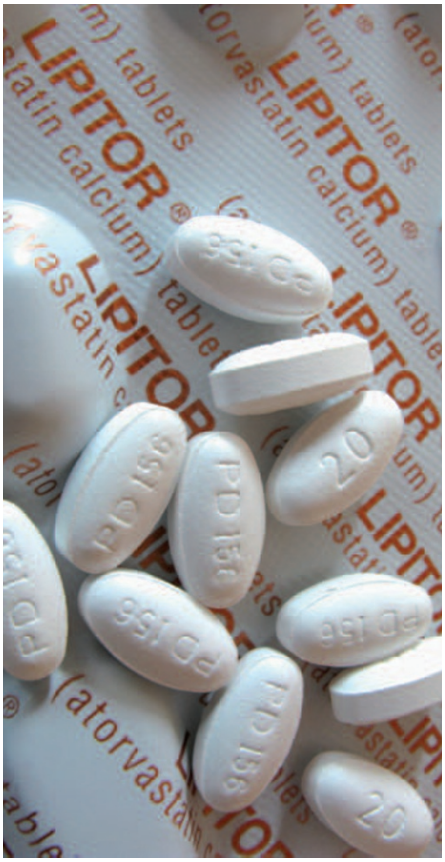
MULTIPLE ROLES

This central role for ApoE is supported by evidence that variants in several other cholesterol-linked genes also increase the risk of Alzheimer's disease. One such gene is *PICALM*, which encodes a protein that assists ApoE in lipid traffic; another is *ABCA7*, which is also involved in cholesterol transport. "We're now talking about six or seven new, strongly replicated genetic factors, all associated with lipid homeostasis in the brain," says Poirier.

ApoE also seems to be a bridge between Alzheimer's disease and other physiological disorders. "The associations with cardiovascular disease and diabetes are strong — you very seldom find a study that doesn't show this association," says Mayeux. "The problem is, a stroke alone or the presence of diabetes alone doesn't cause the disease." But those who carry *APOE4* and have diabetes are twice as likely as non-diabetics with this variant to eventually develop Alzheimer's disease[6].

Another piece of the *APOE4* puzzle is its link to a higher risk of heart attack and stroke. "That alone should be telling us that maybe its role here is actually lipid metabolism instead of some exotic amyloid-β-interacting scheme," says Schellenberg. Accordingly, there is some evidence that taking statins, which lower cholesterol levels, may delay or prevent the onset of the cognitive decline associated with Alzheimer's disease, although clinical trials of statin use have yielded inconclusive results.

The available data fail to tie these various threads together satisfactorily, but several ambitious projects that are underway might help. For example, four of the largest Alzheimer's GWAS groups have joined forces, forming a mega-consortium known as the International Genomics of Alzheimer's Project. The project will draw on data from a total of 40,000 people with Alzheimer's disease and unaffected controls, and will attempt a 'mega-meta-analysis', delving deeper in search of previously overlooked risk factors. "We're working with more than 10 million SNPs," says Amouyel. "That is very dense coverage of the genomic map."



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Genes regulating cholesterol are mutated in Alzheimer's; could statins be a treatment?

The project also aims to identify which pathological features relate to specific genes. But differences in sample collection and storage across different groups are likely to complicate that goal. Van Broeckhoven points out that for many GWAS cohorts, researchers do not have access to a detailed medical history or post-mortem tissue collected using standardized autopsy protocols. This led to a lot of valuable disease data being lost before the study even began. "Knowing what we know today, we have to say that we have missed lots of opportunities in our sampling procedures," says Van Broeckhoven.

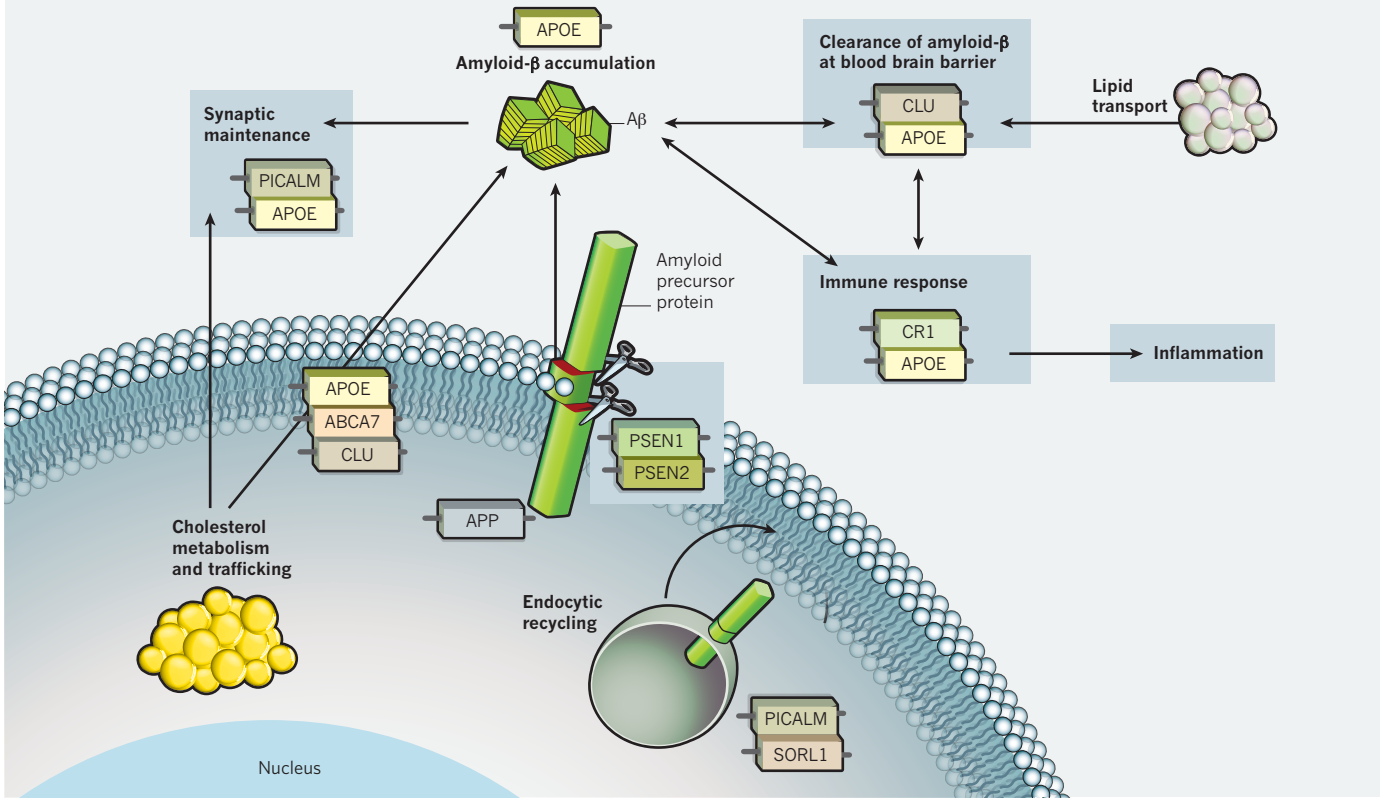
EXPLORING EXOMES

The GWAS studies are inherently limited by the distribution of known SNPs within the genome, leaving gaps that might conceal variants affecting the risk of disease. Because of the challenges of deriving statistically robust data for rare variants, these studies also typically ignore SNPs that are estimated to occur in less than 5% of the population.

However, the falling costs and increasing speed of DNA sequencing have made it easier

GENETIC RISK FACTORS FOR ALZHEIMER'S DISEASE

Several genes implicated in Alzheimer's pathogenesis are involved in multiple cellular pathways, which illustrates the complexity of the disease.



for scientists to comb through entire genomes, and Schellenberg and colleagues are planning to use this approach to fill in the blanks. To save time and money, his team plans to focus initially on the exome — the subset of the genome that contains all the genes that are expressed — in the search for causal mutations. "I'd rather have 2,000 exomes sequenced than 100 genomes," says Schellenberg, "because if you're looking for something rare you need to have a big sample."

Old-fashioned approaches to finding genes haven't died out either, and several researchers are continuing to examine factors that were identified based on a hypothetical association with Alzheimer's disease. For example, Mayeux's group has identified several disease-associated SNPs within the *SORL1* gene, which encodes a protein that participates in the cellular uptake of APP. "There were a lot of doubters because it was a candidate gene, but it holds up in the latest GWAS," says Mayeux. The role of *SORL1* is also supported by functional evidence: mice that produce lower levels of its protein accumulate more amyloid-β in the brain[7].

Montine's group identified another candidate while searching for physiological indicators in the blood or cerebrospinal fluid that might indicate the onset of Alzheimer's disease[8]. Brain-derived neurotrophic factor is linked to several other neurological conditions, and levels of this protein proved to be a powerful predictor of Alzheimer's disease. However, there is no clear evidence of a causative role for variations in this gene. "We looked and couldn't find an association, but we also haven't sequenced the whole gene yet," says Montine.

A LIFETIME OF DAMAGE

A final component of risk is likely to emerge from the interface between genetic predisposition and physiological insults accumulated over the course of a lifetime. "In a disease that's so strongly related to ageing, what we do and what we've been exposed to throughout our lives are likely to figure very importantly," says Montine.

For example, diabetes and stroke can lead to the production of highly reactive compounds known as free radicals, which induce toxic chemical modifications in fats, proteins and nucleic acids. This sort of oxidative stress seems to be a general feature in the brains of people with Alzheimer's disease, and could damage or kill neurons. "It's a normal component of ageing, but there's even more free-radical injury that occurs in people with Alzheimer's," says Montine. Mitochondria, the energy centres of the cell, normally keep oxidative stress in check, and several studies are underway to assess whether mitochondrial DNA also contains risk factors for Alzheimer's disease.

Attempts to understand the environmental aspect face the same problems that confront the geneticists: it is time consuming and expensive to acquire data, analyse it and then construct hypotheses that might prove meaningful for diagnosis, prognosis and treatment. "The genetics defines relevance but not mechanism," says Montine, "and now it's up to experimentalists to try to figure out how things work." ■

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