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The uncertain future of genetic testing

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AnneMarie Ciccarella, a fast-talking 57-year-old brunette with a more than a hint of a New York accent, thought she knew a lot about breast cancer. Her mother was diagnosed with the disease in 1987, and several other female relatives also developed it. When doctors found a suspicious lump in one of her breasts that turned out to be cancer, she immediately sought out testing to look for mutations in the two BRCA genes, which between them account for around 20 per cent of families with a strong history of breast cancer.

Ciccarella assumed her results would be positive. They weren't. Instead, they identified only what's known as a variant of unknown or uncertain significance (VUS) in both BRCA1 and BRCA2. Unlike pathogenic mutations that are known to cause disease or benign ones that don't, these genetic variations just aren't understood enough to know if they are involved or not.

"I thought you could have a mutated gene or not, and with all the cancer in my family, I believed I would carry a mutation. I didn't know there was this huge third category," she says. "I got no information – it felt like a huge waste of blood to get a giant question mark."

Thousands of people have had their BRCA genes tested for increased genetic susceptibility to breast, ovarian, prostate and other cancers. About 5 per cent have learned that they carry a VUS. That number is even higher for other genes: in one study, almost 20 per cent of genetic tests returned a VUS result.

"That's a lot of uncertainty," says Robert Klitzman, a bioethicist at Columbia University in New York. People want genetic tests to be like pregnancy tests, he explains: "You're either pregnant or you're not. Instead, they're more like a weather report." And most people aren't prepared to cope with the probabilities and uncertainties that entails.

When scientists surveyed a group of women one year after they received BRCA gene test results, the women whose results were uncertain or uninformative were feeling much more stress and anxiety than those whose results were clearly either pathogenic or benign. A follow-up study showed that the higher the risk an individual thought her result indicated, and the less tolerant she was of uncertainty, the more likely she was to experience serious long-term distress.

Even before her sequencing results came in, Ciccarella had decided on a bilateral mastectomy based on her family history. For her, the question of whether she would one day develop breast cancer had been answered, and in the worst possible way. But she still wanted information for her son and daughter so they could know whether they had inherited a genetic risk of cancer. Like a number of families, they are learning that genetic sequencing won't deliver answers for everyone.

We are all mutants. The three billion pieces of DNA that make us who we are were long thought to be constant, chiselled in granite like a classical monument, with only tiny changes made here and there. Scientists used to believe that DNA mutations were largely harmful.

By the late 1990s and early 2000s, as the first sequences of the human genome came rolling in, researchers realised that their view of mutations was completely backwards. Instead of being rarities that almost inevitably harm health, mutations litter the human genome. The average human carries around 400 unique mutations, and most of us are none the worse because of them.

This challenged some basic tenets of genetics, as well as the ways that scientists and physicians interpreted genetic tests.

When Robert Resta, a genetic counsellor at the Swedish Medical Center in Seattle, first began examining genetic test results in the late 1980s, he could identify only chromosomal abnormalities or alterations of massive amounts of DNA. When other types of genetic tests were introduced, such as those for detecting the mutations in the CFTR gene that cause cystic fibrosis, interpretation was still reasonably straightforward. Because most of the people who had their CFTR gene sequenced showed clinical signs of cystic fibrosis, Resta could be reasonably confident that an observed mutation in that gene was the one that had led to the disease. In the past few years, however, the price of genetic sequencing has fallen dramatically, and doctors are increasingly requesting DNA testing earlier in the diagnostic process. As more data is gathered, the sheer number of mutations we all carry becomes more significant.

"It turns out mutations are the norm. You expect to find mutations in a gene. It's a very different way of thinking about the human genome. If you don't find a mutation, your machine is probably having technical difficulty," Resta says.

When scientists test for mutations in large numbers of genes with a single test, known as a gene panel, they are virtually guaranteed to find at least one VUS, says Colleen Caleshu, a genetic counsellor at Stanford University's Center for Inherited Cardiovascular Disease. "The more genes you look at, the more variation you'll find," she adds. "We all have tons of variations in our genes, most of which are extremely rare and, by the very nature of rarity, uninterpretable." In short, there isn't enough data to know what you are seeing.

This grey area has only expanded as next-generation DNA sequencing

has led to the growing use of gene panels, to look for mutations in a range of genes that may be related to a patient's symptoms. Of the three possible results – pathogenic, benign, or unknown – pathogenic is the least common, says Resta. You're much more likely to get uncertainty.

If interpreting genetic testing results is difficult for clinicians, it's also tremendously hard for patients. Yvonne Bombard has spent the last several years of her career as a genomics health services researcher at St Michael's Hospital in Toronto, working to understand how families make sense of genetic testing results.

"There's very little research on the impact of uncertain results on families yet – the technology is just too new," Bombard says.

A small study in *Psycho-Oncology* surveyed 24 women with breast or ovarian cancer who had received VUS results for their genetic testing. Many of them had a distorted perception of what those results meant. Although two-thirds correctly remembered three years later that the variants detected by the test were unclassified, 79 per cent interpreted the results as a higher genetic risk for developing cancer. One-third had also made significant medical changes in their lives based only on their test results, which Resta and Caleshu do not recommend.

Families of children with suspected genetic diseases have similar difficulties. Parents tend to interpret any variant that's not classified 'benign' as being the cause of their child's disease, explains Caleshu. But she appreciates how it's hard not to do that, especially when families have been looking for answers for so long.

Families can feel let down by the medical establishment, who often seem to throw up their hands when a patient defies diagnosis, and in the absence of definitive answers it's all too easy to believe that the genetic variants identified on the test must be what's wrong. One of Caleshu's

main jobs is providing pre-test counselling so that patients understand the risks and the limitations of testing. She says her team have changed the way they present results, so that patients and doctors don't read too much into a VUS. Even with the right genetic counselling, however, uncertainty can be agonising.

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Ciccarella had watched her mother endure chemotherapy, and had undergone a similar gruelling regimen herself. If she could get genetic information that might help her children and any future generations to avoid that agony by using improved screening, reproductive planning and prophylactic mastectomies, then she was determined to make that happen.

She decided to get another lab to review the results of her genetic tests, and requested the data from the sequencing company, Myriad Genetics. They refused. Since they owned patents on these genes, no one else could have their proprietary genetic data.

So she followed with interest a lawsuit by the American Civil Liberties Union (ACLU) against Myriad's genetic patent, hoping that if they won, she could get a second opinion on her unclassified variants after all. In 2013, the US Supreme Court found in favour of the ACLU, invalidating Myriad's patents. Myriad still refused to release raw sequencing data, however, saying that doing so would violate the health privacy law known as HIPAA.

Ciccarella teamed up with the ACLU and three other people who wanted access to their full sequencing data and prepared to file a suit against Myriad in 2016, arguing that HIPAA actually guarantees patients the right to their own data. On 18 May, one day before the suit was due to be

filed, Myriad reversed their stance and released the sequencing data to Ciccarella and the others. She found that Myriad had reclassified one of her VUSs to benign, but when she checked this against public databases of genetic variants, she found that no one else had changed this classification.

"So who's right? There are two different opinions – that's exactly the problem. One place says one thing, one place says another, and I'm stuck in the middle with a daughter who just found a suspicious lump," Ciccarella says.

Ciccarella's case may have been settled out of court, but another case is showing that the battles over genetic testing uncertainty are just beginning. In February 2016, Amy Williams filed a lawsuit against Athena Diagnostics, ADI Holding Company and Quest Diagnostics (Athena's parent company) relating to the death of her son, Christian.

Christian was born a seemingly healthy blond-haired, blue-eyed cherub on 23 August 2005. Just before Christmas that year, he had his first massive seizure. Many more followed. Despite countless medications and tests, no one could figure out what was causing his unrelenting seizures. He had a massive battery of tests in early 2007, including the sequencing of a gene called SCN1A. Athena, which performed the genetic tests, reported that he had a VUS there. With no clear genetic answers, his doctors treated him for an undiagnosed mitochondrial disorder, although his treatments had minimal effects on his continuing seizures.

On 5 January 2008, Christian went to bed after celebrating a belated Christmas holiday with his family. Videos taken that day gave no hint that he'd be dead by morning. The official cause of death was listed as a seizure.

Six years later, thinking of starting a family again, Williams wanted to get her own DNA sequenced to learn whether the disease that had affected her son could affect any future children. Again, she turned to Athena, but as well as her own results, she also requested Christian's 2007 lab report. She saw from the revised report they provided that Athena had reclassified Christian's VUS to a disease-associated mutation, which suggested he had a form of childhood epilepsy called Dravet syndrome (also known as severe myoclonic epilepsy of infancy). Several of the medications used to treat seizures in young children, including Christian, are toxic to children with Dravet and can increase the risk of death.

Williams believes this means the treatment Christian received was only making things worse.

What she now wanted to know from Athena was when and why they reclassified the variant. As Williams, a former special education teacher, taught herself the nuances of scientific literature, she found out that the same SCN1A mutation Christian carried had been identified in an Australian family in 2006, before Christian's DNA was tested. Even more concerning was a patent document on the SCN1A gene that listed this mutation (a change in a single amino acid in the gene) as pathogenic. When Athena refused to answer, Williams sued.

Her allegations include that Athena had had enough information to reclassify Christian's mutation before he was tested, and that if they had done so, it would have changed his diagnosis and treatment such that his death from a seizure related to Dravet syndrome could have been avoided.

Athena and the other two companies reject these allegations and argue that the case should be dismissed. They say that the 2007 lab report emphasised the inconclusiveness of the test results, that Dravet could

have been the cause of Christian's seizures without his medication being implicated, that further testing was strongly recommended (in particular testing of his parents, which was offered at no additional charge but not taken up), and that a conclusive diagnosis could be reached only by additional testing. Quest, Athena's parent company, declined to comment on this ongoing legal action, but the case has caused many in the genetic sequencing community to consider what changes may be required in the future.

This case reflects the uncertainties of modern genetic testing and the tension that can cause for patients and their families, and illustrates the increasing scrutiny of clinical genetic sequencing labs, how they share data on variants, and how this data is interpreted. Regulators, researchers, patients and the sequencing labs themselves will have to work together to find ways to improve these processes.

Tess Bigelow is a bubbly seven-year-old with light brown hair that curls forward into her face, framing a pair of bright pink glasses. A few months after Tess was born, in November 2009, her parents, Bo and Kate, noticed that something was wrong. She wasn't rolling over or meeting other developmental milestones. By June 2010, her parents realised that something was very wrong.

"She was not interacting with other people. It was just like she was checked out. We knew she was in there, we just couldn't get to her," her father says.

As she got older, Tess didn't start to speak or communicate, and she continued to have problems walking and standing. A full diagnostic workup revealed nothing, so genetics experts in Boston and the Bigelows' hometown of Portland, Maine, recommended sequencing all of her genes. The team were hopeful that this would turn up results, but they cautioned the Bigelows not to get their hopes up. Tess's sequencing

revealed a mutation in a gene called USP7, but no one could say whether this was the cause of her illness.

"No matter how much they tell you, you believe you're going to get an answer. It's hard to hear that this is where it ends," Bo Bigelow says.

He began learning everything he could about USP7. There wasn't much. Researchers were just starting to learn what the gene did, and he couldn't find any other families with a USP7 mutation. So he decided to see if he could make those other families come to him. In a public Facebook post he drafted late at night in August 2015, Bigelow described his daughter's symptoms, along with her sequencing results. He crossed his fingers and clicked "share".

The post went viral. One person shared it to Reddit, from where a graduate student brought it to the attention of Christian Schaaf, a geneticist at Baylor College of Medicine in Houston, Texas. He was working on USP7 and other genes that had been linked to genetic conditions like Prader–Willi syndrome.

USP7 is part of our cells' protein recycling machinery, making sure that cells dump their garbage quickly enough to prevent the buildup of proteins that are damaged or no longer needed, but not so quickly that it removes healthy proteins. Suspecting that faults in USP7 could lead to disease, Schaaf had searched through Baylor's own genetic sequencing databases and other genome data depositories, and found seven clinical cases of children who had mutations in USP7.

By the end of the day that his post was shared on Facebook, Bigelow had received an email from Mike Fountain, one of Schaaf's co-authors on the research paper about the USP7 mutations and their links to disease. On the phone the next morning, Fountain outlined the array of symptoms experienced by the seven other children, and they all sounded

remarkably like Tess. It looked like they had found the smoking gun, but only the results of more laboratory studies will show for sure whether this was the cause of Tess's condition.

Like many parents of children with rare diseases and special needs, Bigelow has come to live with the uncertainty. But he and other parents and patients have begun sharing their genetic data through portals like MyGene2 to help others. Created by Michael Bamshad and Jessica Chong, MyGene2 lets people share their own sequencing results in the hopes of facilitating research and finding other families with similar medical problems. Other initiatives are springing up, too, and researchers hope they will reduce the uncertainty that continues to plague genetic sequencing.

Heidi Rehm is a clinical medical geneticist at the Broad Institute in Cambridge, Massachusetts. She led teams at the US National Institutes for Health that created two databases helping to improve sharing and curation of genetic data. ClinVar, launched in 2012, links genetic variants with symptoms. ClinGen, introduced the following year, is described as "building an authoritative central resource that defines the clinical relevance of genes and variants for use in precision medicine and research". With these two resources, commercial and academic sequencing labs can combine their expertise to offer people the most accurate description of what their genetic variants mean.

The depositing of results from large sequencing studies, such as the Exome Aggregation Consortium at the Broad Institute, also promises to help reduce genetic uncertainty. Some of the earliest results of this initiative provided some of the largest reclassifications of VUS results yet, according to Rehm. Nearly all of those reclassifications were shifting a VUS to benign, an indication of the sheer volume of normal variation and mutation inherent in all of our genetic blueprints.

To really get a good handle on all the variation in humans, scientists are going to need to sequence tens of millions of people. And the only way to ever get these kinds of large numbers is to share data.

But regardless of how good the databases get and how many people have their genomes sequenced, uncertainty will never completely go away.

Every time our cells divide and copy their DNA, mutations can arise. This uncertainty may be maddening for patients looking for answers, but it's as much stamped into our genetic blueprint as the double helix itself.

More information: Christina G. Selkirk et al. Cancer genetic testing panels for inherited cancer susceptibility: the clinical experience of a large adult genetics practice, *Familial Cancer* (2014). DOI: 10.1007/s10689-014-9741-4