# SEEING DEADLY MUTATIONS IN A NEW LIGHT

*How one of the largest genome resources in the world has quietly been changing scientists' understanding of human genetics.*

BY ERIKA CHECK HAYDEN

Lurking in the genes of the average person are about 54 mutations that look as if they should sicken or even kill their bearer. But they don't. Sonia Vallabh hoped that D178N was one such mutation.

In 2010, Vallabh had watched her mother die from a mysterious illness called fatal familial insomnia, in which misfolded prion proteins cluster together and destroy the brain. The following year, Sonia was tested and found that she had a copy of the prion-protein gene, *PRNP*, with the same genetic glitch — D178N — that had probably caused her mother's illness. It was a veritable death sentence: the average age of onset is 50, and the disease progresses quickly. But it was not a sentence that Vallabh, then 26, was going to accept without a fight. So she and her husband, Eric Minikel, quit their respective careers in law and transportation consulting to become graduate students in biology. They aimed to learn everything they could about fatal familial insomnia and what, if anything, might be done to stop it. One of the most important tasks was to determine whether or not the D178N mutation definitively caused the disease.

Few would have thought to ask such a question in years past, but medical genetics has been going through a bit of soul-searching. The fast pace of genomic research since the start of the twenty-first century has packed the literature with thousands of gene mutations associated with disease and disability. Many such associations are solid, but scores of mutations once suggested to be dangerous or even lethal are turning out to be innocuous. These sheep in wolves' clothing are being unmasked thanks to one of the largest genetics studies ever conducted: the Exome Aggregation Consortium, or ExAC.

ExAC is a simple idea. It combines sequences for the protein-coding region of the genome — the exome — from more than 60,000 people into one database, allowing scientists to compare them and understand how variable they are. But the resource is having tremendous impacts in biomedical research. As well as helping scientists to toss out spurious disease–gene links, it is generating new discoveries. By looking more closely at the frequency of mutations in different populations, researchers can gain insight into what many genes do and how their protein products function.

ExAC has turned human genetics upside down, says geneticist David Goldstein of

ILLUSTRATION BY DARREN HOPES

Columbia University in New York City. Instead of starting with a disease or trait and working backwards to find its genetic underpinnings, researchers can start with mutations that look like they should have an interesting effect and investigate what might be happening in the people who harbour them. "This really is a new way of working," he says.

ExAC is also providing better information for families facing genetic diagnoses. D178N, for example, was strongly suspected of causing prion disease because it had been seen in several people with the condition and seldom elsewhere. But before ExAC, no one really had the power to see just how rare it was. If it shows up in people more frequently than prion disease does, that would mean Vallabh's risk of getting the disease is much lower than predicted.

"We needed to find out if this mutation had ever been seen in a healthy population," Minikel says.

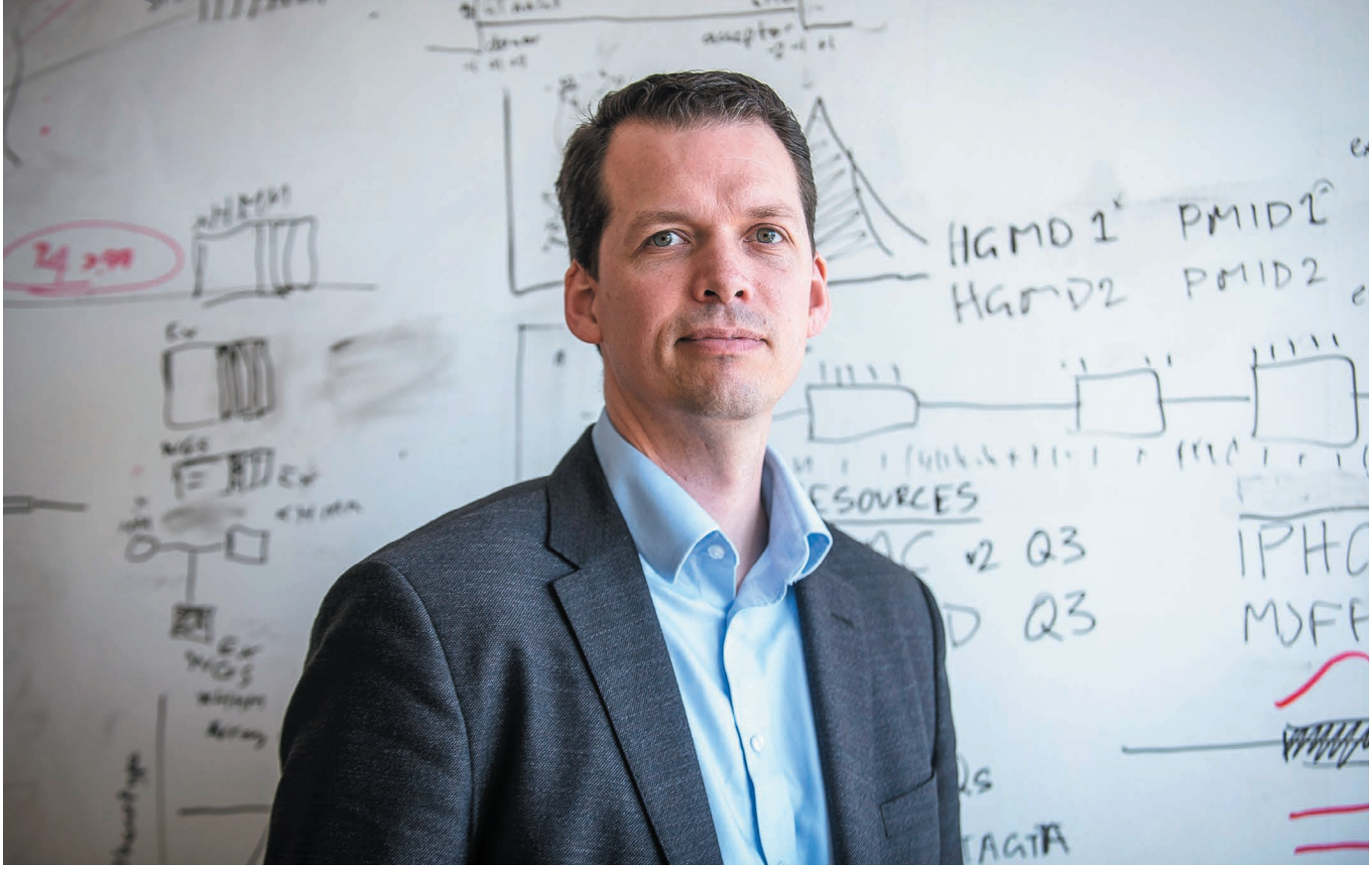
## DATA GATHERING

ExAC was born of frustration. In 2012, geneticist Daniel MacArthur was starting his first laboratory, at Massachusetts General Hospital (MGH) in Boston. He wanted to find genetic mutations that caused rare muscle diseases, and needed two things: genome sequences from people with these disorders, and genome sequences from people without them. If a mutation was more common in people with a disorder than in healthy controls, it stood to reason that the mutation was a likely cause.

The problem was that MacArthur couldn't find enough sequences from unaffected people. He needed lots of exomes, and although researchers had been sequencing them by the thousands, existing data sets weren't large enough. No one had pulled enough together into one combined, standardized resource.

So MacArthur started asking his colleagues to share their data with him. He was well suited to the task: an early adopter of social media, his lively blog posts and acerbic Twitter feed had made him unusually popular and authoritative for a young scientist. He also had a position with the Broad Institute in Cambridge, Massachusetts, a genome-sequencing powerhouse. MacArthur convinced researchers to share data from tens of thousands of exomes with him; most were in some way connected to the Broad.

All that remained was to analyse the data, but that was no trivial task. Although the genes had




**Daniel MacArthur convinced researchers to share genetic data on tens of thousands of people.**

SAM GORESH FOR *NATURE*

been sequenced, the raw data had been analysed using different types of software — including some that were out of date. If one individual in the collection showed a rare mutation, it could be real — or it could be an artefact of how different programs 'called' the bases within, judging whether they were As, Cs, Ts or Gs. MacArthur needed something that would standardize this gigantic data set. The Broad had developed genome-calling software, but it wasn't up to the task of churning through the tremendous amount of data included in ExAC. So MacArthur's team worked closely with the Broad programmers to test the software and scale up its abilities. "That was a pretty horrific 18 months," MacArthur recalls. "We ran into every obstacle imaginable and had nothing to show for it."

## PERSONAL STAKE

While this was going on, in April 2013, Vallabh was learning how to work with stem cells at MGH while Minikel studied bioinformatics. Minikel met MacArthur for lunch and explained his and Vallabh's curiosity about whether D178N existed in healthy people. He admits to being a bit star-struck by MacArthur's reputation. "I thought if I could get him to think about my problem for half an hour, that would probably be the most important thing that happened in my whole month," Minikel says. The pair went upstairs to MacArthur's lab, where bioinformatician Monkol Lek ran a search on the ExAC data that had been analysed so far — about 20,000 exomes. They didn't see Vallabh's mutation. That wasn't good news, but, optimistic about exploring the data further, Minikel joined MacArthur's lab.

By June 2014, MacArthur's team and its collaborators had a data set that they were confident in — exomes from 60,706 individuals representing various ethnic groups, who met certain thresholds for health and consent. They released ExAC that October at the annual meeting of the American Society of Human Genetics (ASHG), in San Diego, California. Immediately, researchers and physicians recognized that the data could help to recast their understanding of genetic risks.

Many disease-association studies, particularly in recent years, have identified mutations as pathogenic simply because scientists performing analyses on a group of people with a disorder found mutations that looked like the culprit, but didn't see them in healthy people. But it's possible that they weren't looking hard enough, or in the right populations. Baseline 'healthy' genetic data has tended to come mainly from people of European descent, which can skew results.

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In August this year, MacArthur's group published[1] its analysis of ExAC data in *Nature*, revealing that many mutations thought to be harmful are probably not. In one analysis, the group identified 192 variants that had previously been thought to be pathogenic, but turned out to be relatively common. The scientists reviewed papers about these variants, looking for plausible evidence that they actually caused disease, but could find solid evidence for only nine of them. Most are actually benign, according to standards set by the American College of Medical Genetics and Genomics, and many have now been reclassified as such.

Similar work promises to have direct impacts on medical practice. In a companion paper[2], geneticist Hugh Watkins of the University of Oxford, UK, looked at genes associated with certain types of cardiomyopathy that cause gradual weakening of the heart muscle. Undetected, they can lead to sudden death, and it has become fairly common to check relatives of people with the conditions for genetic mutations associated with them. Those found to have a genetic risk are sometimes counselled to get an implanted defibrillator, which delivers electrical shocks to the heart if it seems to be beating abnormally. Watkins checked the ExAC database for information on genes that have been associated with these heart conditions, and found that many mutations are much too common among healthy people to be pathogenic. About 60 genes had been implicated as harbouring pathogenic mutations that cause one form of the disease; Watkins' analysis revealed that 40 of these probably bear no link.

This was troubling. "If you have a genetic risk that you believe is predicting disease but isn't, you can end up doing drastic things that can harm someone," says Watkins.

Even some of the mutations that seem to be reliably linked to disease aren't a sure bet — such as those in *PRNP*. There are definitely mutations in the gene that cause the disease, but some

variants might not be pathogenic or might elevate the risk only slightly (see 'The deadly mutations that weren't'). To find out the status of D178N, Vallabh and Minikel gathered genetic data from more than 16,000 people who had been diagnosed with prion diseases, and compared them with data from almost 600,000 others, including the ExAC participants[3].

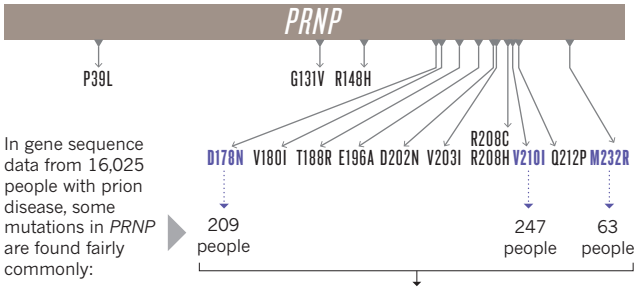
The pair found that 52 people in ExAC had *PRNP* mutations that have been linked to prion diseases, but based on the prevalence of the disease, they would have expected to see maybe two. Minikel calculated that some of these supposedly lethal mutations elevated a person's risk of prion disease slightly; some seemed not to be linked to prion disease at all.

This work provided insight for people such as Alice Uflacker. In 2011, Uflacker's father, Renan, died from Creutzfeldt–Jakob disease, a prion illness that causes rapid mental and physical deterioration. He was 62. Alice found out that she carried a mutation in *PRNP* called V210I, which had been linked to her father's disease in previous studies. Three years later, she learned from Minikel that the mutation confers, at most, a small risk of disease. The information was helpful, and the result made sense; her grandmother had lived to 93 despite having the same mutation.

Vallabh and Minikel would find no such relief, however. D178N was absent from the other genomes they looked at, and is still highly likely to cause prion disease. Minikel and Vallabh had already begun to suspect as much, as Minikel dug into the data. "All along the way was gradual confirmation of what we were assuming anyway," Minikel says. "There wasn't any moment where we said, 'Ah, this is the worst news.' We'd already gotten the worst news."

## THE DEADLY MUTATIONS THAT WEREN'T

Prion diseases are rare neurodegenerative disorders caused by misfolded prion proteins. About 63 mutations in the gene *PRNP* have been linked to them. But until now it has been difficult to estimate how likely it is that a given variant will result in disease, a measure known as penetrance. Data compiled by the Exome Aggregation Consortium (ExAC) can help.



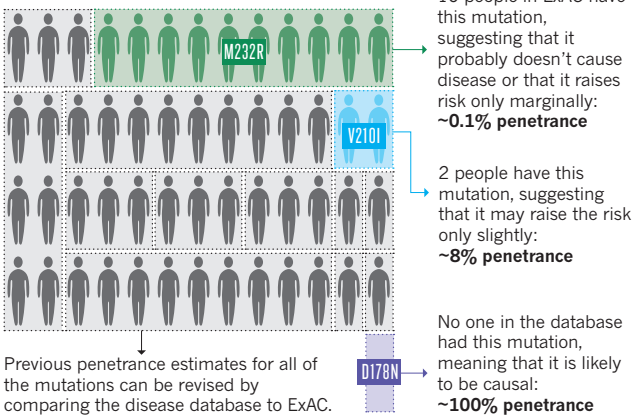

### ExAC DATABASE STUDY

Total prion disease occurence: in every 1,000,000 per year.

ExAC contains the protein-coding sequences of 60,706 people.

Number of people with *PRNP* mutations expected in ExAC: 1.7

Actual number of people with mutations: 52




### HUMAN KNOCKOUTS

ExAC is revealing a lot about genes through the frequency of mutations. MacArthur and his team found[1] 3,200 genes that are almost never severely mutated in any of the ExAC genomes — a signal that these genes are important. And yet 72% of them have never before been linked to disease. Researchers are eager to study whether some of these genes play unappreciated parts in illness.

Conversely, the group has found nearly 180,000 instances of mutations so severe that they should render their protein products completely inactive. Scientists have long studied genes by knocking them out in animals such as mice, so that they don't work. By looking at the symptoms that develop, they can study what the genes do. But that has never been possible in humans. Now, researchers are eager to study these natural human knockouts to understand what they can reveal about how diseases develop or may be cured. MacArthur and other researchers are gearing up to prioritize which human knockout genes to study and how best to contact the people carrying them for further study.

But it will have to wait until he completes the second phase of ExAC. Due to be unveiled at the ASHG meeting in Vancouver, Canada, this month, it will double the data set's size to 135,000 exomes and include some 15,000 whole-genome sequences, which should allow researchers to explore mutations in regulatory regions of the genome that are not captured by exome sequencing.

ExAC is quietly becoming a standard tool in medical genetics. Clinical labs around the world now check it before telling a patient that a particular glitch in their genome might be making them ill. If the mutation is common in ExAC, it's unlikely to be harmful. Geneticist Leslie Biesecker at the US National Human Genome Research Institute in Bethesda, Maryland, says that his lab uses ExAC daily in patient care. "It's a critical factor that we take into consideration for every variant," he says. He and other geneticists are now embarking on a painstaking reckoning with the genetics literature that will probably take years.

ExAC has also driven home a point that Goldstein and other researchers have made repeatedly: that failing to include people from Asian, African, Latino and other non-European ancestries is holding back understanding of how genes influence disease by limiting the view of human genetic diversity (see page 161). There is now a fresh impetus to include under-represented groups in planned studies linking genetics and health information on large numbers of people, such as the US Precision Medicine Initiative.

For Vallabh and Minikel, ExAC provided a disheartening confirmation, but also some promising insight. Minikel's studies have identified[3] three people in ExAC with mutations that should silence one of the two copies of the prion protein gene. If they can live with a limited amount of functioning protein, perhaps a drug could be made that would silence the defective protein in Vallabh, preventing prion aggregation and disease progression without dangerous side effects. Minikel got in touch with one of the individuals, a man in Sweden, who agreed to donate some cells for research. Minikel and Vallabh have now joined the lab of biochemist Stuart Schreiber at the Broad Institute, where they are working full-time to find candidate drugs to treat prion disease.

The couple exemplifies the challenge of translating ExAC data into real medical benefits. "We can't go back from this," Vallabh says. "We have to go through it." Their situation couldn't be more illustrative of what is at stake: Vallabh is now 32 — just 20 years younger than her mother was when she died. She has no time to waste. ■ SEE EDITORIAL P.140

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1. Lek, M. *et al. Nature* **536,** 285–291 (2016).
2. Walsh, R. *et al. Genet. Med.* http://dx.doi.org/10.1038/gim.2016.90 (2016).
3. Minikel, E. V. *et al. Sci. Transl. Med.* **8,** 322ra9 (2016).