disease or Parkinson's disease should expect to see some signs of degeneration in control tissue. "Finding completely healthy control tissue, without any pathology, in anyone over 60 years old is very rare," Nolan says.

The lack of uniformity on what defines a control tissue may hinder large cohort studies that rely on sourcing tissues from multiple institutions. "Some studies require tissue from multiple continents; for example in the case of rare diseases, or to investigate differences between geographical populations," says Nolan. "Additionally, the decreasing cost of genomic methods such as high-throughput sequencing means more and more studies are being conducted using large amounts of tissue from across the world."

At their brain bank, Nolan and Al-Sarraj stain control tissue using five different antibody stains to analyze eight different sections of the brain. "This protocol is the most likely to reveal any disease," says Al-Sarraj. "But other brain banks may use fewer stains or test on different sections of the brain."

Banner Sun Health Research Institute in Sun City, Arizona is one such brain bank that differs in protocol. "We use five stains over about 35 different sections of the brain," says Thomas Beach, director of the Brain and Body Donation Program at the institute. "But that may vary from bank to bank."

Many brain banks even have different standards for accepting donations. All the brain banks within the US National Institutes of Health (NIH) network, for instance, require that patients enroll in an autopsy program before death and complete clinical evaluations so that there are thorough clinical records to go along with the brain donation. Others, such as the MRC brain bank, accept donations without extensive or, in some cases, any clinical history.

Costly banking

The differences in tissue analysis among institutions might be in part because of cost. Processing a single brain—from dissection to staining and classification—can cost anywhere from £2,250 to £2,600 ($3,300 to $3,800), according to Nolan, and relying on the latest stains available is expensive. But for autopsy programs, like the one at Banner Institute, in which donors undergo thorough neuropsychiatric evaluations before they die and donate their brains, the price tag for following these patients from the time of enrollment to collecting the organ, processing and banking can cost more than $10,000.

"Underlying a lot of the problems with brain banking is insufficient funding," says Beach. "It's very expensive to run brain banks and there's not enough funding to do it properly."

With limited resources, the gap between different types of control tissue is even wider. In the US, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) was created in 1986, when it also established guidelines for the classification of Alzheimer's tissue. In 2012, the National Institute on Aging (NIA), along with the Alzheimer's Association (AA), issued its latest guidelines for the classification of brain tissue from individuals with Alzheimer's and other related diseases (*Acta Neuropathol.*, **123**, 1–11, 2012). The new guidelines included more detailed approaches for the assessment of related conditions such as Lewy body disease and vascular brain injury. Perhaps most importantly, the guidelines were expanded to reflect the current understanding that cognitive decline as seen in the clinic is not necessarily a reflection of the presence of disease in the brain.

The nearly 30 brain banks in the US that receive funding through the NIH are required to follow this mandate. But there is no standardization among the non-NIH-funded brain banks.

Beach, who is also one of the authors of the 2012 NIA-AA guidelines, says this doesn't create a huge problem. "My sense is that a lot of people, even elsewhere in the world, look to NIH protocols for direction," he says. "So they are more or less on the same page." Meanwhile, BrainNet Europe, a consortium of brain banks around the continent, laid out protocols similar to the NIA's for identifying disease tissue in 2008.

Yet none of these guidelines, including the 2007 MRC guidelines, offer sufficient instruction and consensus on classifying control tissue, according to Nolan. "There is no such standardization for control tissue," he says. "The protocols for dealing with control tissue are largely decided upon by brain banks themselves based on size, resources, or the disease being studied."

"The guidelines are only concerned with identifying disease," Beach says. "Their purpose is not really to define what a control is."

Ultimately, Nolan and Al-Sarraj hope that their results will stimulate a conversation about the best way to establish standards among brain banks for classifying control tissue. They do not plan to get in touch with researchers who may have relied on pre-2007 tissues to conduct their research. "Our paper only provides a brief snapshot of the problems associated with classifying tissue as control," Nolan says. "It is not meant to undermine the validity of previously conducted studies which have used this tissue."

Shraddha Chakradhar

After flu vaccine mismatch, calls for delayed selection intensify

When a World Health Organization panel met in late February to select the strains for the 2015–2016 influenza vaccine for the Northern Hemisphere, the annual gathering took place about a week later than usual. The difference was small, but it was symbolic: global flu experts had asked for a little more time to collect data on circulating flu strains in the wake of a vaccine mismatch this past year.

The first signs of trouble had emerged just weeks after the WHO had settled on its strain selections for the Northern Hemisphere last year: tests indicated that one of the circulating influenza virus strains had genetic changes that produced small but significant differences in its surface proteins. Such mutations can affect whether the body's immune system can recognize and thwart the flu. By May, the drifted strain made up 17% of circulating influenza A (H3N2) viruses, one common subtype that causes illness. The drifted strain began to predominate in the summer and caused more than half of the illness in this past flu season.

The mismatch between the predominant circulating virus strain and the inactivated or attenuated H3N2 strain used in the vaccine meant less protection during a moderately severe season. Perhaps as a result, the flu hit hard, particularly among the elderly. In the US, about 266 out of every 100,000 people over 65 years of age were hospitalized owing to influenza complications–the highest rate recorded since surveillance began in the 2005–2006 flu season[1]. Additionally, the flu vaccine was only 18% effective against the prevailing H3N2 strain, the country's Centers for Disease Control and Prevention (CDC) reported[2].

Although there are more formulations of influenza vaccine than ever, reflecting new ways of making or administering the vaccine and the addition of a second B strain in some vaccines, the timeline for strain selection and production has barely budged. Instead, it follows a predictable schedule, After the WHO analyzed data from 112 countries and announced its strain selections for the Northern Hemisphere's 2015–2016 season in February, a US Food and Drug Administration (FDA) advisory panel

met on 4 March and approved those strains as the country's vaccine targets for the coming flu season. This kicked off the lengthy processes needed to produce millions of doses that can be tested and approved by the FDA.

At the March meeting, members of the FDA panel were clearly concerned about improving the timing and avoiding future mismatches. Pedro Piedra, professor of molecular virology and microbiology at the Baylor College of Medicine in Houston, asked if they could delay the selection of just the H3N2 strain, which is associated with more-severe disease. Choosing the right strain is essential to maintain public trust in the vaccines, he said. "In today's modern society, it's hard for me to believe that we cannot do better than what we're doing," he said.

Others beyond the FDA advisors have voiced similar concerns to *Nature Medicine*. "To get a better vaccine, what we really need is a more predictable match between the vaccine we produce and circulating strains," says Andrew Pavia, chief of the division of pediatric infectious diseases at the University of Utah in Salt Lake City, who has served on vaccine and infectious disease advisory panels not related to strain selection. Moving back strain selection by four months would allow for a consistently good match, he says. "Being able to make decisions later in the year is probably the best tool we could have right now for getting a better match." Yet officials with Sanofi Pasteur, a leading vaccine manufacturer, told the FDA panel that pushing back even the selection of one strain would lead to a later start in fall vaccinations—and possibly result in fewer schoolchildren being protected.

Pandemic imperative

Timing played a critical role in 2009, when producing a vaccine even one month faster would have saved an estimated 2,000 lives[3]. The CDC identified the novel influenza A (H1N1) pandemic virus on 15 April, about two months after selection of the strains for the seasonal vaccine, yet a separate pandemic vaccine was not widely available until November or later—after the peak of the second wave of the pandemic.

In a 2010 report, the US President's Council of Advisors on Science and Technology suggested ways to improve each step of influenza vaccine production with the goal of shortening the time needed to create the first doses of a pandemic vaccine from about 20 weeks to 10–14 weeks. "[I]n a serious pandemic, saving weeks could translate into saving tens of thousands of lives," the council wrote.

Now even politicians have weighed in on vaccine timing. At a 3 February hearing before an Energy and Commerce subcommittee of the US House of Representatives, Republican Congressman David McKinley of West Virginia vented to the nation's flu vaccine experts. "This whole process [of] designating which vaccine we are going to come up with in September just seems archaic," McKinley said. "In fact, it seems more of a game of chance and probability." The chairman and ranking Democrat of the committee later sent letters to the heads of five US public health agencies, asking how they could improve their influenza response.

Much of the problem lies with the mercurial nature of the influenza virus itself, but continued reliance on old manufacturing methods has a role. Of the 148 million doses distributed this year in the US, all but about 10 million were grown in chicken eggs in a refined version of a technology licensed in 1945. For these vaccines, a 'seed strain' must be developed that is capable of growing in eggs. Cell-based vaccines begin with egg-grown seed viruses that then reproduce in mammal cells. Recombinant versions are developed without eggs and thus are faster, but they still require time for virus replication.

"When we pick the strains, we are mindful of whether the strain is known to be a good grower or not," says Robert Daum, professor of pediatrics, microbiology and molecular medicine at the University of Chicago and chair of the FDA's Vaccines and Related Biological Products Advisory Committee. "There's no point in picking a strain that is a good match clinically and grows poorly."

A longstanding quandary

Speeding up production so more time could be spent tracking strain changes has been a topic of discussion for at least 25 years, says Nancy Cox, who retired in November as director of the CDC's influenza division. She was director of the World Health Organization Collaborating Centre for the Surveillance, Epidemiology and Control of Influenza at the CDC from 1992 to 2014.

Flu vaccines grown in mammalian cells (such as Novartis's Flucelvax) or recombinant versions grown in insect cells (as done for Flublok from Protein Sciences Corporation) allow somewhat faster production, but they still face the roadblock of potency testing to determine how much is required to produce adequate immunity. Each new strain requires a new potency test reagent, even if the vaccine is not egg based.

"There are really significant technical hurdles," Cox says. "There's still work going on, but after four years of pretty intensive work we still don't have a better test available at the present time." The CDC has improved its surveillance and methods for analyzing strains, and the WHO has held three international meetings seeking to improve strain selection, she notes. Researchers are working out new ways to predict strain changes and even developing synthetic viruses that could be used in vaccines. "I think the end result is that there are a lot of things on the horizon," Jerry Weir, director of the FDA's division of viral products, told the FDA panel on 4 March. "I think they will be incorporated at some point when they're ready."

In addition to targeting the H1N1 virus, the 2015 influenza vaccine approved for the Southern Hemisphere in September is also designed against the new H3N2 strain and an influenza B strain that is different from the one in last year's vaccine. The changes required two new potency tests—and led to a six-week delay in vaccine supply. (The 2015–2016 Northern Hemisphere vaccine will be the same as the Southern Hemisphere vaccine.)

Meanwhile, just a week after the FDA meeting, researchers at the Massachusetts Institute of Technology warned of changes in an H1N1 strain detected in India that could make the virus more virulent. The information was culled from the genetic sequencing of only two viruses from 2014 that had been submitted to a public surveillance database, and Ram Sasisekharan, professor of biological engineering, and research scientist Kannan Tharakaraman urged greater monitoring of circulating strains[4]. The WHO and CDC said no major changes have been detected in the currently circulating H1N1 viruses, but surveillance continues.

Ultimately, with genetic sequencing alone, it is hard to assess the impact of strain mutations on the protective value of existing vaccines, says Cox. "We would all like the situation to be different," she says of difficulties with strain selection. "We can't change influenza viruses and their natural history. The only thing we can do is work on new vaccine approaches to try to counter that enemy."

Michele Cohen Marill

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