nature OUTLOOK

ALZHEIMER'S DISEASE

14 July 2011 / Vol 475 / Issue No. 7355

COVER ART: NIK SPENCER

Editorial
Herb Brody, Michelle Grayson, Apoorva Mandavilli, Tony Scully, Nick Haines

Art & Design
Alisdair MacDonald, Barbara Izdebska

Production
Karl Smart, Emilia Orviss, Leonora Dawson-Bowling, Stephen Russell

Sponsorship
Gerard Preston, David Bagshaw, Yvette Smith

Marketing
Elena Woodstock, Hannah Phipps

Project Managers
Helen Anthony, Claudia Deasy

Art Director
Kelly Buckheit Krause

Magazine Editor
Tim Appenzeller

Editor-in-Chief
Philip Campbell

Editorial advisor
Marie-Thérèse Heemels

The fog that envelops so many people as they age, severing them from their memories and thus from their identity, used to be considered a normal part of growing old — along with sore joints, needing reading glasses and losing touch with popular music. However, to anyone who has seen a loved one slip behind the heavy curtains of what we now call Alzheimer's disease, the decline seems anything but natural. What kind of massive malfunction in the brain can send an alert, robust, witty person into a state of persistent confusion?

The theory that plaques of amyloid-β in the brain trigger the disease has been called into question (page S12); Alzheimer's disease is a subtler foe. And without a handle on the disease's cause or genetic underpinnings (page S20), the developers of drugs (page S9) and vaccines (page S18) are working in a fog of their own. Moreover, Alzheimer's disease cannot be definitively diagnosed without an autopsy of the brain — at which point the information is rather academic, at least for that individual. So the search is intensifying for biomarkers — clues that indicate reliably whether a person who is still alive and healthy is destined for Alzheimer's disease (page S5).

The stakes are high. Alzheimer's disease is a drain not only on individuals and families, but also on societies, with the costs of care and lost productivity exceeding US$300 billion per year, which will only increase with rising incidence. More people than ever are making it to old age, but dementia is the reward for 6 out of every 100 individuals who get past 60 years (page S2).

We can take some encouragement from the findings that there may be non-medical steps that people can take to ward off the disease (page S16) — and that the prescribed activities, such as dancing and playing games, are pleasant enough in their own right.

We thank Eli Lilly and Company for the financial support that has made this *Outlook* possible. As always, *Nature* retains sole responsibility for all editorial content.

Herb Brody
Supplements Editor, Nature Outlook.

Nature Outlooks are sponsored supplements that aim to stimulate interest and debate around a subject of interest to the sponsor, while satisfying the editorial values of *Nature* and our readers' expectations. The boundaries of sponsor involvement are clearly delineated in the Nature Outlook Editorial guidelines available at http://www.nature.com/advertising/resources/pdf/outlook_guidelines.pdf

CITING THE OUTLOOK
Cite as a supplement to *Nature*, for example, Nature **Vol XXX**, No. XXXX Suppl, Sxx–Sxx (2011). To cite previously published articles from the collection, please use the original citation, which can be found at the start of each article.

VISIT THE OUTLOOK ONLINE
The *Nature Outlook Alzheimer's disease* supplement can be found at http://www.nature.com/nature/outlook/Alzheimers/

All featured articles will be freely available for 6 months.

SUBSCRIPTIONS AND CUSTOMER SERVICES
For UK/Europe (excluding Japan):Nature Publishing Group, Subscriptions, Brunel Road, Basingstoke, Hants, RG21 6XS, UK. Tel: +44 (0) 1256 329242. Subscriptions and customer services for Americas – including Canada, Latin America and the Caribbean: Nature Publishing Group, 75 Varick St, 9th floor, New York, NY 10013-1917, USA. Tel: +1 866 363 7860 (US/Canada) or +1 212 726 9223 (outside US/Canada). Japan/China/Korea:Nature Publishing Group — Asia-Pacific, Chiyoda Building 5-6th Floor, 2-37 Ichigaya Tamachi, Shinjuku-ku, Tokyo, 162-0843, Japan. Tel: +81 3 3267 8751.

CUSTOMER SERVICES
Feedback@nature.com
Copyright © 2011 Nature Publishing Group

CONTENTS

S2 DEMENTIA
A problem for our age
An ageing global population means dementia is a rapidly growing disease

S5 BIOMARKERS
Warning signs
New ways to track cognitive decline, from brain imaging to simple patient tests

S8 PERSPECTIVE
In search of biomarkers
Neil S. Buckholtz

S9 DRUGS
A tangled web of targets
Obscure pathology is keeping drug development in the dark

S12 AMYLOID
Little proteins, big clues
Fibril clumps and tau tangles are the usual suspects, but are they guilty?

S15 PERSPECTIVE
Prevention is better than cure
Sam Gandy

S16 PREVENTION
Activity is the best medicine
A healthy lifestyle might ward off the symptoms of Alzheimer's

S18 VACCINES
Chasing the dream
Setbacks and successes in the pursuit of disease-modifying treatments

S20 GENETICS
Finding risk factors
A diverse range of genes are implicated in the disease, complicating the picture

COLLECTION

S23 **Amyloid-β and tau — a toxic pas de deux in Alzheimer's disease**
L. M. Ittner & J. Götz

S29 **The ethics of informed consent in Alzheimer disease research**
Scott Y. H. Kim

S34 **Recollection of lost memories**
R. C. Malenka & R. Malinow

S35 **Gamma-secretase activating protein is a therapeutic target for Alzheimer's disease**
He et al.

S39 **Clinical trials of disease-modifying therapies for neurodegenerative diseases: the challenges and the future**
Anthony E. Lang

JUSTIN JIN/PANOS

Rising life expectancy in developing countries such as China will bring with it an increase in the number of people with dementias.

DEMENTIA

A problem for our age

As the number of Alzheimer's cases rises rapidly in an ageing global population, the need to understand this puzzling disease is growing.

BY ALISON ABBOTT

The world is getting richer. But wealth brings its own burdens. Prosperous people live longer and old age carries a high risk of dementia — a condition that is so far neither preventable nor curable.

In 2000, for example, 4.5% of the population of the United States was older than 65 years, and there were 411,000 new cases of Alzheimer's disease. Ten years on, those numbers had risen to 5.1% of the US population and 454,000 cases, according to the Alzheimer's Association in the United States.

This same trend is happening across the world. In fact, when Alzheimer's disease is conflated with other dementias with similar clinical profiles, it covers an estimated 35.6 million people — around 0.5% of the global population. And these figures are about to get worse: the number of people with dementia is set to double in the next 20 years, according to the *World Alzheimer Report 2010*, a global assessment of the economic impact of dementia.

Commissioned by Alzheimer's Disease International (ADI) — a federation of Alzheimer's associations around the world — the report gathered numbers on a range of Alzheimer's-like dementia. Dozens of teams are working to find ways to predict, prevent, diagnose and treat the condition, but so far their efforts have achieved only limited success. As a result, the economic costs of dementias will likely be crippling, the report says.

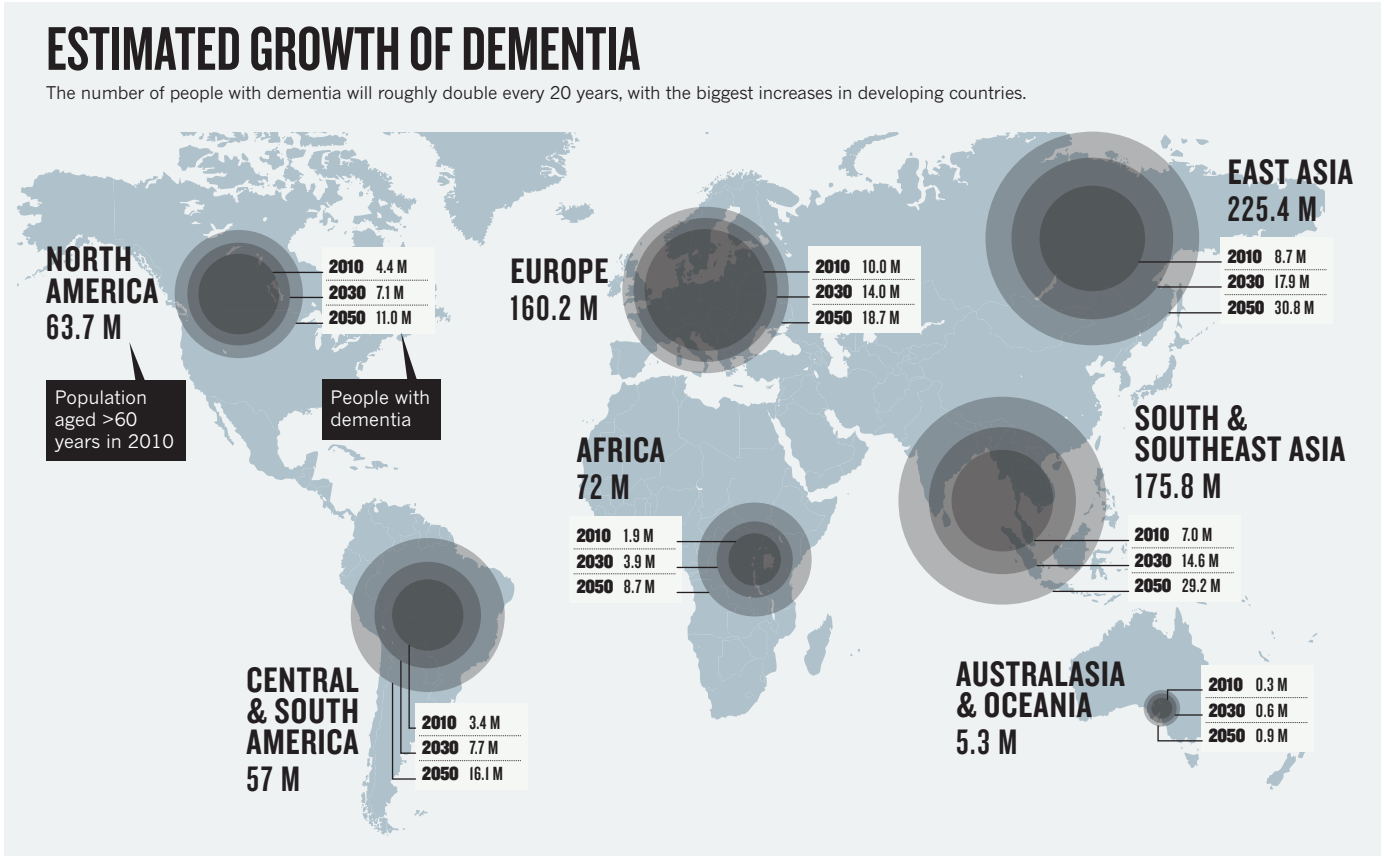
In 2010, the global economic impact of dementias was US$604 billion. This figure dwarfs the costs of cancer or heart disease. Based on demographics, the ADI report foresees an 85% increase in cost by 2030, with developing countries bearing an increasing share of the economic burden.

"We are seeing a linear increase in prevalence in rich countries, but an exponential increase in low-income countries," says report co-author Anders Wimo, an epidemiologist at the Karolinska Institute in Stockholm. "The need for solutions is urgent."

The ADI report used the best available data to determine the direct medical and social care costs, as well as the indirect costs, which mostly relate to family care and reduced productivity. Nearly 90% of the global costs in 2010, it says, are borne by rich countries — about 70% in Western Europe and North America — and less than 1% by low-income countries, where there is greater reliance on unpaid home care (see 'Global costs of dementia'). There is a fiftyfold difference in the cost of care per person between the richest countries and the poorest.

AGEING IN ASIA

Just under half of people with dementia live in high-income countries, 39% live in middle-income countries, and only 14% live in low-income countries, the report says. But these



proportions are forecast to change dramatically in the coming decades, particularly in rapidly developing countries such as China and India, for two important reasons.

The first reason is demographic. In compiling the ADI report, Wimo and co-author Martin Prince of the Institute of Psychiatry at King's College London reviewed the available epidemiological studies. They found that the prevalence of dementias in people aged over 60 is fairly uniform across the world — between 5% and 7%.

As living standards increase in countries such as India and China, this will lead to increased life expectancy. Given that the biggest risk factor for dementia is age, a longer-living global population means there will be more people with dementia. The report predicts that the number of people with dementia will roughly double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050 (see 'Estimated growth of dementia'). Most of this increase will be in developing countries.

Second, as wages rise, demand for more costly professional care will also increase — at least, that is what happened in wealthier countries where the Alzheimer's epidemic hit earlier. China has particular reason to worry: its one-child policy took effect in 1978, meaning that parents who reach old age in the next 20 years may not be able to rely on home care.

There are no comparable detailed global analyses for other chronic diseases. But *Dementia 2010*, a report commissioned by the UK Alzheimer's Research Trust, estimated that the annual national cost of dementias was £23 billion (US$38 billion), nearly twice that of cancer (£12 billion) and far more than the costs of heart disease (£8 billion) and stroke (£5 billion) (see 'Comparing costs').

The allocation of public research funds to these diseases does not reflect this hierarchy, however. In 2008, UK public spending on cancer research was 12 times higher than on dementia (see 'Comparing Investment'). In the United States, the National Institutes of Health spends 13 times more on cancer than on Alzheimer's-like dementias. "We can't fund all the good ideas we have in grant applications," says Neil Buckholtz, chief of dementia research at the US National Institute on Aging (NIA) in Bethesda, Maryland.

TACKLING THE DISEASE

As the scale of the threat looms large, some countries are launching programmes to tackle dementia on several fronts. For example, in 2009, Germany opened the German Centre for Neurodegenerative Diseases (DZNE) in Bonn at a cost of €66 million (US$95 million) per year. Developing treatment and preventive strategies will depend on clearly defining the disease and learning more about its clinical manifestations, says DZNE director Pierluigi Nicotera.

But these researchers will be aiming at a maddeningly elusive target. Fundamental questions about the disease — such as what its main cause is, and even what pathologies define it — remain unanswered (see 'Common types of dementia'). The label 'Alzheimer's disease' was not widely used to describe dementia until 1976, when Robert Butler, the founding director of the NIA, coined the term, partly to make it easier to attract research funds to study the condition. At the time, the syndrome wherein some elderly people became forgetful and child-like was known as senile dementia. This was not viewed as a disease that might be prevented or cured, but as an intrinsic part of getting old.

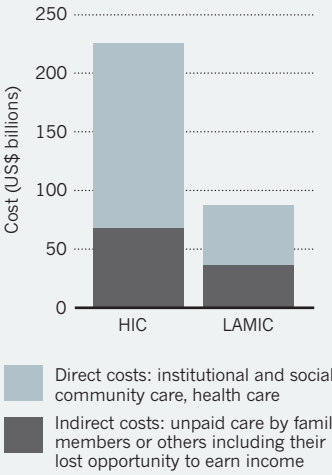
Alzheimer's disease is widely thought to be driven by amyloid pathology, in which peptides of amyloid-β are generated in the brain and clump together into plaques. The plaques release toxic fragments of amyloid-β, which wreak havoc by a mechanism that is not yet completely understood (see 'Little proteins, big clues', page S12).

Another form of dementia with similar symptoms is driven by vascular pathology. Leaking blood vessels deprive small areas of the brain of blood and oxygen, and these 'microstrokes' damage brain tissue and eventually result in cognitive defects. Scientists are still arguing about what proportions of dementias are driven by plaques and by vascular pathology. Post-mortem analyses of brains from people with dementia suggest that there is no simple answer: Alzheimer's-type pathology is more common, but it nearly always coexists with vascular pathology.

A 2011 investigation of more than 450 brains

GLOBAL COSTS OF DEMENTIA

There is a vast difference in the cost of care per person between high-income countries (HIC) and low- and middle-income countries (LAMIC).

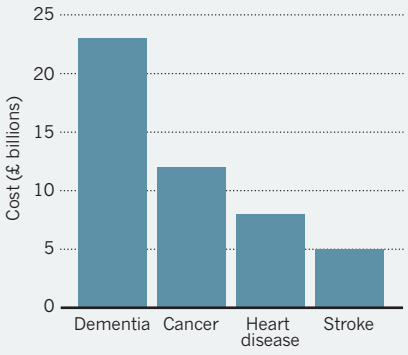


Direct costs: institutional and social/community care, health care

Indirect costs: unpaid care by family members or others including their lost opportunity to earn income

COMPARING COSTS

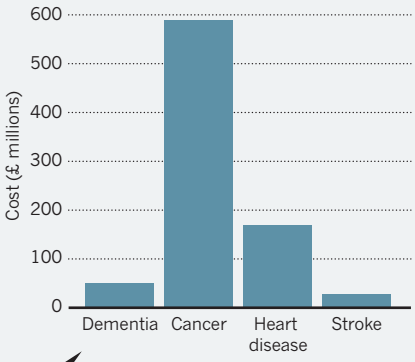
In the United Kingdom, the economic impact of dementias dwarfs the costs of other diseases.



Total costs, comprising direct and indirect costs

COMPARING INVESTMENT

In the United Kingdom, annual government and charity spend on dementia research is 12 times lower than on cancer research.



For every person in the UK with dementia just £61 is spent on research, compared to £295 for every person with cancer.

Sources: Alzheimer's Disease International / Alzheimer's Research Trust and Dementia 2010.

from the UK Cognitive Function and Ageing Studies identified vascular damage in four-fifths of brains from individuals with dementia, and found plaques in nearly all of them (Wharton, S. B. *et al. J. Alzheimer's Disease*, in press). Scientists suspect that vascular pathology usually accelerates the damage driven by amyloid pathology. But the same study found that three-quarters of brains from individuals without dementia also had vascular pathology, and some of those from the oldest individuals showed a significant burden of plaques.

AIMING AT AMYLOID

Amid this confusion, companies interested in developing therapies have primarily been targeting amyloid pathology, encouraged by the fact that the heritable, early onset form of Alzheimer's disease is mostly caused by mutations in the genes responsible for the production and metabolism of amyloid-β. These familial cases account for fewer than 5% of total dementia (see 'Finding risk factors', page S20), but the companies hope that a significant proportion of later-onset dementia will be, one way or another, driven by amyloid-β. "There is a level of wishful thinking in this," says Nicotera. But so far none of the amyloid-based strategies has been successful (see 'A tangled web of targets', page S9). Yet drug developers have not given up on the concept. More reliable biomarkers of Alzheimer's disease are being developed (see 'Warning signs', page S5), potentially making it possible to carry out trials on patients before symptoms, and irreversible damage, set in.

Some scientists are also wondering whether it might be valuable to target vascular pathology as well. In fact, drugs such as statins, which lower cholesterol levels in the blood, and drugs to reduce blood pressure are now routinely given long term to patients at high risk of heart attack or stroke. If vascular pathology drives a significant proportion of dementias, those who have benefited from the long-term cardiovascular treatment introduced in the past two or three decades might be protected from dementias as well.

Few epidemiological surveys have so far backed this up, but the authors of the most rigorous survey to date, the Rotterdam Study, announced at the Alzheimer's Disease International conference in Toronto in March 2011 that they have observed a slowing in the number of people being diagnosed with dementia.

Launched in 1990, the Rotterdam Study is considered to be a model for epidemiology trials. Intended to pinpoint the factors that contribute to various diseases, including dementia, in the elderly, it has recruited nearly 15,000 middle-aged individuals from a local population in three cohorts — in 1990, 2000 and 2006 — and is following their progress. Preliminary results have shown a small decrease in the age-specific incidence of dementias, and fewer plaques and less vascular damage among undiagnosed individuals, says epidemiologist Monique Breteler, head of the neurological and imaging part of the survey.

If dementias were ever to come under control, other medical problems of the elderly would become more prominent, notes Rudi Westendorp, who studies healthy ageing at Leiden University Medical Centre in the Netherlands. Because people with dementia are either less aware of pain or are unable to express their distress, "painful illnesses like herpes zoster [shingles] are probably being masked by dementia", he says. "Sight and hearing fail distressingly when we get old — we need to invest more heavily in research aimed at circumventing this, like developing neural implants to bypass damaged retinas."

Westendorp is an optimist who believes that solutions will be found to these problems, including the dementias, in the foreseeable future if countries invest in research now. Most of the problems that come with old age, he says, will have a medical solution — so living to a grand old age need not carry such a social and economic burden. ■

Alison Abbott *is* Nature*'s senior European correspondent.*

COMMON TYPES OF DEMENTIA

There is a great deal of overlap between the symptoms of various dementias.

Dementia type	Symptoms	Neuropathology	Proportion of dementia cases
Alzheimer's disease	Impaired memory, depression, poor judgement and confusion	Amyloid plaques and neurofibrillary tangles	50–80%
Vascular dementia	Similar to Alzheimer's disease, but memory less affected	Decreased blood flow to the brain owing to a series of small strokes	20–30%
Frontotemporal dementia	Changes in personality and mood, and difficulties with language	Damage limited to frontal and temporal lobes	5–10%
Dementia with Lewy bodies	Similar to Alzheimer's disease, also hallucinations, tremors	Cortical Lewy bodies (of the protein α-synuclein) inside neurons	<5%

Source: Alzheimer's Association, 2010

BIOMARKERS

Warning signs

The hunt is on for biomarkers that signal the descent into Alzheimer's disease. One initiative is leading the pack.

ILLUSTRATION BY GRACIA LAM

BY RUTH WILLIAMS

Each week for the past six years, box after delivery box of blood, cerebrospinal fluid (CSF) and urine samples have arrived at a lab in the University of Pennsylvania in Philadelphia. Researchers there have documented, divided, labelled and stored the samples, row after row, in seven enormous freezers.

Some 14,000 samples have been divided into 160,000 tubes — and each one is precious. "We have back-up freezers and alarm systems in case of electrical failures," says John Trojanowski, director of the Alzheimer's Disease Center at the University of Pennsylvania.

There's good reason for these precautions. The specimens, accompanied by detailed medical histories, cognitive and clinical measures, and high-resolution brain images, are among the "most highly annotated biological samples in the entire history of Alzheimer's disease research" — at least, that's the claim of the Alzheimer's Disease Neuroimaging Initiative (ADNI). Trojanowski is co-leader of ADNI's biomarker division.

At the moment, definitive diagnosis of Alzheimer's disease requires post-mortem analysis of the brain. While someone is still alive, the best bet is to assess their behaviour and memory, and rule out other disorders. Doctors are desperate for a marker that can reliably tell them who will get Alzheimer's disease, and what stage of the disease someone is going through.

A marker like that would, of course, be useful in the clinic, but it would also help researchers test drugs designed to slow the decline. The prevailing hypothesis in Alzheimer's disease is that deposition of the amyloid-β protein leads to the formation of insoluble amyloid plaques between brain cells, and that these plaques are implicated in the dysfunction and death of brain cells (see 'Little proteins, big clues', page S12).

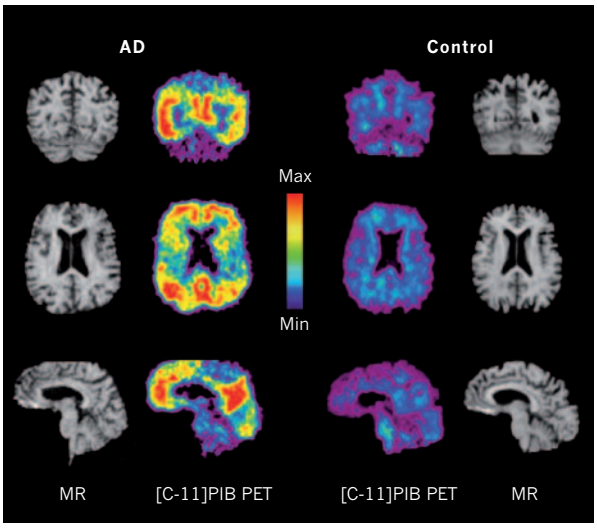
"Pharmaceutical companies were making drugs aimed at pulling the amyloid out, or reducing the amyloid, and they needed measures to monitor the effects of these treatments," says Michael Weiner, professor of medicine, radiology and psychiatry at the University of California, San Francisco, and ADNI's principal investigator. "Obviously, imaging and biomarkers were going to be important tools in all of this."

WORLDWIDE NETWORK

Launched in 2004, ADNI is one of the largest and longest-running studies of Alzheimer's disease[1]. Its goal is to find biological markers that can help determine how advanced someone's disease is and predict how well they will respond to treatment. The effort has already validated a few sensitive markers found by smaller studies.

This US$160-million project is funded jointly by the US National Institutes of Health (NIH), 20 of the biggest pharma-ceutical companies in the world, including Merck, AstraZeneca, Pfizer and GSK, and two non-profit partners, the Alzheimer's Association and the Alzheimer's Drug Discovery Foundation. "It is the largest public–private partnership that the NIH has," says Weiner.

So far, ADNI has recruited 1,000 volunteers at 59 centres across the United States and Canada. Collaborative centres have also been set up in Europe, Japan, Australia and elsewhere. "What we are trying to do is establish a worldwide network of sites that are all using similar methods and sharing data," says Weiner. "This makes it much easier to do international treatment trials and also allows us to look at differences between countries."



Pittsburgh compound B (PiB) lights up amyloid plaques in positron emission tomography (PET) images of the human brain.

UNIVERSITY OF PITTSBURGH, PENNSYLVANIA

ADNI is the best-funded effort in the hunt for Alzheimer's biomarkers, but it is by no means the only one (see 'Finding risk factors', page S20). Dozens of research teams are analysing brain images, DNA sequence variations and patterns in the expression of genes, proteins and immune molecules. In each case the aim is to identify measurable differences that either aid the diagnosis of Alzheimer's disease or reflect its progression.

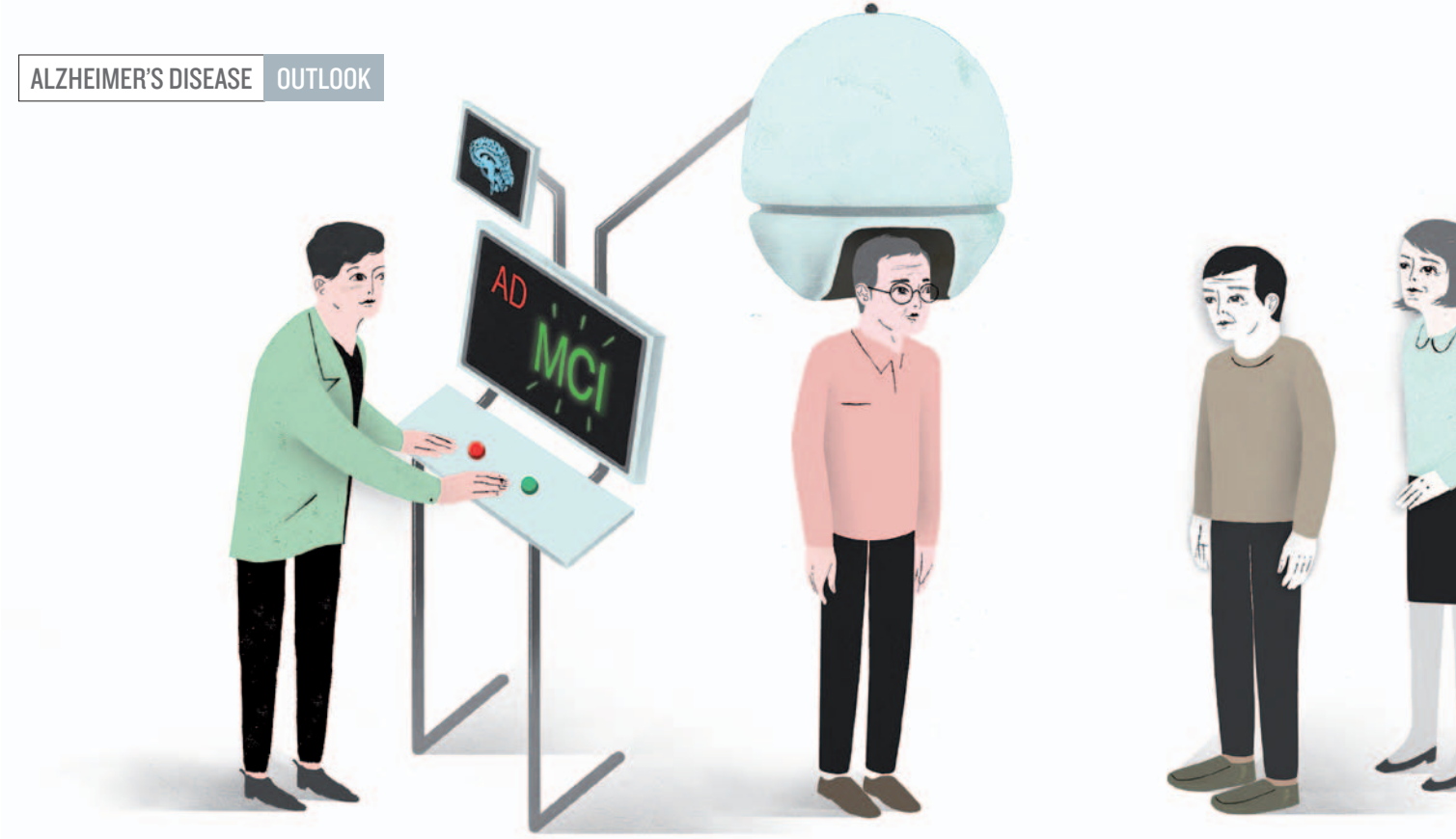
A CLEAR PICTURE

Weiner says he wanted to do a multi-site study to compare different brain imaging techniques, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), which could be used to detect changes in brain structure and metabolism associated with Alzheimer's disease. He approached several pharmaceutical companies, but the project was too expensive for any company to do it alone.

He then contacted Neil Buckholtz, chief of the Dementias of Aging Branch at the US National Institute on Aging (NIA). Buckholtz had been pondering a similar idea, so they began a series of discussions that led to the launch of ADNI a year later.

Of the 800 volunteers originally recruited, 200 had Alzheimer's disease, 400 had mild cognitive impairment (MCI) — a condition with high risk for progression to Alzheimer's disease — and 200 were healthy age-matched controls (including Weiner himself). After spending about a year standardizing operations and techniques, the team began using PET with a radioisotope of glucose known as FDG to measure brain metabolic activity, and using MRI to measure the volume of specific brain regions.

They also recorded levels in blood and CSF of various chemicals, including amyloid-β, tau protein, sulphatides (components of nerve cell membranes), isoprostanes (markers of oxidative stress) and homocysteine (an amino acid), all of which had been shown to be altered in Alzheimer's disease. "ADNI's main goal has been to validate discoveries that were made in other smaller studies," says Weiner, "and to show that these results really are replicable and clinically useful."



SENSITIVE MARKERS

As the data from these analyses emerged, some measures began to look quite promising while others fell by the wayside. The levels of sulphatides, isoprostanes and homocysteine in CSF, for example, turned out not to correlate to either Alzheimer's risk or disease progression. Another potential marker — the level of homocysteine in plasma — could help distinguish between MCI and healthy controls, but not between MCI and Alzheimer's disease.

Eventually, the researchers identified two sensitive biomarkers in CSF for detecting Alzheimer's disease, and for predicting the transition from MCI to Alzheimer's. One is the total level of tau protein; the other is the level of amyloid-β — a 42-amino-acid peptide cleaved from amyloid precursor protein. The best CSF marker for indicating functional decline in healthy controls turned out to be P-tau, which is tau protein with additional phosphate groups[2,3].

Imaging technologies are helping to identify changes in the brain that correlate with cognitive decline. MRI scans of people with advancing Alzheimer's disease reveal shrinkage of the temporal lobe and the hippocampus — the brain region used for storing memories and spatial navigation — and enlarged ventricles[2], the brain cavities that contain CSF. The FDG-PET studies show that cognitive decline is most closely associated with reduced brain metabolic activity.

Shortly after the launch of ADNI, researchers at the University of Pittsburgh, Pennsylvania, developed a new form of PET. Using a radiolabelled compound called Pittsburgh compound B (PiB), they generated scans that lit up amyloid plaques in the living human brain[4] (see image). ADNI quickly added this technique to its repertoire. In combination with CSF measurements, it confirmed that as levels of aggregated amyloid-β in the brain increase, soluble amyloid-β in the CSF diminishes. This not only established PiB–PET as a technique for detecting biomarkers but also further validated CSF amyloid-β measures as reliable markers of brain pathology.

"I think we are still a little premature to say that these are validated biomarkers of prediction and progression, but it certainly is moving in that direction," says Ronald Petersen of the Mayo Clinic in Rochester, Minnesota, who heads the ADNI clinical core.

Despite Petersen's cautious endorsement, pharmaceutical companies are already using ADNI's measures in clinical trials. Meanwhile, ADNI continues to validate biomarkers in centres across the globe.

EARLY STAGE

Kaj Blennow, professor of clinical neurochemistry at the Universtity of Gothenburg, Sweden, and a member of the European ADNI, says that even if the biomarkers are robust enough to use, there are no reliable drugs to test them. "We need biomarkers in drug development," he says. "But at the same time, we need to have an approved drug that affects [amyloid-β] pathology or neurodegeneration so that we can use the drug to validate the biomarkers."

ADNI may have started out with the aim of validating and standardizing biomarkers, but its scope has grown well beyond. "ADNI has clearly shown that Alzheimer's pathology in the brain exists in people long before they have dementia," says Weiner. The study has indicated that seemingly healthy people aged 70 years or above who have amyloid-β in their brains might have a higher risk of developing dementia.

Indeed, new NIH guidelines for diagnosing Alzheimer's disease have expanded the definition of the disease to include MCI and a presymptomatic phase[5]. The presence of amyloid-β at even this early stage could explain why trials of anti-amyloid-β vaccines (see 'Chasing the dream', page S18) have been unsuccessful. Blennow says that the trials were carried out on patients with disease that was too advanced. "Perhaps the drug is not that effective when you have so much pathology, so you need to go earlier."

Whatever the reason, more long-term studies are needed that follow healthy people until a subset of them develops symptoms of Alzheimer's disease. With NIH funding secured for another six years, this is exactly what ADNI plans to do. The team has already recruited 200 new participants with early MCI. So far, "they are falling between the normal controls and the late MCI subjects", says Petersen. "It really is lining up somewhat as we expected and hoped."

SIMPLE TEST

If pathology is present before subjects experience cognitive decline, then the logical next step would be the routine scanning of older adults to identify the telltale signs of the disease. But this is easier said than done. MRI is expensive and PET even more so and not readily available. "You only have PET

instruments in specialized large hospitals or research institutes," says Blennow.

The lumbar punctures used to obtain CSF may be routine, but they are still much more invasive than drawing blood and carry a small risk of infection and damage to the spinal cord. "We cannot puncture healthy people or MCI patients," says Christian Humpel, professor of psychiatry at Innsbruck University, Austria. "It's not ethical."

An ideal biomarker would show up in a simple blood test, and new markers that meet this criterion are regularly suggested. Candidates proposed in the past few years include clusterin, carbonyl proteins, angiotensin-converting enzyme, lipid peroxidation products and gene expression patterns. The ideal marker could be proposed next week, Humpel says, or it might not even exist. "We might have to use a combination of biomarkers."

Humpel says he has unpublished evidence of two potential biomarkers — an immune molecule and a tumour-suppressor protein — found in blood monocytes, a type of immune cell. If other groups replicate his findings, these markers might end up in clinical screens, he says.

"We need biomarkers in drug development and we need to have an approved drug to validate the biomarkers."

Like Humpel, Stanford University neurology professor Tony Wyss-Coray also thought that a combination of biomarkers might work best. In 2007, his team came up with a set of 18 plasma proteins that, measured together, differentiate people with Alzheimer's disease from healthy controls[6]. But even this approach did not lead to reproducible results.

"One reason you may not be able to reproduce a finding is because you use different tools," says Wyss-Coray. His team used antibody arrays, which can be highly variable in the way they recognize and bind proteins, he explains. If all researchers used exactly the same array kit and plasma preparation techniques, they should get the same results, he says. Unfortunately, the kit his team used is no longer available.

ANTIBODY APPROACH

This lack of reproducibility has sounded the death knell for many promising biomarker studies, and it underscores the importance of ADNI's efforts to standardize them. It also suggested to Thomas Kodadek, professor of chemistry and cancer biology at the Scripps Research Institute in Jupiter, Florida, that a different approach was required.

Instead of using an array of antibodies to look for proteins in the blood, Kodadek and his team decided to do the reverse: they are using an array of 15,000 synthetic proteins to look for antibodies in the blood. Antibodies are produced by the body's immune system in response to foreign — or, in some cases, the body's own — molecules, or antigens. "You are much better off trying to study antibodies rather than the antigens," says Kodadek. "The antibodies shouldn't be there at all in the absence of disease, but in the presence of disease they're going to be amplified millionfold."

His approach assumes that the pathology of Alzheimer's disease includes an immune response — an idea that is not generally shared among researchers. But his gamble seems to have paid off. His team has found two antibodies that are robustly expressed in 14 of 16 people with Alzheimer's disease and just 2 of 16 control subjects[7]. Because the controls were age-matched, the two with high antibody levels might have preclinical disease, Kodadek says, in much the same way that amyloid plaques emerge well before cognitive symptoms. He has extended his study to about 200 people. "The results are holding up quite beautifully," he says. "There are strong indications that our test is capable of picking up very early stage Alzheimer's."

Kodadek says he would like to test whether these antibodies are also amplified in blood samples from ADNI, and be able draw on all the associated imaging and other data. He's not alone. ADNI is bombarded by requests from researchers who would like access to the samples, but cannot honour them all. After all, 160,000 tubes may sound like a lot, but they would quickly dwindle if every new candidate biomarker were tested. ■

Ruth Williams *is a science writer based in Brooklyn, New York.*

1. Alzheimer's Disease Neuroimaging Initiative www.adni-info.org
2. Trojanowski, J. Q. *et al. Alzheim. Dement.* **6**, 230–238 (2010).
3. Shaw, L. M. *et al. Ann. Neurol.* **65**, 403–413 (2009).
4. Klunk, W. E. *et al. Ann. Neurol.* **55**, 306–319 (2004).
5. Jack, C. R. Jr *et al. Alzheim. Dement.* **7**, 257–262 (2011).
6. Ray, S. *et al. Nature Med.* **13**, 1359–1362 (2007).
7. Reddy, M. M. *et al. Cell* **144**, 132–142 (2011).

ILLUSTRATIONS BY GRACIA LAM

DRUGS

A tangled web of targets

Drugs in development for Alzheimer's disease take aim at a variety of neural mechanisms. But despite a wealth of possibilities, there have been few successes.

BY LAUREN GRAVITZ

For research into Alzheimer's disease, 2003 was a good year. The US Food and Drug Administration (FDA) had just approved memantine, the first in a class of drugs that reduces abnormal brain activity. Scientists had identified several potential targets, various academics and companies were developing therapies based on each, and the field seemed to be moving in the right direction.

Memantine is one of several drugs on the market in Europe and the United States that can slow the mental and physical decline of patients already in the throes of Alzheimer's disease. These drugs boost the activity of healthy neurons in the brain, masking the progression of dementia for a limited time (memantine, for example, seems to be effective for at least six months). But none of them can stop Alzheimer's disease in its tracks. So researchers began to shift their emphasis from treating symptoms to attacking the underlying cause of disease.

Eight years on, multiple therapies are in late-stage testing (see 'Selected drugs in clinical trials 2011'), including four that have the potential to modify the biological roots of Alzheimer's disease[1]. And yet, despite these seemingly imminent improvements in Alzheimer's therapeutics, a vague pall of scepticism hangs over the field. There is no clear evidence that these approaches will work, and many indications that they may not.

"Progress in the basic science of disease has been so substantial for the last few decades that many of us were quite optimistic," says Paul Aisen, director of the Alzheimer's Disease Cooperative Study and a researcher at the University of California, San Diego. But a sense of stasis has now set in. "We haven't had a new drug since 2003," says Aisen, "and the result of every major trial that's reported since then has been very disappointing."

A TWISTED TALE

A big part of the problem is that researchers don't know enough about the biology of Alzheimer's disease to identify the right targets. The disease is the result of a long chain of events, but some of the links in that chain are still a mystery — nobody is certain which link to cut to stop disease progression.

In a field with limited funding, the multitude of theories and possible targets has made for a difficult, albeit stimulating, challenge. "The therapeutic landscape for Alzheimer's disease is wide open — and it's wide open because we don't have a good definition of the disease, we don't have validated drug targets, and we have too many unvalidated ones," says Lon Schneider, a gerontologist and neurologist at the University of Southern California in Los Angeles.

But despite the wide variety of potential approaches, three of the four drugs in phase III trials share one main target: an improperly folded peptide called amyloid-β. In people with Alzheimer's disease, this protein fragment is sequestered into hard plaques nestled between neurons in the brain. Although few researchers doubt that amyloid-β is at least partly to blame for the disease (see 'Little proteins, big clues', page S12), many are beginning to wonder whether it is the right molecule to target.

Amyloid plaques are one of the hallmarks of Alzheimer's disease. Indeed, imaging studies have shown that plaques can start to accumulate 10–15 years before symptoms

emerge, prompting researchers to suggest that amyloid-β may be a good target for prevention (see 'Prevention is better than cure', page S15). Eliminating amyloid-β might not halt the disease, however. By the time Alzheimer's becomes symptomatic, attacking amyloid-β could have no perceptible effects.

So maybe a different type of drug is needed to halt or reverse cognitive decline. It might be better, some researchers suggest, to target another characteristic of the disease: the twisted clumps of fibrous protein inside neurons called neurofibrillary tangles. These are caused by the accumulation of a toxic form of the tau protein and correlate closely to the timing of symptom onset.

Other researchers champion wholly different approaches, ranging from brain surgery to repurposing drugs approved for a host of conditions including diabetes and arthritis. "This is a messy illness, and there are many, many ways of potentially cleaning up the mess," says Schneider. "That's what's so frustrating."

PREVENTING CLEAVAGE

The evidence pointing to amyloid-β as a cause of Alzheimer's disease seems overwhelming. Genetic studies reveal abnormal amyloid-β production in familial Alzheimer's disease, and cell-culture and animal studies implicate the misfolded protein in everything from neuronal death to behavioural and memory problems. For nearly two decades, most of the therapeutic research has focused on finding ways to reduce amyloid-β production and dissolve amyloid plaques in the brain.

But targeting amyloid-β is far from simple. "There's a slew of uncertainty about where in the disease course one would have to intervene" to target amyloid-β, says Jeffrey Cummings, director of the Cleveland Clinic's Lou Ruvo Center for Brain Health in Las Vegas, Nevada. "Its biology is very complex, the pathways for amyloid-β metabolism are multiple, and it may prove to be very difficult to work with."

Two different enzymes — γ- and β-secretase — cleave the amyloid precursor protein (APP) in two different spots, separating the short amyloid-β peptide from its progenitor. These peptides aggregate into small, stable clusters called oligomers, which then clump together to form larger plaques. Every step of the process, from the first snip to the final plaque, presents an opportunity to arrest the disease.

One approach involves γ- and β-secretase inhibitors. If γ- and β-secretase can be prevented from cleaving APP in the first place, there will be no amyloid-β. But targeting these enzymes has proven tricky, partly because γ-secretase is not specific to APP but also cleaves other proteins — including the vital protein Notch. One of the greatest disappointments in Alzheimer's therapeutics so far came last year when Eli Lilly, of Indianapolis, Indiana, abruptly halted a phase III trial of its γ-secretase inhibitor, semagacestat,

when an interim analysis revealed that the drug actually accelerated the progression of disease rather than slowing it down.

The reasons for this failure are still being investigated. "One possibility is that maybe anything you do that manipulates amyloid-β is bad for the brain," says Eric Seimers, the Eli Lilly senior medical director who oversaw the trial. "A more likely possibility, though, is that the worsening is not because we reduced amyloid-β, but because γ-secretase does something else that the brain needs." Several of the trial subjects also developed skin cancer, perhaps because of the drug's effects on Notch.

"Amyloid-β biology is very complex and it may prove to be very difficult to work with."

So what about β-secretase? It is more specific to APP than γ-secretase, and pharmaceutical companies are in dogged pursuit of drugs that inhibit it. But the enzyme's shape has turned out to be problematic. Researchers have had a difficult time creating a molecule that is large enough to inhibit the enzyme's active binding site but small enough to pass through the blood–brain barrier so it can be taken orally. Despite some early setbacks, many companies — Eli Lilly included — are continuing to target β-secretase, and a few compounds are in early stage trials.

AIMING AT AMYLOID

All eyes, however, are trained on a passive immune therapy that leaves both secretase enzymes alone and goes after amyloid-β directly. Two candidates, Eli Lilly's solanezumab and Janssen and Pfizer's bapineuzumab (originally developed by the Dublin-based company Elan), are monoclonal antibodies that work with the immune system, binding to amyloid-β and helping to clear accumulated amyloid-β peptides in the brain. Both are being tested in phase III trials on thousands of participants with mild-to-moderate Alzheimer's disease (see 'Chasing the dream', page S18).

"Probably every big company and even a number of smaller companies have products that will eliminate amyloid in an amyloid-producing mouse," says William Thies, chief medical and scientific officer at the Alzheimer's Association, a nonprofit organization in Chicago, Illinois, dedicated to patient care and research funding. "But they're not going to move them on to a phase III study until they see the results of the ongoing trials. They would like to be convinced that the amyloid hypothesis is correct."

There are some hints that it might not be — or at least that targeting amyloid-β will not work once symptoms are apparent. The 18-month phase II trial of bapineuzumab left many researchers feeling sceptical. Although imaging studies showed that the antibody decreased amyloid plaques in the brain[2], it seemed to have little, if any, effect on cognition. With vaccine studies yielding similar results, many are growing uneasy with the approach, and suggest that it might work only as a preventive measure and should be tested in people without symptoms[3]. Attacking amyloid plaques in symptomatic patients may be like cleaning up the mess inside a house after a flood: the structure remains, but all the personal effects are long gone.

Others say that plaques could be the body's way of sequestering the toxic amyloid-β oligomers. Elan, which led the field in immune approaches, has a candidate called *scyllo*-inositol. "It binds to amyloid-β at some intermediate structure, blocking its ability to form plaques and also blocking its ability to cause toxicity to neurons," says Dale Schenk, Elan's chief scientific officer.

But some worry that researchers are spending too much time and resources on something that might never pan out. "I think amyloid-β is proving to be a very intractable target," says Cummings. "The great danger to the field is that if bapineuzumab fails, some pharmaceutical companies will decide that Alzheimer's disease is too tough a target to yield stockholder value and will redirect their resources toward more tractable diseases."

TAUIST PHILOSOPHY

With the amyloid-β issue still unresolved, more researchers are looking to the second major target: tau protein.

Tau protein normally stabilizes structural elements, called microtubules, in healthy neurons. In Alzheimer's disease and other 'tau-opathies', however, tau acquires too many phosphate groups and becomes dysfunctional. It aggregates inside neurons, the microtubules collapse, and the resulting neurofibrillary tangles block neuronal signalling.

Neither amyloid plaques nor tau tangles are solely responsible for causing Alzheimer's disease, but of the two, tangles show a better correlation with clinical symptoms, says Peter Davies, director of Alzheimer's research at the Feinstein Institute for Medical Research in Manhasset, New York. "You can have a lot of amyloid in your

SELECTED DRUGS IN CLINICAL TRIALS 2011

Drug	Trial status	Mode of action	Developer
Bapineuzumab	Phase III, ongoing	Humanized monoclonal antibody to amyloid-β; targets the peptide's N-terminus	Pfizer/ Janssen
Solanezumab	Phase III, ongoing	Humanized monoclonal antibody to amyloid-β; targets the centre of the peptide	Eli Lilly
Intravenous immunoglobulin (IVIg)	Phase III, ongoing	Isolated from pooled human blood, believed to have anti-amyloid-β and anti-inflammatory properties	Baxter
Latrepirdine (Dimebon)	Phase III, ongoing	Thought to stabilize mitochrondria, thereby protecting neurons and preventing them from malfunctioning	Pfizer/ Medivation
Scyllo-inositol / ELND 005	Phase II completed, Phase III in planning	Prevents or inhibits amyloid-β aggregation	Elan
Methylthioninium chloride (Rember)	Phase II completed, Phase III in planning	Unclear; thought to inhibit tau aggregation, but may be acting as an anti-amyloid-β disaggregator	TauRx Pharmaceuticals
CERE-110	Phase II, ongoing	Adenovirus-aided delivery of a nerve growth factor gene that helps protect neurons; delivered via surgery	Ceregene
PBT2	Phase IIb in planning	Metal chelator, small molecule that inhibits tau hyperphosphorylation and amyloid-β aggregation	Prana Biotechnology
Davenutide/AL-108	Phase II completed	Microtubule stabilizer, preventing tau hyperphosphorylation and tangle formation	Allon
BMS-708163	Phase II, ongoing	Inhibits formation of γ-secretase, thereby inhibiting formation of amyloid-β	Bristol-Myers Squibb
PF-04494700/ TTP488	Phase II, ongoing	RAGE inhibitor, modulates glial activity and reduces amyloid-β plaque formation	Pfizer
Tideglusib/NP-12 (Nypta)	Phase II, ongoing	GSK-3 inhibitor, preventing tau hyperphosphorylation	Noscira

brain and be absolutely fine," he says. "If you have a lot of tau pathology, you're never fine."

Self-dubbed 'tauists', who believe that tau protein is the key to Alzheimer's disease, are studying whether interfering with the extra phosphate groups[4] or the enzyme that attaches them could slow or even reverse the symptoms of disease. "Until you can undo tau pathology and show that it undoes symptoms, you won't know for sure," Davies says.

Tau research has progressed more slowly than work on amyloid-β, partly because of scant funding and the overwhelming interest in amyloid-β, and partly because of tau's essential role in maintaining healthy cells. But a few groups have persisted, and at least one drug candidate has made it to phase II trials. In April, Madrid-based biopharmaceutical company Noscira began European efficacy trials on a compound that inhibits GSK-3, the enzyme that adds phosphate groups to tau. This is actually the second GSK-3 inhibitor to go to human testing — lithium inhibits the same enzyme, but small trials of lithium were inconclusive.

One of the most hyped therapies in the tau class is a repurposed drug. In 2008, researchers from TauRx Pharmaceuticals in Singapore made an announcement that sent small tremors of excitement through the field. They tested a modified version of methylene blue — an outdated treatment for malaria, urinary tract infections and bipolar disorder — in a dosing and efficacy trial of 321 people with mild-to-moderate Alzheimer's disease. After 84 weeks, the cognitive decline of those on the drug appeared to be 81% slower than those taking a placebo.

At the time, TauRx scientists claimed that their drug, which they call Rember, worked by preventing tau aggregation. But the company never published the data — the compound is old, with a proven safety record, so they had no regulatory incentive to do so. Their reported results still remain a mystery. An independent team of researchers found that, in animal models at least, methylene blue clears amyloid-β but has no effect on tau whatsoever[5]. Despite these contradictory interpretations, the company is seeking patents in both Europe and the United States, and announced in December that it plans to press ahead with a large-scale phase III trial.

SLOWING THE DECLINE

Alzheimer's disease is one of dying neurons — of incomplete circuits, of neural impulses left unfinished, of thoughts, memories and ideas that are lost with the dying nerves. Thus, other than those therapies that target amyloid-β or tau protein, most of the drugs in the pipeline aim to protect some of these neurons and to slow the course of disease, not reverse it.

Among the more radical approaches is CERE-110 from Ceregene in San Diego. The product delivers a gene for nerve growth factor (NGF) — a large protein that helps nurture neurons — where it is most needed. Because NGF cannot pass through the blood–brain barrier, and because the gene must be incorporated by the specific subset of neurons most affected by the disease, precise delivery requires brain surgery.

The seriousness of brain surgery cannot be overlooked, but this particular procedure takes just a few hours and no safety problems have been reported from more than a hundred operations done so far, says Mark Tuszynski of the University of California, San Diego, the researcher who devised the approach. "It won't be a cure," he says, "but the hope is that it can meaningfully enhance neurons enough to slow decline and have a useful impact on quality of life."

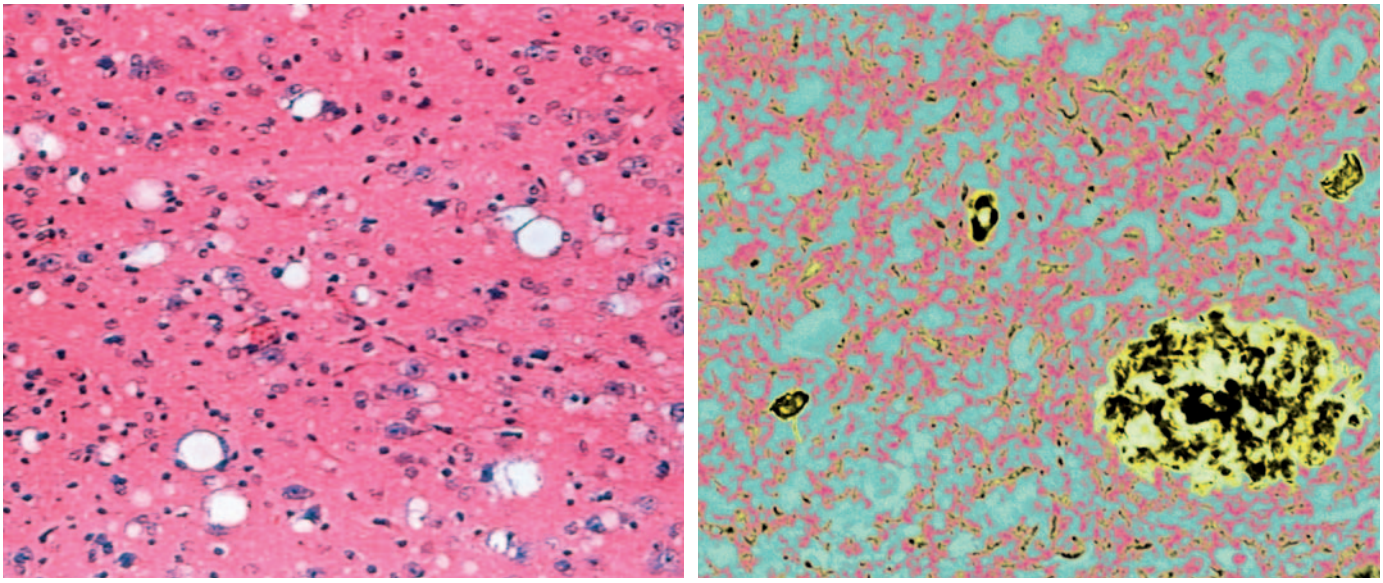
Dimebon, which has been used as an antihistamine in Russia since 1983, has also shown an ability to protect neurons. In 2008, a small phase II trial of dimebon in 155 people with mild-to-moderate Alzheimer's disease yielded surprisingly good results: those taking the drug appeared to improve in cognition and daily function for up to 12 months.

But the excitement over the drug's potential abated when a larger trial failed to elicit the same results. In 2010, the first large phase III trial of dimebon showed no significant difference between the test and control groups. A second phase III trial is underway. "Something like dimebon comes along, with no rational reason about why it works the way it's supposed to work, and people go gaga and suspend their ordinary scientific scepticism," says Schneider at the University of Southern California.

Alzheimer's disease hides its secrets well. So although researchers may disagree on the best approach for halting it, most agree that the current range of targets provides a good starting point. "There are lots of things in the pipeline — lots of different possibilities. And that's what we need at this time," says Thies of the Alzheimer's Association. "The reality is that nobody knows which approach will be best." ■

Lauren Gravitz *is a science writer based in Los Angeles, California.*

1. Mangialasche, F. *et al. Lancet Neurol.* **9**, 702–716 (2010).
2. Rinne, J. O. *et al. Lancet Neurol.* **9**, 363–372 (2010).
3. Golde, T. E. *et al. Neuron* **69**, 203–213 (2011).
4. Mazanetz1, M. P. & Fischer, P. M. *Nature Rev. Drug Discov.* **6**, 464–479 (2007).
5. Medina, D. X., Caccamo, A. & Oddo, S. *Brain Pathol.* **21**, 140–149 (2011).



SIMON FRASER/JAMES KING-HOLMES/SCIENCE PHOTO LIBRARY

At autopsy, the brains of Alzheimer's patients (right) are filled with amyloid plaques, reminiscent of the plaques seen in the brains of animals with scrapie (left).

AMYLOID

Little proteins, big clues

After a quarter of a century, the amyloid hypothesis for Alzheimer's disease is reconnecting to its roots in prion research.

BY JIM SCHNABEL

In September 1984, a group of prominent researchers from around the world met in Scotland to discuss a disease that afflicted sheep and goats.

Scrapie, as they called it, was important for more than agricultural reasons — it was also the most easily studied example of an emerging class of diseases that destroyed the brain. The illnesses jumped infectiously from animal to animal, yet yielded no trace of a virus or other microorganism. One big clue was that these diseases left behind insoluble clumps, or plaques, made from millions of tiny fibrils, each of which comprised hundreds or thousands of proteins. A striking new hypothesis proposed that these fibrils and their plaques marked the toxic passage of infectious proteinaceous particles, or prions.

On the first night of the conference, several researchers gathered for dinner. Among them were Colin Masters, a neuropathologist from the University of Western Australia, and Konrad Beyreuther, a protein-sequencing expert from the University of Cologne in Germany. Masters began telling Beyreuther about a human disease that featured plaques like those seen in scrapie and seemed to be very common. It was called Alzheimer's disease.

"Until then I had never heard of Alzheimer's disease," Beyreuther recalls.

It is easy to forget how recently Alzheimer's disease entered the public consciousness. For many decades after it first appeared in the medical literature, the term referred only to an obscure, early onset form of dementia. What we now know as common, late-onset Alzheimer's was then called 'senile dementia' — and it was so prevalent among the elderly that it hardly seemed worth classifying as a disease (see 'A problem for our age', page S2).

THE MYSTERY PROTEIN

It is also easy to forget that at the dawn of Alzheimer's research, the disease was suspected of being a prion disease — we tend to think of the connection between prions and Alzheimer's as being much more recent. In late 2010, for example, a team led by Mathias Jucker at the University of Tübingen, Germany, reported that they could, in essence, transmit Alzheimer's-type brain pathology in a prion-like manner by injecting Alzheimer's brain matter into the bodies of mice (Eisele, Y. S. *et al. Science* **330**, 980–982, 2010). Such findings have contributed to a major rethink of the cause of Alzheimer's disease. But this rethink is partly a renaissance because, as the story of Masters and Beyreuther's early interest in Alzheimer's reminds us, the prion connection is not new. "It was there at the beginning," says Masters.

As Masters knew in 1984, autopsies of Alzheimer's patients revealed brain plaques resembling those seen in scrapie, often surrounded by dying neurons and their twisted axons and dendrites. When doused with Congo red, a standard pathology stain, and illuminated with polarized light, the Alzheimer's plaques — just like scrapie plaques — displayed an apple-green shimmer, a prismatic sign of the hydrogen bonds that held their fibrils tightly together. Protein aggregates that had this peculiar property were called amyloids.

Earlier that year George Glenner, an amyloid researcher at the University of California, San Diego, reported isolating a small protein from amyloid deposits in brain blood vessels in people with Alzheimer's disease. Was the protein embedded in Alzheimer's brain plaques the same as the one in Glenner's vascular deposits? Or was it more like the scrapie protein?

Masters and Beyreuther, at their dinner in Scotland, agreed to collaborate to find out, and their partnership probably did more than any other to launch modern Alzheimer's research and its central idea: the amyloid hypothesis.

Masters had already painstakingly purified a quantity of Alzheimer's amyloid, in a process akin to bomb-grade uranium enrichment. When a sample arrived in Germany, Beyreuther and his colleagues broke it down with formic acid and sifted through

➲ NATURE.COM
research the role of amyloid-β in Alzheimer's disease
go.nature.com/qpcyez

EYE OF SCIENCE/SCIENCE PHOTO LIBRARY

the debris to find the smallest stable protein. This turned out to be a tiny peptide of roughly 40 amino acids, and Masters and Beyreuther called it A4. Sequencing A4 showed that it was not the scrapie protein, or indeed anything like it, but was essentially the protein Glenner had isolated from blood vessels.

Beyreuther's team quickly determined that A4 is a fragment of a much larger neuronal protein, amyloid precursor protein (APP). They found the gene that encodes APP on chromosome 21. This was a big clue, as people with Down's syndrome, who have an extra copy of chromosome 21, were known to develop Alzheimer's-like brain plaques by 40 years of age. The overproduction of APP and A4 was now revealed as the likely reason for the plaques in Down's syndrome — and probably in Alzheimer's disease too.

TOO MUCH AGGREGATION

Other Alzheimer's investigators readily pursued the APP lead. But three other important clues from this initial burst of research by Beyreuther and Masters would be almost entirely overlooked for most of the next decade.

The first was an observation by Beyreuther about the forms of A4 in different solvent mixes. He noted the presence of stable clusters, or oligomers, made of two, four or more copies of A4. So strong was the peptide's tendency to form these oligomers that in certain solutions, dimers made of two copies of A4 were more prevalent than monomers.

The second clue was that full-length A4 is extremely prone to aggregate. After obtaining the full A4 sequence, Beyreuther began to synthesize various lengths of it in his lab, including a series that started at the 42nd (and terminal) amino acid of its longest variant and worked towards the opposite end. "When we came close to the end of the peptide and took it off the resin, we saw it getting aggregated," he remembers. "I thought '*Mein Gott*, it's snowing!' It was aggregating so quickly. It was horrible."

The third clue came after Beyreuther and Masters raised the first antibodies to A4 and used them to detect amyloid deposits with unprecedented sensitivity in autopsied brains. The deposits were much more extensive than anyone had realized and were almost always present in people older than 80 years of age. In younger brains the plaques tended to be sparser and more diffuse, but they were still detectable in about 20% of cognitively normal people who had died in their fifties. The implication was that Alzheimer's disease is almost inevitable, with plaques beginning to form in the brain three decades before symptoms develop. "I thought that was amazing," says Beyreuther.

THE AMYLOID HYPOTHESIS

By the end of the 1980s, Beyreuther and Masters had largely completed their discovery work on A4. Other scientists, mostly from the United States, took the lead on Alzheimer's research,

Like prions (above), amyloid-β might spread in an infectious manner within tissues.

and one of their first acts was to rename the A4 protein amyloid-β, where the β referred to the classic β-sheet molecular structure of amyloids. They also put much less emphasis on the original prion connection. "Some of these young guys who came after us didn't seem to know what a prion was," says Masters.

Even so, they seemed to move swiftly towards an understanding of how amyloid-β causes Alzheimer's disease. In the early and mid-1990s, *in-vitro* studies indicated that amyloid-β becomes toxic to neurons when it begins to aggregate. Genetic studies of families with early onset Alzheimer's disease detected mutations within the gene that encodes APP, and analysis of one of these mutant APP genes found that it causes a sevenfold overproduction of amyloid-β (see 'Finding risk factors', page S20). Transgenic mice that overproduced human APP and amyloid-β developed plaques resembling those seen in Alzheimer's disease, and their behaviour in standard tests suggested some cognitive deficits. The amyloid hypothesis seemed straightforward: when the amyloid-β concentration in the brain becomes too high, the protein aggregates into fibrils and plaques, and begins killing neurons.

It eventually became clear that the situation was not quite that simple. Further genetic studies showed that familial, early onset Alzheimer's is usually caused not by the overproduction of total amyloid-β, but by the relative overproduction of a less common variant of amyloid-β known as Aβ42, the full-length, 42-amino-acid variant whose extreme proneness to aggregation had so alarmed Beyreuther.

The Aβ42 findings were still consistent with the plaque hypothesis, particularly once researchers recognized in the mid-1990s that the variant in most plaques is Aβ42. The problem was that mouse models with an overdose of Aβ42 — like the first Alzheimer's mouse models that overexpressed APP — lacked the heavy neuronal losses and cognitive decay associated with the human disease. "These models have some cognitive decline, but it's not as much as a person with full-blown Alzheimer's disease, by any stretch," says Harvard neurologist Bruce Yankner, a long-time Alzheimer's researcher.

Some researchers suspected that mice, with their small brains and short lives, cannot accurately model such a slow-burning, big-brain disease. But another possibility, which gained currency in the late 1990s, is that amyloid-β plaques are not the real drivers of dementia. Autopsy studies showed, for example, that the progress of Alzheimer's dementia does not correlate well with the development of plaques. As Beyreuther and Masters had initially observed, the plaques become dense in the brain long before any signs of cognitive decline.

Unfortunately, the major pharmaceutical companies had already placed their bets on the amyloid-β plaque hypothesis, and numerous drug-development programs would go on to fail in clinical trials. But in the meantime, a small group of researchers had begun to develop a new hypothesis that encompassed Alzheimer's and a variety of other amyloid-forming diseases.

OLIGOMERS REVISITED

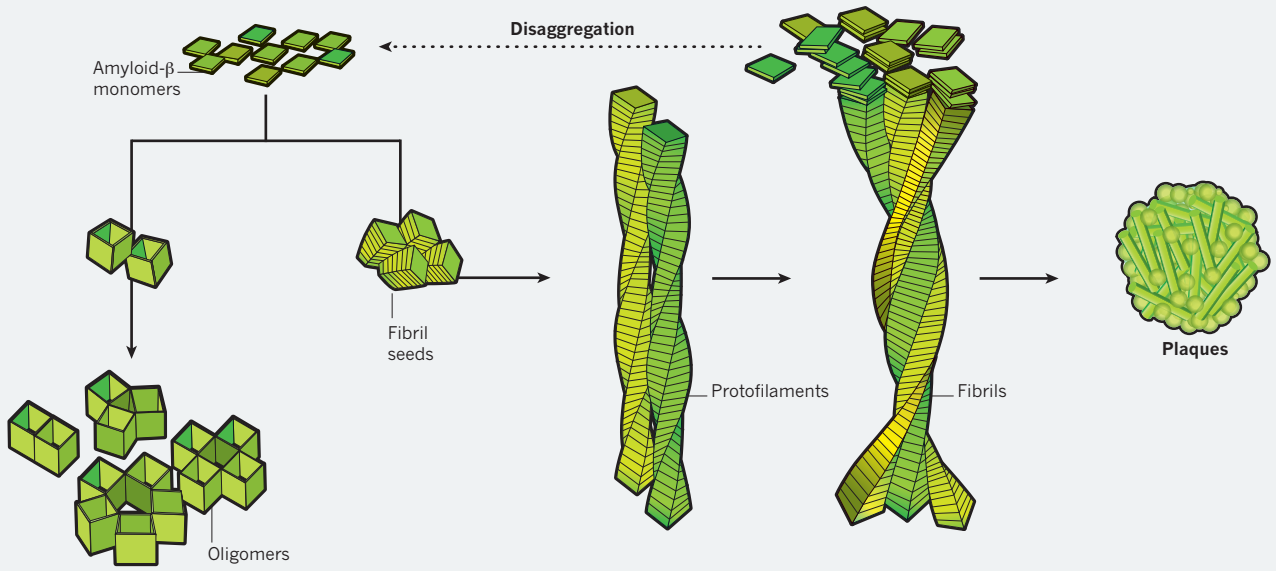
The genetic evidence made it almost certain that the aggregation of amyloid-β somehow leads to Alzheimer's disease. The fibrils in plaques were the most obvious type of aggregate, and therefore the most obvious suspect. Only after the plaque hypothesis began to fail did researchers return to the other aggregates: the amyloid-β oligomers first seen by Beyreuther and his colleagues in Cologne.

In the early and mid-1990s, Charles Glabe at the University of California, Irvine, and Dennis Selkoe at Harvard University reported finding oligomers in experiments with amyloid-β. They saw them as briefly existing intermediates on the way to disease-causing fibrils, rather than fully fledged drivers of disease. But in 1998, William Klein's lab at Northwestern University in Evanston, Illinois, reported that oligomers could be the true culprits in Alzheimer's disease. When Klein's team added a chemical to a solution of amyloid-β to stop it forming fibrils, the amyloid-β instead formed oligomers, which then began to kill nearby neurons. At least some of this toxicity seemed to be the result of the oligomers weakening the synapses — the junctions between neurons — and impairing their ability to contribute to learning and memory (see 'Two pathways of aggregation'). Similar results soon followed from the Selkoe and Glabe labs, and in time mouse models also demonstrated oligomer toxicity.

In the 2000s, a new consensus began to emerge: that amyloid-β fibrils are weakly toxic on their own, that they seem to provoke harmful inflammation, and that they are prone, especially

TWO PATHWAYS OF AGGREGATION

Individual amyloid-β peptides, which are produced normally by neurons, can assemble in at least two ways. One pathway leads to insoluble, plaque-forming fibrils. The other pathway leads to soluble oligomers, which are small enough to enter synapses. These oligomers are suspected to be the main toxic species in Alzheimer's disease.



when their plaques become especially dense, to slough off soluble amyloid-β that can then reform into oligomers. But in this model, amyloid-β oligomers are the more worrisome neurotoxins. Indeed, the amyloid-β fibrils are probably protective to the extent that they trap aggregating amyloid-β in a less harmful form.

Amyloid-β oligomers are now thought to exert their harmful effects by binding directly to the membranes of neurons, or to specific receptors — the insulin and NMDA glutamate receptors are suspects — needed for neuronal signalling. But if amyloid-β oligomers were merely toxic to neurons, they might never overwhelm the clearance mechanisms of the brain and cause disease. To do that, they seem to need another deleterious property that is associated with prions: infectiousness.

PRIONS REVISITED

The idea that Alzheimer's might be a prion disease was first suggested in 1984 by the future Nobel laureate Stanley Prusiner of the University of California at San Francisco. His idea was widely dismissed after amyloid-β was found to be different from scrapie protein. But by the mid 2000s, it was clear that Prusiner had been essentially correct. Both amyloid-β and prion-disease proteins could fall into a state that was both toxic and self-replicating.

Prusiner, who was also at the dinner in Scotland with Masters and Beyreuther, was apparently wrong about the replication mechanism. He had initially proposed that an infectious prion is a protein monomer with a misfolded shape that can induce the same misfolding in normal versions of the protein.

But as the chemist Peter Lansbury, then at Massachusetts Institute of Technology, showed in a series of *in-vitro* experiments in the mid-1990s, the key self-replicator in prion diseases and Alzheimer's disease appears to be an oligomer, not a monomer. Once formed, the oligomer becomes a template, or 'seed', that attracts new monomers, and aggregation around that nucleus proceeds rapidly. "This is one of those nonlinear phenomena in which small changes can have big effects," says Lansbury, now chief scientist at Link Medicine, a biotechnology company in Cambridge, Massachusetts.

One type of nucleus would serve as a template for new oligomers. Another would seed ever-lengthening fibrils. Lansbury showed that this initial nucleation event happens faster with a particularly sticky stretch of amino acids found on both prion proteins and Aβ42. Adding this stretch from Aβ42, or even adding full-length Aβ42, can trigger the runaway aggregation of all the amyloid-β in the vicinity. Beyreuther's snow metaphor was apt: a similar nucleation phenomenon lies at the heart of ice and snow crystallization.

Alzheimer's disease is almost inevitable, with plaques beginning to form in the three decades before symptoms develop.

More recently, Jucker and others have shown that brain matter containing amyloid-β from Alzheimer's patients can nucleate plaques in mice. Amyloid-β is less hardy than prion proteins and so is much less likely to jump from one person to another, but it does seem to spread in an infection-like manner within tissues. "I was away from amyloid-β research for years, but I've become interested again since Jucker showed that the stuff is infectious," says Beyreuther, who is now at the University of Heidelberg.

Similar infectious properties have been observed for aggregates of tau protein, which appear in Alzheimer's-affected cells late in the disease, as well as for α-synuclein protein in Parkinson's disease. Researchers suspect that numerous other amyloid-linked diseases feature the same toxic, oligomeric mechanisms and involve a slow spread of pathology starting in the regions of the brain most vulnerable to the disorder. "We know, for example, that people who present with Parkinson's motor signs are almost always going to have Parkinson's dementia 20 years later," says Lansbury. In contrast, Alzheimer's disease affects memory and cognition quite early on.

In principle, according to Beyreuther, there could be protein structures in our food, air and water that get into the brain and promote disease-causing spirals of protein aggregation "like the little bit of dust that seeds the ice crystals in the windows", he says. "If that's true, then we are in trouble."■

Jim Schnabel *is a science writer based in Miami, Florida.*

ILLUSTRATION BY GRACIA LAM

PREVENTION

Activity is the best medicine

Can exercise, social interaction and the Mediterranean diet really help to keep the cognitive decline of Alzheimer's disease at bay?

BY SARAH DEWEERDT

Rhumba. Lindy hop. Cha-cha. Ballroom dancing may not be the first preventive treatment for Alzheimer's disease that springs to mind, but it is an ideal prescription for those concerned about their declining memory. In fact, says Perminder Sachdev, a neuropsychiatrist at the University of New South Wales in Sydney, Australia, dancing has a perfect blend of elements that help stave off dementia. "There's cognitive activity, there's also physical activity, and there's social interaction as well."

A healthy Mediterranean-style diet is also thought to be protective — so that dance class could be topped off with a big Greek salad and a glass of red wine.

Over the past decade, epidemiological studies have shown that exercise, intellectual activity, social relationships and a healthy diet all lead to a lower risk of dementia. Such findings have to be interpreted with caution, however, because many researchers are sceptical about the benefits, and because withdrawing from social relationships and other activities can be an early symptom of dementia, not just a risk factor for it.

➲ NATURE.COM
more research on the links between lifestyle and dementia
go.nature.com/ivcdp8

Even so, "we have enough suggestive observational data now from several studies" to conclude that lifestyle factors are important in Alzheimer's disease, much as they are in cardiovascular disease, says Ronald Petersen, director of the Alzheimer's Disease Research Center at the Mayo Clinic in Rochester, Minnesota.

FIT AND HEALTHY

The task now is to move from lifestyle factors to interventions — to find out how much exercise, what kind of intellectual activity and at what stage each could influence the course of the disease. "We need to do more [clinical trials] where we actually intervene" with cognitive activity, training programmes and exercise, and with an appropriate control group, Petersen says.

Some of these trials are already under way. For example, in the Fitness for the Aging Brain Study, researchers[1] in Australia recruited 170 people who were worried that their memory had deteriorated or who had mild cognitive impairment (MCI), a condition that is considered a precursor to Alzheimer's disease. The researchers assigned half of the participants to a six-month exercise programme, either walking or doing other aerobic exercise for 50 minutes, three times a week. The other half, in the control group, carried on with their usual level of activity.

After six months, those in the exercise group slightly improved their scores on the cognitive section of the Alzheimer's Disease Assessment Scale (ADAS-Cog), a series of short memory, language and reasoning tests, whereas control subjects declined at a rate consistent with normal ageing. What's more, the exercise had lasting effects, leading to better scores 12 months after the programme ended.

ADAS-Cog is commonly used in clinical trials of Alzheimer's disease drugs, so the researchers were able to compare the effects of exercise with those of drugs called acetylcholinesterase inhibitors, which reduce the breakdown of the neurotransmitter acetylcholine. For people with MCI, regular exercise "can help your brain more than taking the medication that is currently available for Alzheimer's disease", says one of the researchers, Nicola Lautenschlager, who studies geriatric psychiatry at the University of Melbourne.

PHYSICAL CHANGES

How does this connection between body and mind work? Studies in rodents have suggested at least two different mechanisms[2]. First, exercise increases the activity of an enzyme called neprilysin that metabolizes amyloid-β — the protein that makes up the characteristic plaques of Alzheimer's disease — and might

help clear it from the brain. Physical activity also turns on the production of brain chemicals such as nerve growth factors, which promote the formation of nerve cells and the connections between them. This process is thought to make the brain better able to cope despite the pathological changes of Alzheimer's disease.

In the past few years, the development of biomarkers (see 'Warning signs', page S5) that can indicate Alzheimer's-related brain changes in living people have allowed researchers to explore more fully the mechanisms of the mind–body connection. For example, one study this year of 120 sedentary but healthy older adults without any memory problems assigned half the participants to a 3-days-a-week programme of physical exercise[3]. After a year, researchers performed magnetic resonance imaging (MRI) on several brain areas, including the hippocampus, the brain structure responsible for memory formation.

In older adults, the hippocampus typically shrinks by 1–2% each year, and this is what happened in the control group. But in the exercise group, the volume of the hippocampus actually increased by 2%. "That's probably millions of cells," says research team member Kirk Erickson, a psychologist at the University of Pittsburgh, Pennsylvania. With one year of exercise, "we are in essence rolling back the clock by one to two years".

BRAIN TRAINING

Another report[4], also published this year, suggests that similar mechanisms are at work when people exercise their brains. Canadian researchers used functional MRI to analyse brain activity in 15 people with MCI. After a one-week programme designed to teach the participants new memory strategies, there was activation in several additional brain regions during memory tests, suggesting that intact areas of the brain were able to take over from damaged areas. The participants also scored better on the tests.

Many studies of cognitive stimulation and dementia make use of computer games designed to boost mental skills. Although such 'brain training' interventions do not generally make healthy people smarter, they produce positive results in people with Alzheimer's disease and related conditions. One 2006 trial funded by the US National Institutes of Health showed that brain training can counteract some of the cognitive decline expected with ageing[5]. In that study — known as Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE) — people over 65 years of age who did a five- to six-week brain training programme focusing on memory, reasoning or speed of processing skills were better at these skills than control participants even five years later.

Computerized brain training programs are popular among researchers because these interventions are controllable and predictable, especially compared with intellectual pursuits in the real world. But this doesn't mean that people need to play computer games to stay mentally agile, says Sachdev. Instead, he argues, people are likely to benefit from any intellectual pursuit that both requires effort ("something where you challenge your brain") and is enjoyable ("so that you can sustain it"). That could mean anything from taking up the clarinet to doing Sudoku puzzles.

FOOD FOR THOUGHT

Meanwhile, other lifestyle factors that can modify the risk of Alzheimer's disease are continuing to emerge through epidemiological research. These types of studies, involving observation of thousands of people and their habits, underpin our knowledge about the Mediterranean diet, which includes a relatively high consumption of fruits, vegetables, whole grains and olive oil, relatively low consumption of red meat and saturated fat, and a glass of red wine with dinner.

Eating these foods has already been shown to reduce the risk of cardiovascular disease, hypertension and diabetes. In the past few years, three independent epidemiological studies conducted in New York[6], Chicago[7] and Bordeaux, France[8], have shown that those who eat mostly Greek peasant food also stay the sharpest mentally. "There has been converging evidence that adherence to such a diet is related to lower risk of cognitive decline or Alzheimer's disease," says Nikolaos Scarmeas, a neurologist at Columbia University in New York.

A team of Columbia University researchers including Scarmeas asked 1,880 New Yorkers detailed questions about their eating habits, then studied them for an average of five-and-a-half years. They found that the people with the most Mediterranean diet have up to a 40% lower risk of developing Alzheimer's disease than those who eat less Mediterranean food[6]. Results like these are so promising that several groups around the world are planning randomized trials of the Mediterranean diet as a way of preventing Alzheimer's disease.

Evidence that social engagement helps to prevent dementia also comes primarily from observational studies. For example, among more than 6,000 people aged 65 or older in Chicago, those with the most extensive social networks and the highest levels of social engagement have the lowest rates of cognitive decline[9].

It can be difficult to measure people's level of social engagement and it is even harder to design randomized trials to investigate it. Disentangling the effects of social engagement from those of other lifestyle elements is far from straightforward. Still, social engagement is a form of intellectual engagement, argues Linda Teri, professor of psychosocial and community health at the University of Washington in Seattle. Teri has designed programmes to encourage physical activity and social connections in people with MCI and dementia. "When we are with other people, we are listening to the conversation, we're tracking ideas, we're forming our own ideas," she says. "We're actually engaging in quite a bit of cognitive skills."

So people who exercise in groups may benefit from both the social stimulation and the physical activity. For example, consider Erickson and colleagues' research into exercise and brain changes in healthy older people[3]. Instead of aerobic exercise, the control group met three times a week to do stretches. This did not increase the size of their hippocampus, but it did improve their scores on a simple computerized test of memory, similar to the improvements in the exercise group. Erickson suggests that this social stimulation benefits other parts of the brain that the study did not measure.

A LITTLE BIT BETTER

In some parts of the research community, the argument that lifestyle can help to delay Alzheimer's disease is a tough sell. Last year, the US National Institutes of Health organized a consensus panel on preventing Alzheimer's disease. It concluded that it is too soon to tell whether lifestyle changes — or any other prevention strategy — can affect the development or the course of Alzheimer's disease.

Even those who are more bullish about the evidence say that lifestyle changes are likely to have only a limited benefit. But because Alzheimer's disease develops late in life, even small changes in risk or slight delays in the development of symptoms could greatly reduce the burden of disease, as people would be more likely to die from other causes before becoming mentally impaired.

As Erickson says: "If we can at least prevent some of the normal age-related decline from happening, even if it doesn't eliminate the risk — if it just reduces the risk of developing Alzheimer's disease or makes the quality of life a little bit better — I think we've gone a long way." ■

Sarah DeWeerdt *is a science writer based in Seattle, Washington.*

1. Lautenschlager, N. T. *et al. J. Am. Med. Assoc.* **300**, 1027–1037 (2008).
2. Savica, R. & Petersen, R. C. *Psychiatr. Clin. N. Am.* **34**, 127–145 (2011).
3. Erickson, K. I. *et al. Proc. Natl Acad. Sci. USA* **108**, 3017–3022 (2011).
4. Belleville, S. *et al. Brain* **134**, 1623–1634 (2011).
5. Willis, S. L. *et al. J. Am. Med. Assoc.* **296**, 2805–2814 (2006).
6. Scarmeas, N. *et al. J. Am. Med. Assoc.* **302**, 627–637 (2009).
7. Tangney, C. C. *et al. Am. J. Clin. Nutr.* **93**, 601–607 (2011).
8. Féart, C. *et al. J. Am. Med. Assoc.* **302**, 638–648 (2009).
9. Barnes, L. L., Mendes de Leon, C. F., Wilson, R. S., Bienias, J. L. & Evans, D. A. *Neurology* **63**, 2322–2326 (2004).

VACCINES

Chasing the dream

After a decade of disappointments, hopes for a successful Alzheimer's vaccine that ameliorates symptoms and ultimately prevents the disease are rising again.

BY JIM SCHNABEL

Using the formidable powers of the immune system to attack one of the body's own proteins seems like a risky approach. But this is what nearly all vaccines, or immunotherapies, against Alzheimer's disease aim to do. Their target is amyloid-β, a tiny protein produced by neurons. Scientists do not know what function amyloid-β evolved to have in its ordinary, free-floating form. But they do know that it is unusually prone to sticking to copies of itself, and that this aggregation process seems to be the principal trigger for Alzheimer's disease.

The first vaccine against Alzheimer's disease — Dublin-based Elan Pharmaceuticals' AN-1792 — was based on a particularly aggregation-prone form of amyloid-β known as Aβ42. In mice that had Alzheimer's-like deposits, or 'plaques', of amyloid-β in their brains, it seemed enormously promising: it provoked a storm of anti-amyloid-β antibodies that dissolved the plaques in older mice and stopped plaques from forming in younger ones. But in humans, AN-1792 was a disaster. Elan halted its first large clinical trial in 2002, after patients developed meningoencephalitis, an inflammation of the brain and its membranes that was apparently caused by rogue immune cells[1].

Most subsequent efforts have fared little better. Milder, second-generation active vaccines against amyloid-β are still in clinical trials, but many researchers suspect that these will not be strong enough to provoke a sufficient antibody response in elderly patients with weak immune systems. Passive vaccine infusions of lab-grown anti-amyloid-β antibodies are meant to get round this problem, but they haven't performed well in clinical trials.

"We in the field have had to look back and say, what did we do wrong?" says Norman Relkin, a neurologist at Weill Cornell Medical College, part of Cornell University in New York.

But despite these disappointments, there are hints of clinical success from a surprising direction — one that could lead to a better understanding of Alzheimer's disease and to therapies and preventives that really work.

➲ NATURE.COM
vaccines against Alzheimer's disease
go.nature.com/wattos

The vaccine that has raised some researchers' hopes is a mix of antibodies pooled from donated human blood. Known as intravenous immunoglobulin (IVIg), it has long been marketed as a general booster for antibody-based immunity in people who lack it for genetic reasons, and as a moderator of some rare autoimmune conditions.

The idea of using IVIg to treat Alzheimer's disease occurred to Relkin and his colleague Marc Weksler after they found, in 2002, that people with Alzheimer's disease have lower levels of anti-amyloid-β antibodies in their blood than cognitively normal people of the same age. They decided to set up a small, 6-month study of IVIg in eight of Relkin's patients. "The concept simply was to give back these antibodies, since IVIg is derived from the plasma of young individuals who tend to have higher levels," Relkin says.

The results were surprisingly good: six patients improved their cognitive scores, and a seventh stabilized. In a larger trial of 24 patients, Relkin again found signs that IVIg was working: the eight-person placebo group worsened as expected, but nearly all the 16 treated patients improved moderately on both cognitive and quality-of-life measures over the first 6 months (ref. 2). Their improvements were roughly equivalent to turning back the clock by 6–18 months. What's more, they stayed at those levels for as long as the treatment continued — more than two years in some cases.

INJECTION OF REALISM

The results of small trials often fail to hold up in larger trials. But Relkin's results have inspired some optimism — and some off-label prescribing of IVIg for Alzheimer's disease — because the improved cognitive and behavioural scores were dose dependent and have been backed up by changes in biological markers, including lower levels of amyloid-β in cerebrospinal fluid and reduced brain shrinkage. In fact, Relkin says, brain shrinkage is "towards the normal range in individuals who got the best dose, which is a very provocative finding".

The US National Institute on Aging, along with Baxter BioScience of Deerfield, Illinois, one of several producers of IVIg, is sponsoring a follow-up trial in 400 individuals with Alzheimer's disease. The results could be ready by the end of 2012. If the trial is successful, it could lead to the first Alzheimer's therapy approved by the US Food and Drug Administration that modifies the disease, rather than just treats the symptoms.

But this would not be the end of the Alzheimer's story, merely the end of the beginning. IVIg has several shortcomings. First, those who seemed to benefit from treatment had only modest gains. "I have not seen anyone re-enrol in adult education classes," says Weksler. Second, there seems to be a limited window of time when the therapy is effective. In the small trials carried out so far, the patients who started IVIg treatment later in the disease course seemed more likely to keep worsening.

There are also problems of cost and availability. IVIg is infused at high doses every two weeks in these studies, and patients might need them for the rest of their lives, at a cost of thousands of US dollars per infusion. Worse still, the production capacity for blood products from human donors is limited, and demand for IVIg from Alzheimer's patients and their families would swiftly outstrip supply. "We need next-generation products that are easier to produce and are based on IVIg's mechanisms of action," says Relkin.

Unlike most other Alzheimer's vaccines, IVIg has several plausible mechanisms. Although some of its antibodies may keep aggregates of amyloid-β in check, others may counter brain inflammation and reduce aggregates of tau protein, which also contribute to dementia. "You're talking about a complex disease that has many different pathological processes occurring either sequentially or in parallel," says Relkin. "So IVIg in this respect is ideally suited."

By contrast, AN-1792 and other big pharma Alzheimer's vaccines have aimed squarely at amyloid-β in its natural, single-copy form, as well as in fibrils — the long, insoluble, plaque-making aggregates that show up prominently in the brain and cerebral blood vessels of Alzheimer's patients. The lack of success with these vaccines suggests that single-copy and fibril amyloid-β might not be the best targets in patients who already have dementia.

So far, for all these vaccines, there has been only one published efficacy study: a phase II trial of bapineuzumab, Elan's passive anti-amyloid-β antibody infusion. The beneficial effects of bapineuzumab seemed weak to non-existent and, even worse, at high doses it caused brain swelling and associated

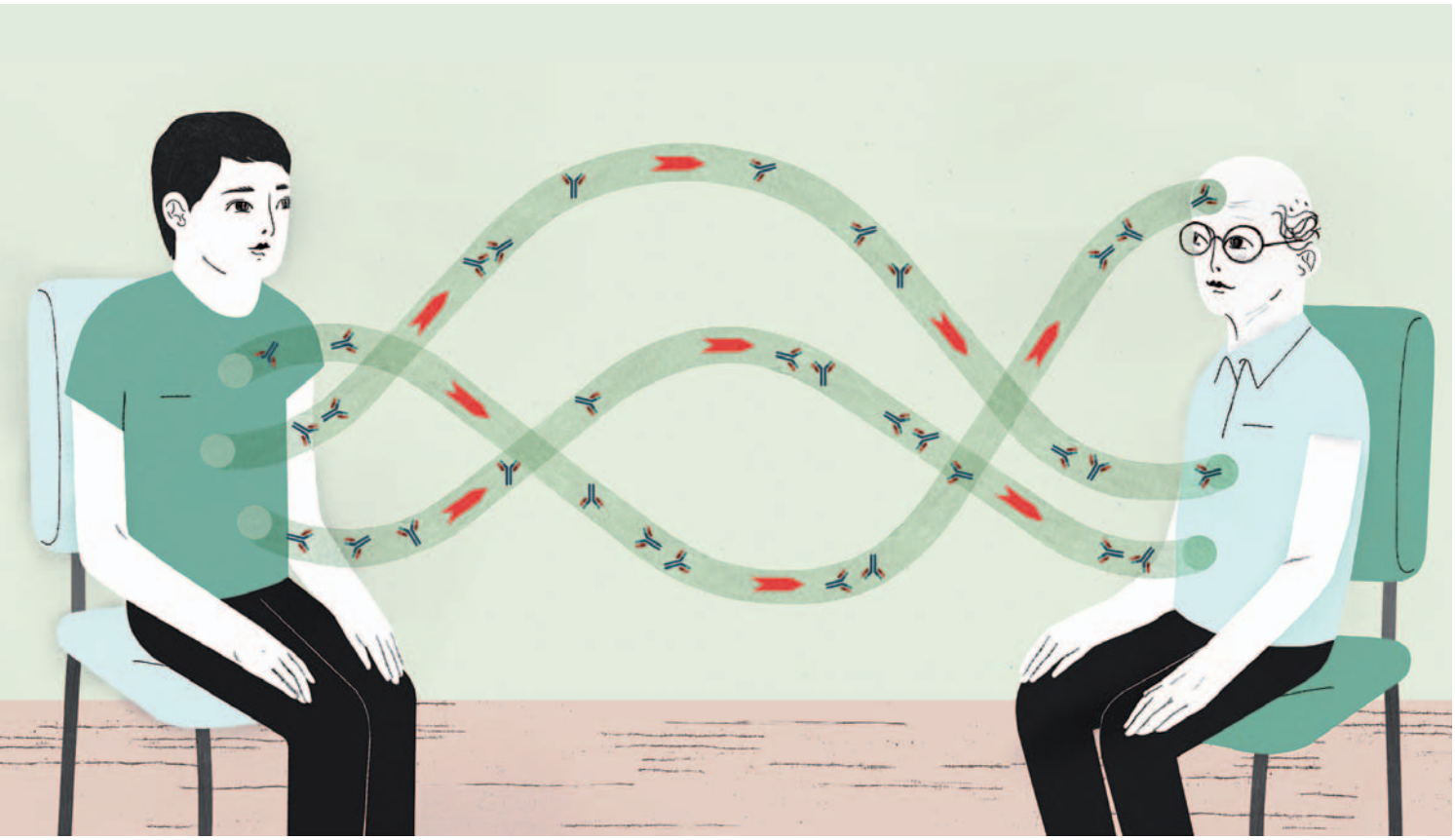


ILLUSTRATION BY GRACIA LAM

microbleeds in some patients with heavy vascular amyloid-β deposits[3]. Autopsy and brain imaging studies of selected bapineuzumab and AN-1792 recipients suggest that these vaccines can fail to slow the progress of dementia even when they succeed in reducing plaques of amyloid-β in the brain[4].

One reason for these disappointing results may be that the vaccines address only amyloid-β and do nothing to counteract brain inflammation or tau aggregates. Another possibility is that they are less effective at clearing the small, soluble clusters of amyloid-β known as oligomers, which are now seen as far more toxic than fibrils and which seem to promote the appearance of tau aggregates[5] (see 'Little proteins, big clues', page S12).

The short-term effects of IVIg could be due to its ability to clear amyloid-β oligomers, Relkin says. "Studies have suggested that you can reverse signs of memory impairment in mouse models within 24 hours of giving anti-oligomer antibodies," he says. "It's wonderful that we have a potential therapeutic as well as something that is directing us towards new avenues, new mechanisms, in studying the problem."

DREAM VACCINES

In the future, vaccines may also be used to treat people who have less advanced disease and so might get more benefit. "We're all moving towards the idea of treating patients with very mild dementia or even before they develop symptoms," says Dennis Selkoe, a neurologist at Harvard Medical School and long-time Alzheimer's researcher.

"The ultimate dream is to be able to give people a vaccine when they're still in their 20s or 30s, to prevent the disease process from even starting," says Cynthia Lemere, a Harvard neurobiologist who tests active anti-amyloid-β vaccines in monkeys.

Lemere, Selkoe and others believe that until dementia sets in, amyloid-β is the main driver of disease. Even the existing vaccine candidates might work well in this presymptomatic phase by keeping amyloid-β, in all its forms, within manageable levels.

Other researchers favour a universal Alzheimer's vaccine that leaves ordinary, single-copy amyloid-β alone and instead targets structures found only on amyloid-β aggregates, particularly oligomers and incipient fibrils. According to Relkin, the natural anti-amyloid-β antibodies found in IVIg seem to target these shapes, rather than single-copy amyloid-β.

"I see these as pathology-specific structures, so they're ideal targets," says Charles Glabe, an Alzheimer's vaccine researcher at the University of California, Irvine. "I think you'd have your best therapeutic effect this way, and the fewest side effects."

To elicit antibodies against these targets, Glabe and others have vaccinated animals with synthetic peptides that have the desired shapes but contain non-human amino-acid sequences, lowering the risk of autoimmune reactions. These vaccines reduce brain pathology and improve memory-related behaviours in mouse models of Alzheimer's disease, just as broader anti-amyloid-β vaccines do[6]. In principle, some of the aggregate-specific antibodies evoked by these vaccines would bind to aggregates of other disease-linked proteins, such as α-synuclein in Parkinson's disease or prion proteins in Creutzfeldt–Jakob disease (CJD), so the same approach could be used against all such diseases.

So far, none of these third-generation vaccines has had the corporate backing to reach clinical trials, but that could change quickly. "If one of the existing vaccines shows a strong effectiveness profile in clinical trials, then I think interest will go way up," says Glabe. He would particularly welcome a success for IVIg, because it is widely believed to work on the same principle as an oligomer vaccine. "But investors tend to lump all immunotherapies together," he says, "so they rise and fall together even though they may have very different targets." ■

Jim Schnabel *is a science writer based in Miami, Florida.*

1. Gilman, S. *et al. Neurology* **64**, 1553–1562 (2005).
2. Hughes, R. A. C. *et al. Clin. Exp. Immunol.* **158** (Suppl 1), 34–42 (2009).
3. Salloway, S. *et al. Neurology* **73**, 2061–2070 (2009).
4. Rinne, J. O. *et al. Lancet Neurol.* **9**, 363–372 (2010).
5. Jin, M. *et al. Proc. Natl Acad. Sci. USA* **108**, 5819–5824 (2011).
6. Goñi, F. *et al. PLoS One* **5**, e13391 (2010).

BSIP, MENDIL/SPL

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."

CAROL & MIKE WERNER, VISUALS UNLIMITED /SPL

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.

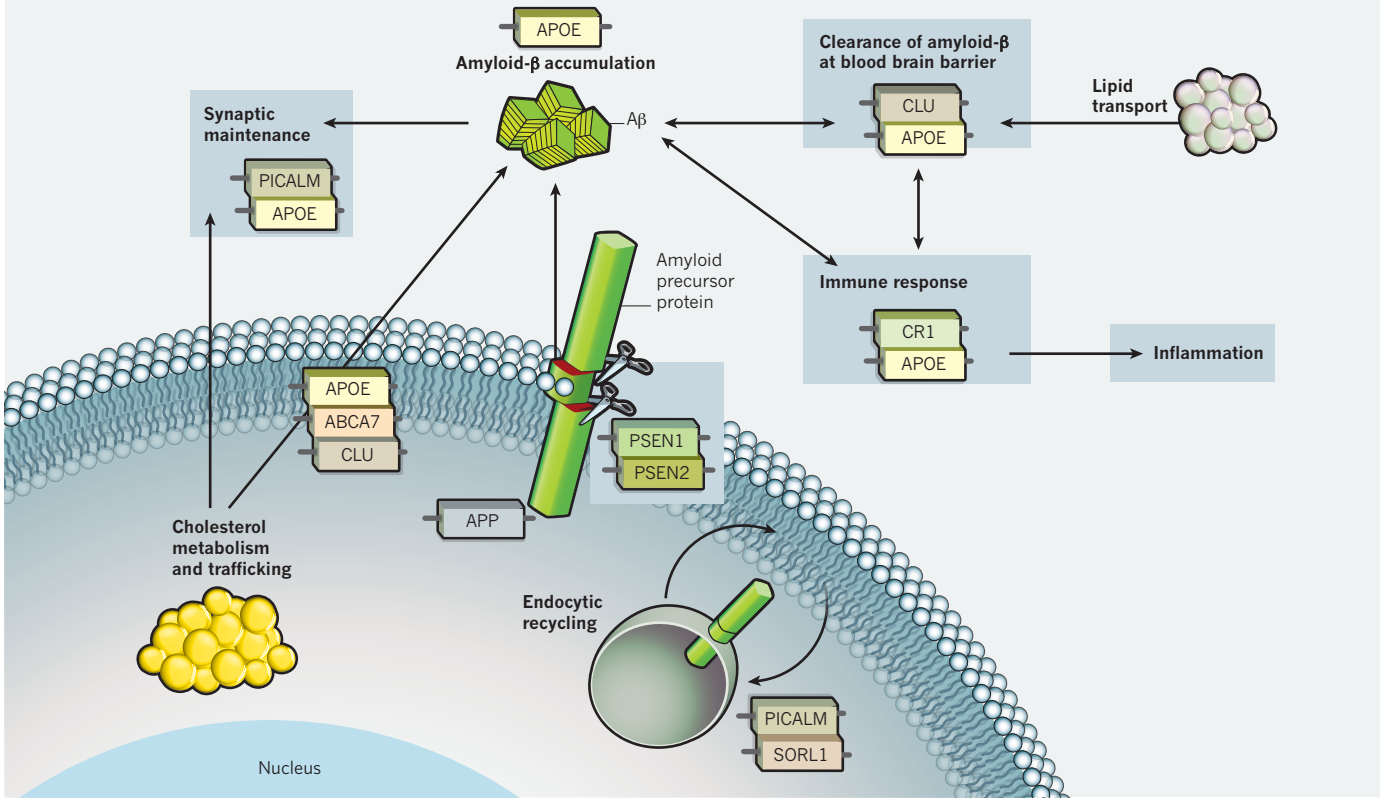
EXPLORING EXOMES

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." ■

Michael Eisenstein *is a science writer based in Philadelphia, Pennsylvania.*

1. Gatz, M. *et al. Arch. Gen. Psychiatry* **63**, 168–174 (2006).
2. Strittmatter, W. J. *et al. Proc. Natl Acad. Sci. USA* **90**, 1977–1981 (1993).
3. Bertram, L., Lill, C. M. & Tanzi, R. E. *Neuron* **68**, 270–281 (2010).
4. Hollingworth, P. *et al. Nature Genet.* **43**, 429–435 (2011).
5. Naj, A. C. *et al. Nature Genet.* **43**, 436–441 (2011).
6. Peila, R. *et al. Diabetes* **51**, 1256–1262 (2002).
7. Andersen, O. M. *et al. Proc. Natl Acad. Sci. USA* **102**, 13461–13466 (2005).
8. Zhang, J. *et al. Am. J. Clin. Pathol.* **129**, 526–529 (2008).