'Outbred' mice are used to reveal the genetic diversity that underlies disease.

CAROLYN A. MCKEONE/SPL

DEEP PHENOTYPING

The details of disease

Precision medicine demands precise matching of deep genomic and phenotypic models — and the deeper you go, the more you know.

BY CATHRYN M. DELUDE

Twenty years ago, amid an explosion of optimism that sequencing the human genome would lead to precision medicine, Isaac Kohane sounded a note of caution. Yes, gene sequencing was a major step forward. But wringing clinical value from the flood of genomic information, he said, would depend on the more pedestrian practice of phenotyping — clinically characterizing traits that signify health or disease, such as a fever, a rash, a limp or an irregular heartbeat.

"Science is informed by what it is possible to measure, and it takes a great leap forward when we can measure something new," says Kohane, a bioinformatician at Harvard Medical School in Boston, Massachusetts. "Previously it was hard to measure differences in genome sequences among individuals. Now that's been reduced to a commodity."

But measuring different phenotypes in diabetes, for instance, still requires someone to comb medical records for data on metrics such as weight, blood pressure and blood glucose levels — a tedious and expensive exercise. Moreover, new forms of measurement, such as continuous glucose monitoring, that may provide valuable clues to disease may not be included in these records.

Precision medicine requires an understanding of the precise relationship between gene and phenotype, and the stratification of diseases into subtypes according to their underlying biological mechanisms. But researchers do not know the functions of most genes, and what they do know is limited to a few cell types, tissues or physiological contexts. Furthermore, descriptions of disease phenotypes often fail to capture the diverse manifestations of common diseases or to define subclasses of those diseases that predict the outcome or response to treatment. Phenotype descriptions are typically "sloppy or imprecise", according to a 2012 review[1].

Overcoming these difficulties requires an exhaustive examination of the discrete components of a phenotype that goes beyond what is typically recorded in medical charts. Such 'deep phenotyping', as it is known, gathers details about disease manifestations in a more individual and finer-grained way, and uses sophisticated algorithms to integrate the resulting wealth of data with other kinds of information.

Historically, phenotyping has not represented big data. It has been partial, generic and time-consuming to gather. Information about individual phenotypes has not been matched to genetic variations among individuals. Deep phenotyping will provide more specificity, new types of big data, and potential connections between disease subtypes and genetic variations.

This approach will allow researchers to address new questions. What is the specific pattern of protein expression or gene regulation in the diseased cells? What about the cells' metabolites and other biochemistry? Are there unusual gut bacteria? Does the patient have other seemingly unrelated conditions, such as autoimmunity or a psychiatric disorder, that might share a biological pathway? This comprehensive deep-phenotyping information, in combination with other big data such as genomic data, can reveal the precise underlying mechanisms of each individual's disease. As Kohane says, deep phenotyping "shows the different dimensions of the disease".

DIVIDING DIABETES

Diabetes exemplifies the problem of imprecise phenotypes. "There are a hundred ways to be diabetic, involving different processes in the pancreas, liver, muscle, brain and fat," says Gary Churchill, a mouse geneticist at the Jackson Laboratory in Bar Harbor, Maine. "Genetic studies lose statistical power by looking at a conglomeration of underlying causes." Different genes are responsible for particular subtypes of diabetes, so mixing them together obscures the reasons why people with the same genetic mutation respond differently to the same treatment.

"There are many steps between causal gene and phenotype at the level of body weight and blood sugar," says Alan Attie, a biochemist at the University of Wisconsin-Madison who collaborates with Churchill. "Each step is subject to genetic variation, which can weaken links between gene and phenotype."

Attie is looking at how individual genomic differences affect one particular phenotype of diabetes: insulin secretion by islet cells. He is isolating islet cells from genetically diverse mice and testing their response not just to

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glucose, but also to fatty acids, amino acids and other molecules that affect insulin secretion. Preliminary data reveal significant variation among islet cells.

Churchill says that studying 'outbred' mice, rather than inbred strains that have identical genomes, better mirrors human diversity in diseases such as diabetes that have many genetic contributors. For instance, B6 mice, a commonly used inbred strain, would all get diabetes when they become obese for the same reason. "If we only studied that mouse, the findings would translate to some human patients but we wouldn't see the breadth of other causes," he says.

BRAIN WORK

Combining deep phenotyping with big '-omic' data is far from straightforward. And the link between gene and phenotype is particularly precarious in neuropsychiatric disorders such as autism.

"Precision medicine? That's not about us. We barely know how to do medicine," says Steven Hyman, a neuroscientist at the Broad Institute in Cambridge, Massachusetts. "In psychiatry, we only have descriptive phenotypes," he says, not mechanistic ones that reveal what has gone awry in the brain. Taking a deep-phenotyping approach to neuropsychiatric disease might break the current impasse in progress to better treatments, says Hyman.

Most brain disorders are polygenic, with different combinations of gene mutations causing disease in individual patients, so identifying genes still fails to explain the majority of cases. For autism, fewer than 10% of cases are linked to genes that might explain the underlying disease mechanism. And an autism gene could also be involved in schizophrenia, obsessive–compulsive disorder and bipolar disorder, says Guoping Feng, a neuroscientist at the Massachusetts Institute of Technology in Cambridge. "Some symptoms are unique to each disorder, but other symptoms overlap."

Furthermore, although most people with autism share core symptoms (such as repetitive behaviours and social deficits), some also have irritable bowel syndrome, infections, seizures, schizophrenia or attention deficit hyperactivity disorder. "We should consider not just neurology and behaviour, but other diagnoses the patient has, such as inflammation and heart disorders," says Kohane. "Defining these subclasses is a prerequisite for precision medicine."

Steve Brown, a mammalian geneticist at the Medical Research Council centre at Harwell, UK, hopes that his work with the International Mouse Phenotyping Consortium can untangle such complications. The consortium is systematically phenotyping a knockout mouse strain for every gene in the mouse genome.

"We can't look at just one or two phenotypes because we don't know the function of most genes," Brown says. "We can't make assumptions about what to look for." Researchers test each mouse for sensory perception, cardiovascular and lung functions, metabolism, morphology and pathologies, and record environmental conditions and diet. They also record behavioural data on activity, social interactions, grooming, sleeping and feeding.

The consortium's knockout mice are all from an inbred strain, which limits the exploration of natural diversity but enables comparative studies and replication of findings. "We never expect to create a model of autism or schizophrenia," Brown says. Instead, the goal is to establish baselines for what each gene does and how it might affect behaviour.

THE LIMITATIONS OF MODELLING

Those who are performing deep phenotyping in animal models acknowledge the fundamental limitations of modelling disorders in non-human species, however. "Human neuropsychiatric disorders involve the prefrontal cerebral cortex, which is a recent arrival in evolution," Hyman says. "Many important cells and circuits in the human cerebral cortex simply aren't there in mice." Scientists should focus on cells and molecular mechanisms that are shared by mice and humans, he says.

"Too many studies start with a transgenic mouse that is, say, lousy at building nests, decide it models schizophrenia or autism, and draw conclusions about the molecular mechanisms of disease," he adds. "It should work the other way round."

Walker Jackson, a prion-disease researcher at the German Center for Neurodegenerative Diseases in Bonn, Germany, studies how single amino-acid mutations in the human prion protein cause the pathologies of Creutzfeldt–Jakob disease and fatal familial insomnia in mice. Jackson measures behaviours to understand the natural history of the diseases, but stops short of seeking a genetic link. "I'm not trying to see how a mutation connects to behaviour because it's hard to know what is changing behaviour," he says.

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He finds that the same mutation affects some neurons but not others, and wants to understand how non-diseased neurons compensate for the mutation to reveal targets for therapy. These effects occur in the hippocampus, cerebellum and thalamus — all regions linked to the behavioural symptoms seen in these disorders. "The data are showing us that the disease is more complex than we thought," Jackson says. "Affected neurons show dysfunction in different ways, so therapy that works in one type of neuron may not work in others."

Similarly, researchers at Stanford University Medical School in California started with a single mutation in the *NL3* gene that has been directly linked to some cases of human autism — a rare occurrence in psychiatric illness. They inserted this mutation in mice and traced its effect on motor behaviour to impaired dopamine inhibition in certain neurons in an unexpected brain region[2].

Feng used a similar approach to identify neural circuitry imbalances caused by another autism gene (*Shank3*) in mice[3]. But this method cannot be widely used because most disorders involve myriad genes, each with a small effect. "I don't think deep phenotyping a mutant mouse's behaviour alone will give us great insight," Feng says. But studying cells derived from humans might help, he suggests, because "these cells already have the perfect combination that can cause disease in a person."

THE HUMAN TOUCH

Given the limitations of animal studies, and the advantages of studying illnesses directly in human cells, deep phenotyping is now extending to research on new human cell models of complex diseases. Neuropsychiatric researchers, for example, can induce skin cells to form stem cells, and can differentiate them into neurons or self-assembled clusters of cells called organoids, so they can study the connections between phenotypes, genomics and related biological data.

Kohane is leading one such project, called N-GRID, which collects cells from patients with neuropsychiatric disorders to look for links between individual genomes and transcriptomes, proteomes, patterns of DNA methylation and other epigenetic markers that affect gene expression, responses to small molecules, and clinical features. The project's deep-phenotyping approach includes "whatever we can measure, to see if distinctive subsets emerge", Kohane says. The aim is to build a "more robust scheme of classifying neuropsychiatric disease — one that is more reliable with regard to prognosis of these diseases, more insightful as to the biological aberration in each category and, therefore, more effective in treating the patient".

Hyman proposes that researchers should consider reserving animal models for safety and pharmacokinetics studies. The efficacy of a new therapy could be tested instead in engineered human cell cultures or organoids. "What if we can't have a mouse model of schizophrenia?" he asks. This should not stop the quest for safe, effective therapies — and if animal models cannot provide good readouts on efficacy, deep phenotyping of human cells might well fill the gaps. ■

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1. Robinson, P. N. *Hum. Mutat.* **33,** 777–780 (2012).
2. Rothwell, P. E. *et al. Cell* **158,** 198–212 (2014).
3. Peça, J. *et al. Nature* **472,** 437–442 (2011).