Tisianna Kamba and Bruce Lamb work with mouse models of Alzheimer's disease.

ANIMAL MODELS

A model challenge

Developing better models of Alzheimer's disease could be key to stemming the continued clinical failure of treatments.

BY ANTHONY KING

In the past three decades, scientists' knowledge of the biology that underlies Alzheimer's disease has advanced tremendously. In particular, the roles of aberrant accumulation in the brain of the peptide amyloid-β, which is derived from amyloid precursor protein (APP), and microtubule-associated protein tau are now much better understood (see page S2). But efforts to convert this insight into gains in the clinic have floundered. Although researchers have conducted more than 400 trials in people of potential treatments for Alzheimer's disease, almost no drugs have been brought to the market. And despite the blame being placed on a variety of factors, one of the main sources of researchers' concern is the animal models that are used in the initial stages of drug development.

The proposal that amyloid-β peptides aggregate into structures known as plaques, from which other features of Alzheimer's disease stem, became the leading hypothesis of the condition in the 1990s. Ever since, removing these peptide deposits has been viewed as a promising therapeutic strategy. However, no animal model of Alzheimer's disease was available in which such experiments could be performed. This is because researchers could find no evidence of the condition in organisms other than humans, which hampered the discovery and development of potential drugs.

Then, in 1995, researchers made a breakthrough — the creation of transgenic mice carrying a single gene mutation associated with the uncommon, inherited form of Alzheimer's disease, which is characterized by an early onset, at around the age of 45. Known as PD-APP mice, these animals express high levels of mutant APP in their brains and develop many hallmarks of Alzheimer's disease, including plaques and cognitive deficits. Researchers drew confidence from the structural similarity between the mouse plaques and those found in people. Subsequent models similarly focused on the expression of mutant APP. In 2006, several research groups generated mice containing multiple gene mutations associated with familial Alzheimer's disease. The mice accumulated amyloid-β deposits faster than did AD-APP mice, with plaques appearing in the brain after only around 2 months, compared with at least 6 months in the single-mutation mice.

Such models provided researchers with important insight into Alzheimer's disease.

They showed, for instance, that mutations associated with inherited Alzheimer's disease favour the production of a variant of amyloid-β called amyloid-β(1–42), which is two amino acids longer than the usual form and aggregates more readily. The models also served as crucial preclinical test beds for drugs. In 1999, an experimental vaccine was able to clear amyloid-β from the brains of PD-APP mice. Several monoclonal antibodies have since been shown to clear plaques in mouse models. But all have failed to provide benefit to people with Alzheimer's disease in trials. Even in cases in which the drugs cleared plaques from the brain, participants did not show improvements in memory.

"While mouse models have provided astounding new insights into disease mechanisms," says Bruce Lamb, a neuroscientist at Indiana University School of Medicine in Indianapolis, "they don't reflect the entire biology of the disease." Researchers are now seeking to create mouse models that better reflect the more common, sporadic form of Alzheimer's disease, as well as to develop alternative animal models.

STARTING SMALL

Alzheimer's disease is generally considered to be a disease of old age — unless a person has inherited a rare mutation, the average age of onset is around 80. But in many mouse models of the condition, plaques can appear in animals that are only a few months old. This difference provides a possible explanation for why drugs that successfully treat mice do not work in people. "A lot of the data in mice is from young animals, and their immune system is quite different from an aged individual," says Matthew Campbell, a geneticist at Trinity College Dublin, Ireland. The mouse models, therefore, might best reflect the early stages of Alzheimer's disease, including the period before a patient's diagnosis. If that were the case, clinical trials of drugs that target amyloid-β could be failing because people are treated too late to halt the death of brain cells. "Most of the animal work has seen mice treated at the beginning of plaque build-up, which is more a prevention than treatment paradigm," says Cynthia Lemere, a neuroscientist at Brigham and Women's Hospital in Boston, Massachusetts. Work to discover biomarkers that could help doctors to identify Alzheimer's disease in people before they show symptoms is now under way (S10).

If clearing amyloid-β from the brains of such individuals was shown to prevent them from developing the condition, the mouse models would be partially exonerated. But researchers know that there are areas in which the condition, as modelled in mice, deviates considerably from Alzheimer's disease in humans. The first mouse models were designed to overexpress proteins containing mutations that lead to greater aggregation of amyloid-β. These include APP and PSEN1, a subunit of an enzyme called γ-secretase that is involved in APP processing. However, although this strategy reliably produces cellular hallmarks that

mimic those of Alzheimer's disease in people, the mechanism by which they are created is not the same. "Humans do not overexpress APP or PSEN1," says Takaomi Saido, a neuroscientist at the RIKEN Centre for Brain Science in Saitama, Japan. The profusion of mutant protein generated by the overexpression throws grit into the metabolic cogs of the brain cells of mice, causing problems that are unrelated to Alzheimer's disease. "Many of these mice show cognitive defects before amyloid-β pathology starts, and many die early," Saido explains.

In 2014, he developed a mouse model that overproduces amyloid-β(1–42) without overexpressing APP. In these mice, mutant human APP is produced at physiologically normal levels, as well as in the expected regions of the brain. The animals show memory impairment that Saido thinks could reflect early cognitive decline in people. "It was hard to tease apart effects due to amyloid and effects due to the overexpression of the precursor proteins," says Lemere, of the first mouse models. Now, she and the members of more than 300 laboratories worldwide use Saido's mice.

But amyloid-β represents only part of the story of Alzheimer's disease. Most mouse models under development still struggle to replicate another important aspect of the disease: dysfunction of tau. In people, amyloid-β aggregation is followed by the formation of insoluble tau aggregates, known as neurofibrillary tangles, inside neurons. But PD-APP mice loaded with amyloid-β plaques do not develop tau tangles, and they show limited loss of neurons. In 2002, Michel Goedert, a neuroscientist at the University of Cambridge, UK, produced one of the first transgenic mouse models to generate tau tangles. The model, and others similar to it, have revealed much about tau tangles and how they propagate, says Goedert (S16). "In human disease, the tau pathology seems to become self-sustaining," he says. Saido and Lamb are now developing mouse models that produce humanized tau. Mice that express physiological levels of both human APP and human tau would be ideal for testing potential drugs, says Lemere.

Unfortunately, no mutation in tau has yet been linked to Alzheimer's disease. "There is probably a pathway from amyloid-β aggregation to tau aggregation," says Goedert. But in the absence of a clear mechanism, current mouse models use tau mutations implicated in another cognitive disorder of humans known as frontotemporal dementia. Sometimes these mutations are combined with others that generate amyloid-β plaques to achieve models that get even closer to mimicking Alzheimer's disease. Still, many researchers question the value of such models. "That combination is not seen in people. When you force together mutations, you have to ask what that model is representing," says Lamb.

In 2016, the US National Institute on Aging assigned a group of researchers, led by Lamb, the task of developing mouse models that more closely reflect Alzheimer's disease.

Called MODEL-AD, the consortium — a collaboration between Indiana University, the Jackson Laboratory in Bar Harbor, Maine, Sage Bionetworks of Seattle, Washington (a non-profit organization that promotes open science) and the University of California, Irvine — aims to accurately represent the sporadic, late-onset form of Alzheimer's disease in mice, which has been largely overlooked by current models. "That to me is the biggest disconnect with the models," says Lamb.
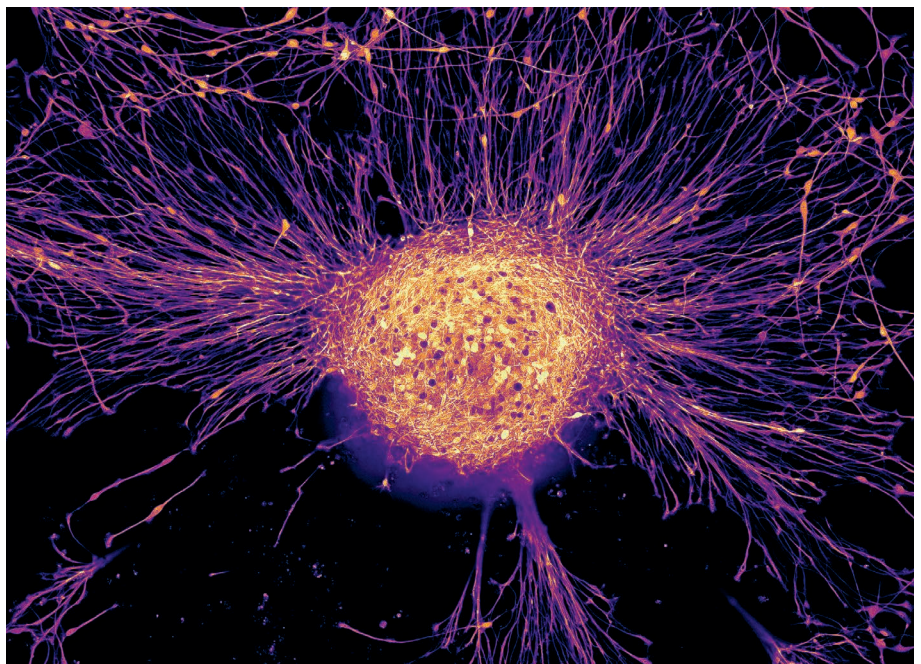
The consortium has already created a mouse model that incorporates the ε4 variant of the gene APOE, which is closely associated with an increased risk of the sporadic form of Alzheimer's disease. It encodes apolipoprotein E, a protein that transports cholesterol in the central nervous system and influences amyloid-β aggregation and clearance in the brain. People with a single copy of APOE ε4 have a 3-fold greater risk of developing the condition; those with two copies have a 12-fold greater risk. The model also expresses a variant of the gene TREM2, which encodes a cell-surface receptor on microglia (the immune cells of the brain). That variant increases the risk of developing sporadic Alzheimer's disease by up to four times. The consortium plans to introduce up to ten new mouse models per year during the project's first five years of funding. It will also use the genome-editing technology CRISPR in mice to stack up gene variants identified as risk factors in humans. "APOE ε4 is the biggest risk factor for Alzheimer's disease beyond ageing," says Lemere, who will soon begin to use mouse models expressing the ε4 variant — as well as another APOE variant called ε3, which has a more neutral effect on risk. "If you put the APOE ε4 variant into a mouse model, it causes earlier and more robust plaque deposition — just like in humans," she says.

STANDARD SPECIES

Although mice have been productive workhorses for research on Alzheimer's disease, the difficulties that researchers have experienced in translating promising findings from mice into successful trials in people are driving the field to explore other options for animal models. "The predictive value of transgenic mouse models for therapeutics has been limited," says Lary Walker, a neuroscientist at Emory University School of Medicine in Atlanta, Georgia. One route to closing the gap between people and animal models is to start with a species more-closely related to humans, such as one of the non-human primates. "If a genetically modified primate can be generated that develops bona fide Alzheimer's disease within a reasonable time frame, this would be a boon to the field," Walker says.

Non-human primates, which include rhesus macaques and marmosets, are not known to develop Alzheimer's disease. However, like humans, they do accumulate deposits of amyloid-β in their brains. The brains of these animals also undergo structural and biochemical changes as they age that mirror those seen in people. However, most such primates do not develop tau tangles, and their use in research is accompanied by a host of practical challenges and ethical considerations.

Macaques are the most popular choice of non-human primate for researchers who work on Alzheimer's disease, but no particular species or model is accepted over others. "Non-human primate models are not used much in academia or in industry because they are not validated," says Erwan Bezard, director of the Institute of Neurodegenerative Diseases at the University of Bordeaux, France. This could soon change. The European Union is sponsoring a consortium of research organizations called IMPRiND that is striving to develop a standardized macaque

Stem-cell-derived brain organoids can provide an alternative to animal models of Alzheimer's disease.

model of Alzheimer's disease.

As part of the project, Bezard is working to recreate the condition's progression in macaques by injecting them with brain tissue from humans, which leads the animals to develop plaques and tau tangles, as well as cognitive impairment. This approach has already produced a primate model of the neurodegenerative disorder Parkinson's disease. Validating a macaque model of Alzheimer's disease could convince a wary pharmaceutical industry that, although expensive, it would be sensible to work with such a model before embarking on clinical trials. Indeed, Bezard argues, had non-human primate models already been in wider use, many failed clinical trials could have been avoided. "It was expected, based on the rodent work, that removing plaques would help the cognitive status of patients," he says. Tests in non-human primates could have shown that this would not be the case.

At RIKEN, Saido is creating a marmoset model of Alzheimer's disease by using CRISPR to insert mutations into the gene *PSEN1* in fertilized eggs. Marmosets hold two big advantages over macaques: they can develop both amyloid-β plaques and tau tangles, and plaques are established more quickly (taking 7 years, rather than around 25 years, to appear). Although researchers will still have to wait for a long time, Saido's mutations will accelerate plaque formation so that the models become useful even faster — in just a year or two, he expects. The first of his modified marmosets is expected to be born later this year.

As researchers learn more about how immunological processes influence the onset and progression of Alzheimer's disease, there will be an increasing need to test potential drugs on non-human primates, which have immune systems similar to those of humans. Saido hopes to take advantage of marmosets' similarity to humans to identify biomarkers that might not be found in mice. But there is no sign that the high costs and ethical controversy associated with research on non-human primates will disappear.

MINI-BRAINS

In the past five years, researchers have been given a further option for use in preclinical testing — 3D tissue models of the brain. These small organoids are grown from stem cells over periods of a few weeks, and they enable large numbers of drugs to be evaluated in a short amount of time.

Rudy Tanzi, a neuroscientist at Harvard Medical School in Boston, Massachusetts, reported in 2014 that his lab had grown human neural stem cells containing *APP* and *PSEN1* mutations in a 3D culture system supported by a gel matrix (S. H. Choi *et al. Nature* **515**, 274–278; 2014). The results were startling: the tissue that developed contained neurons that deposited amyloid-β into the gel, and plaques formed after 5 to 6 weeks. A week or two later, the elusive tau tangles also appeared. The

finding provided considerable support for the idea that amyloid-β accumulation leads to tangle formation in Alzheimer's disease — known as the amyloid hypothesis (S4). "All previous models failed to show the plaques going into tangles," says Doo Yeon Kim, a neurologist at Harvard Medical School who worked on the project with Tanzi. "These are real tau filaments, indistinguishable from those you see in the Alzheimer's-patient brain," Tanzi adds. In mice, the brain generates a single variety of tau known as 4-repeat tau. But the human neurons in 3D culture generated both 4-repeat tau and another type of tau (3-repeat tau) in a 50 : 50 ratio — as occurs in the adult human brain.

Li-Huei Tsai, a cognitive neuroscientist at the Massachusetts Institute of Technology in Cambridge, is also using human brain organoids in her research on Alzheimer's disease. In 2014, her lab transformed cells from people with the condition who had either an *APP* duplication or a *PSEN1* mutation into induced pluripotent stem (iPS) cells. The cells formed small brain-like tissues that developed aggregates of amyloid-β and tau protein. Both hallmarks of Alzheimer's disease were reduced when the secretases that chop up APP were inhibited — a strategy also being pursued in clinical trials.

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"This is a new platform for drug discovery," says Tsai. Earlier this year, Tsai and her colleagues reported that brain cells derived from iPS cells carrying the *APOE* ε4 variant secreted more of the stickier amyloid-β(1–42), which encouraged aggregation of amyloid-β and tau (Y. T. Lin *et al. Neuron* **98**, 1141–1154; 2018). Using CRISPR to edit the cells so that the risk-conferring variant ε4 was replaced with the risk-neutral variant ε3 led to a reduction in signs of the condition, including increased amyloid-β production, in neurons, immune cells and even organoids.

The race is on to add different cell types to organoids, so that they can better represent real brain tissue. Tanzi has already incorporated microglia into his model. Initially held in a reservoir outside the dish in which the organoids grow, the microglia travel down microfluidic channels into the main plate in response to the release of pro-inflammatory compounds by stressed neurons (J. Park *et al. Nature Neurosci.* **21**, 941–951; 2018). The microglia then respond to any plaques by pruning the junctions between neurons — effectively trimming the connections that underpin memory. Tanzi thinks that this is how the immune system responds to plaques and tangles, and inflicts harm on the brain.

A considerable advantage of growing model brains in a dish is that it enables rapid screening of compounds for potential drugs. "We are testing all FDA-approved drugs — about 1,200 of them — in these 3D cell-culture models,"

Researchers are developing marmoset models of Alzheimer's disease.

says Kim. The number of mice needed to conduct such a screen would be prohibitive. "The dish is faster, more effective and less expensive," adds Tanzi. His group has found 38 drugs that reduce tangles by at least 90%, as well as 13 drugs that can curb inflammation of nerve tissue.

THE COST OF VARIETY

Many researchers now use more than one model of Alzheimer's disease in their work. "The availability of transgenic mouse models has been a fantastic boon for neurodegenerative disorders," says Thomas Wisniewski, a neuroscientist at NYU Langone Medical Center in New York City. "But they are a mixed blessing, and should be used with caution." He advocates diversification — at present, he is working with four mouse models, as well as a squirrel-monkey model. "That should be a more-typical approach," Wisniewski says. Lamb agrees: "Different models can give different results."

The cost associated with using several animal models at once is a concern. Even mouse models, which have dominated preclinical research for decades, are not as affordable as some researchers would like. "There are still proprietary mouse models that cost €300 (US$349) to €400 per mouse," says Campbell, noting that such costs stop him from using certain models. For its part, MODEL-AD will make its mouse models and associated imaging and phenotypic data freely available. "The field has been slowed because of restrictions placed on some models," says Lamb, who cites lawsuits initiated against academics and companies for their use of certain strains that contain patented DNA sequences.

As more scientists embrace a multitude of models in their research, Tsai expects the cost of research on Alzheimer's disease to climb. There are signs, however, that funding bodies are taking note — given that an increasing number of people are expected to be affected by dementia. In March, the US Congress gave the US National Institutes of Health an extra $414 million for research on Alzheimer's disease and dementia, bringing the total amount dedicated to work on dementia in 2018 to $1.8 billion. It seems that the human cost of the consistent failure of clinical trials of drugs for Alzheimer's disease outweighs all others. ■

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