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).

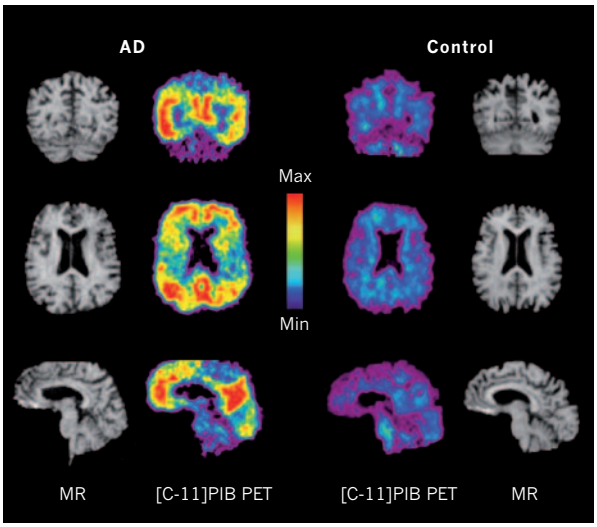
"Pharmaceutical companies were making drugs aimed at pulling the amyloid out, or reducing the amyloid, and they needed measures to monitor the effects of these treatments," says Michael Weiner, professor of medicine, radiology and psychiatry at the University of California, San Francisco, and ADNI's 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."



UNIVERSITY OF PITTSBURGH, PENNSYLVANIA

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

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.

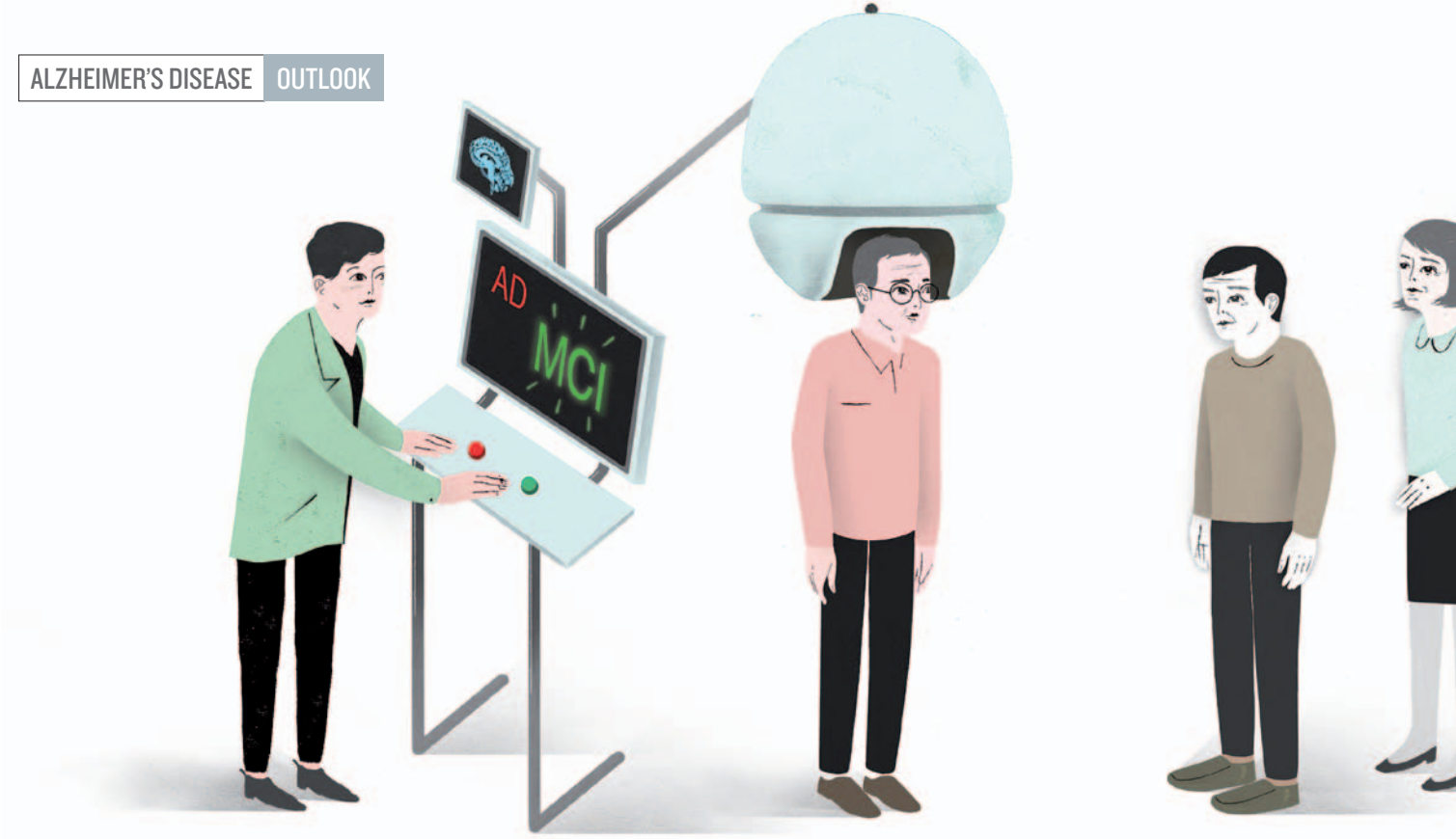
A CLEAR PICTURE

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.*

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7. Reddy, M. M. *et al. Cell* **144**, 132–142 (2011).