GENOMES ON PRESCRIPTION

The first clinical uses of whole-genome sequencing show just how challenging it can be.

BY BRENDAN MAHER

The first thing Debbie Jorde noticed about her newborn daughter was that her arms were bent at unnatural angles. She had other problems, too: a cleft palate, eight fingers, eight toes and no lower eyelids. She would eventually be diagnosed with Miller syndrome, a disease so rare that doctors have long assumed that each case arises through spontaneous mutation, rather than being passed down through families. Doctors told Jorde that her chances of having a second child with the syndrome were less than one in a million.

They were wrong. Jorde's son, born three years after his sister, had the same features. Lynn Jorde, Debbie's current husband and a geneticist at the University of Utah in Salt Lake City, still cringes when Debbie recounts what the doctors had told her. "The right answer for that situation is that there have been so few cases that we really can't predict the risk," he says.

Thanks to next-generation genome sequencing, Debbie and her children now know the family's genetic risks. Lynn and his collaborators had been talking about sequencing the genomes of an entire 'nuclear' family affected by a genetic disease, both to identify the mutation responsible and to investigate how genes are inherited in unprecedented detail. Debbie, her former husband and her now-adult children, Heather and Logan Madsen, were happy to be take part, and in 2009 became the first family in the world to have their genomes fully sequenced[1].

Over the course of six months, the research team cross-compared the whopping amount of DNA data from the four genomes. With the help of a parallel sequencing effort that included others

ILLUSTRATION BY OLIVER MUNDAY

with Miller syndrome[2], the researchers identified the gene involved, called *DHODH*, which encodes a protein involved in the synthesis of nucleotides. The disease, it turns out, is recessive. In this case, both parents carried a single mutated copy of the gene, so their chance of having a child with the syndrome was actually one in four. The analyses also revealed that the children had a second recessive genetic disorder, primary ciliary dyskinesia, which affects lung development. Before that discovery, says Debbie, "We never knew why they kept getting pneumonia."

Families like Debbie Jorde's are part of a small but growing vanguard of people, mostly with rare diseases and cancers, whose genomes have been sequenced to help diagnose or understand their condition. Although knowing the sequence didn't alter treatments for Heather and Logan, some individuals are being sequenced with that intent. A boy in Wisconsin was given a risky but life-saving bone-marrow transplant last year on the basis of a partial genome sequence[3]; a woman with leukaemia was spared a similar procedure after her genome was sequenced[4]; and genome sequencing was used to refine the therapy given to twins with a rare disorder (see '6 billion to one')[5].

Most of those involved so far have been lucky enough to know the right people — researchers with an interest in clinical genetics — or determined enough to seek them out, and many, such as Debbie Jorde's family, were taking part in research projects. But now, with genome sequencing becoming much cheaper and faster, clinical programmes are starting up around the world that will routinely analyse genomes for those who might benefit from the information. Illumina, which is based in San Diego, California, and provided the sequencing machines for many of the programmes, offers whole-genome sequencing for as little as US$7,500 for people with life-threatening disease, and for $10,000 for people with cancers that require the sequencing of both tumour and non-cancer cells.

As prices fall further, some say that prescribing a genome sequence or analysis will become akin to requesting a magnetic resonance imaging (MRI) scan. "It's just like any other test in medicine. There's nothing remotely special about it," says David Bick, a clinical geneticist at the Medical College of Wisconsin in Milwaukee. But, he adds, "people will cry and scream and yell about that statement". That's true: unlike the results of most medical tests, a genome sequence provides a vast amount of difficult-to-interpret data, not all of which will be necessary for diagnosing or treating the patient's condition and which could provide unwanted clues to future health risks. The few success stories published so far also suggest that wringing information from the human genome and counselling patients and their families adequately may be too big a burden for medical systems that are already stretched to their limits. "You can't immediately jump from those few profound but limited stories and think that you can reduce this to practice for clinical care," says Eric Green, director of the National Human Genome Research Institute (NHGRI) in Bethesda, Maryland. Still, from the pioneering cases, much can be learned.

RARE BIRTHS

Take Nicholas Volker. From the time he was born, an undiagnosed condition ravaged his intestines, sometimes causing fistulae: holes that ran from his gut through to the outside of his body, leaking faeces and requiring surgery. By the time he turned three, Volker had been in an operating room more than 100 times. Doctors hypothesized that he had an immune deficiency and that a bone-marrow transplant might correct the problem. But a number of tests, including the sequencing of several genes, were inconclusive. After intense deliberation, a team at the Medical College of Wisconsin was cleared to sequence Volker's exome, the 1–2% of the genome that codes for proteins and key regulatory RNA molecules.

Using computational tools, the team combed Volker's DNA for sequences that vary from person to person. They compared these with known variants in the general population, with variants associated with diseases and with related sequences in other species, looking for a mutation that might have caused the problem, says David Dimmock, a clinical geneticist at the college. It took, "basically one person staring at a computer for three and a half months", he says, but eventually they identified a mutation on the X chromosome in a gene called *X-linked inhibitor of Apoptosis*, or *XIAP* (ref. 3). A deficiency of the protein encoded by this gene is known to put patients at high risk for a deadly immune-cell disorder, and a bone-marrow transplant suddenly became imperative. More than a year later, Dimmock says, Volker is doing well.

What started as an experiment has become a programme at Wisconsin, where Dimmock, Bick and their colleagues now aim to provide comprehensive whole-genome sequencing for patients. The team is focusing on people with rare disorders that are thought to involve a genetic defect, and in whom identifying that defect is likely to inform the course of treatment.

Bick says that of 48 patients evaluated for the programme, 17 have been accepted, and their families have gone through six hours or more of genetic counselling before sequencing. Insurance companies have agreed to foot the bill for at least two of the cases. Their rationale is straightforward, says Tina Hambuch, a senior scientist at Illumina's clinical services laboratory, which has been doing the sequencing for this programme. A full genome sequence can be less expensive than a series of single genetic tests, and might clarify whether a costly treatment is required. "There are cases where it's cost effective," Hambuch says.

GENOME FACTORIES

Other institutions are following suit. In the United Kingdom, the Wellcome Trust Centre for Human Genetics at the University of Oxford has made plans with Illumina to sequence 500 genomes from people — some from the same family — with a wide range of conditions. The Undiagnosed Diseases Program at the National Institutes of Health in Bethesda has been running a sequencing programme since 2008. It has sequenced more than 140 exomes and 5 genomes in its attempts to find the molecular underpinnings of diseases that have eluded diagnosis. The programme was so overwhelmed by interest that it temporarily stopped accepting applications a few months ago.

Green says that "now is the time to push the accelerator". Clinical geneticists often talk about tackling Mendelian disorders: diseases thought to involve a single gene and that roughly obey the rules of inheritance drawn up by Gregor Mendel in the nineteenth century. These conditions may account for as many as 20% of paediatric hospitalizations worldwide and a large share of health-care costs. Yet their genetic basis is often unknown. The compendium of such conditions, called Online Mendelian Inheritance in Man (OMIM), currently contains just under 7,000 disorders, about half of which have been assigned a molecular cause. This autumn, Green says, the NHGRI will announce the winners of its Mendelian Disorders Genome Centers grants, which will fund sequencing centres looking for causes of the rest.

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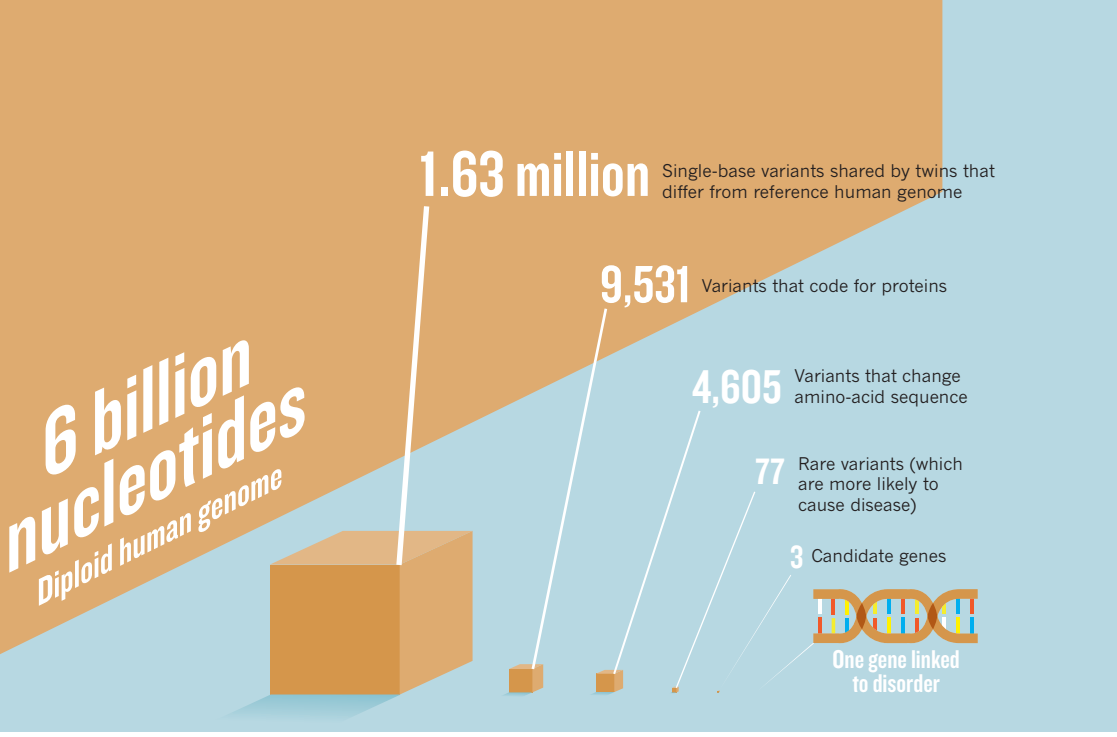
Still, many researchers worry that it will be difficult to make clinical use of most genomes. At the Undiagnosed Diseases Program, the misses have certainly outnumbered the hits so far. "I think we've learned a lot about how hard evaluating an exome is," says Thomas Markello, from the medical-genetics branch of the NHGRI. "I'm most concerned that people don't recognize that what's been published to date are the success stories."

Many researchers say that genome sequencing could be used in diagnosis and therapy of cancer more easily than in rare diseases. Clinicians are already doing sophisticated analyses of some tumours in order to tailor therapies to the patient's genetic characteristics; a genome sequence provides even more molecular detail. For example, an individual's cancer genome sometimes reveals defects in a pathway that might point to use of a known drug, but were not apparent from standard tests.

In 2007, a 78-year-old man in Canada with a rare tongue cancer that had spread throughout his body was being treated at the British Columbia

6 BILLION TO ONE

Sequencing a genome is simple; finding the genetic cause of a disease is not. In one study[5], scientists sequenced the genomes of fraternal twins diagnosed with a movement disorder and whittled down more than 1.6 million variants shared by the twins to the mutations in just one gene. The molecular diagnosis led to improved treatment for the twins.



Cancer Agency in Vancouver. There was no approved treatment for his type of cancer and — being what doctors described as a "savvy sort" — he and his clinician convinced scientists at the agency to sequence the cancer's genome. The scientists also analysed its transcriptome, revealing both the sequence and the amount of RNA that the tumour was producing. The team then compared these data with those for other cancers and for the patient's normal cells.

The researchers homed in on *RET*, a gene known to promote cancer, which was duplicated in the tumour genome and churning out RNA. Several drugs are known to inhibit the protein encoded by this gene. Marco Marra, director of the cancer agency's genome-sciences centre, says that "after much agonizing and hand-wringing", the clinical team prioritized these drugs and tried the top one, sunitinib. The cancer stabilized for several months on this and a second treatment, but eventually started to spread again. An analysis of the recurring tumours showed that different cancer-promoting pathways had been activated[6], making the tumours resistant to the first drug, but possibly responsive to others. Unfortunately, by then it was too late to do more: the man died.

UNSTOPPABLE TRAIN

Marra's group is now setting up a project to better diagnose subtypes of another cancer, acute myelogenous leukaemia, using transcriptome and other sequencing methods. Partly inspired by Marra's efforts, Elaine Mardis, a geneticist at Washington University in St Louis, Missouri, and her collaborators have used genome sequencing to try to help a handful of people with cancer, including the woman with leukaemia[4]. The woman had been treated and had gone into remission, but standard tests were unable show conclusively whether she had acute promyelocytic leukaemia (APL) — which generally has a good outcome with standard therapy — or a type of leukaemia that would require aggressive follow-up treatment, such as a bone-marrow transplantation. Over about seven weeks, the team sequenced the cancer's genome and found a gene fusion that was consistent with APL. Mardis is enthusiastic about the approach, but notes its limitations. "It's another piece of evidence," she says. "It's not going to be the only thing that you're looking at when going to a patient diagnosis."

Moving whole-genome sequencing from research to clinic is beset with challenges. Unlike in research, DNA sequencing that is intended to inform a diagnosis must be done in accredited laboratories, such as those used by Illumina. The institutional review boards that oversee research in humans have not reached a consensus on whether approval is needed for clinical genome sequencing; and the US Food and Drug Administration is yet to work out how to regulate the coming wave of clinical sequencing.

Many researchers and clinicians worry that health systems don't have enough people well versed in genomics or bioinformatics to interpret the flood of data. What's more, say experts, function and disease information for the human genome is scattered across scientific articles and databases that are hard to troll through and aren't always correct. Sequence analysis is where most costs now lie. Hambuch says that for the few research projects on which Illumina has collaborated, just identifying all the variants in a genome has taken two to three weeks. "That's a lot of effort from high-skill people," she says.

The information could also overwhelm patients. Medical geneticists and ethicists have long worried about finding genetic pointers to disease risks that are unrelated to the illness being treated. With a full genome sequence, the likelihood of such incidental findings shoots up. The situation is particularly tricky for young patients. Do parents have the right to decide for them what information is revealed? This is where many of those hours of genetic counselling are spent, says Bick.

For these reasons, Stephen Kingsmore at Children's Mercy Hospital in Kansas City, Missouri, argues that clinical sequencing should be limited in scope. He advocates sequencing just what he calls the Mendelianome, the genetic regions known to be involved in inherited diseases. "Ethically, legally, socially that's going to be more acceptable," he says. His group is developing methods that use a panel of mutations associated with just over 600 recessive diseases for such screening. Doing much more than this, he says, puts research goals ahead of the patient.

But some geneticists think that the train is unstoppable. "Once you demonstrate how informative this technology is, I think this is going to be a widely adopted," says Hakon Hakonarson, who is starting a programme for clinical assessment of genomes at the Children's Hospital of Philadelphia in Pennsylvania.

The members of Debbie Jorde's family still ponder what their genome sequences have meant for them. Although the sequences didn't alter treatment, if they had known about the lung problem earlier it might have prevented a dangerous procedure that both Heather and Logan underwent to reduce the recurrence of pneumonia.

Still, Lynn Jorde thinks that more successes are on the way for genomes in clinical care. "I'd predict some spectacular applications." But, he adds, "I'm a congenital optimist". ■ SEE NEWS P.17 & P.19

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