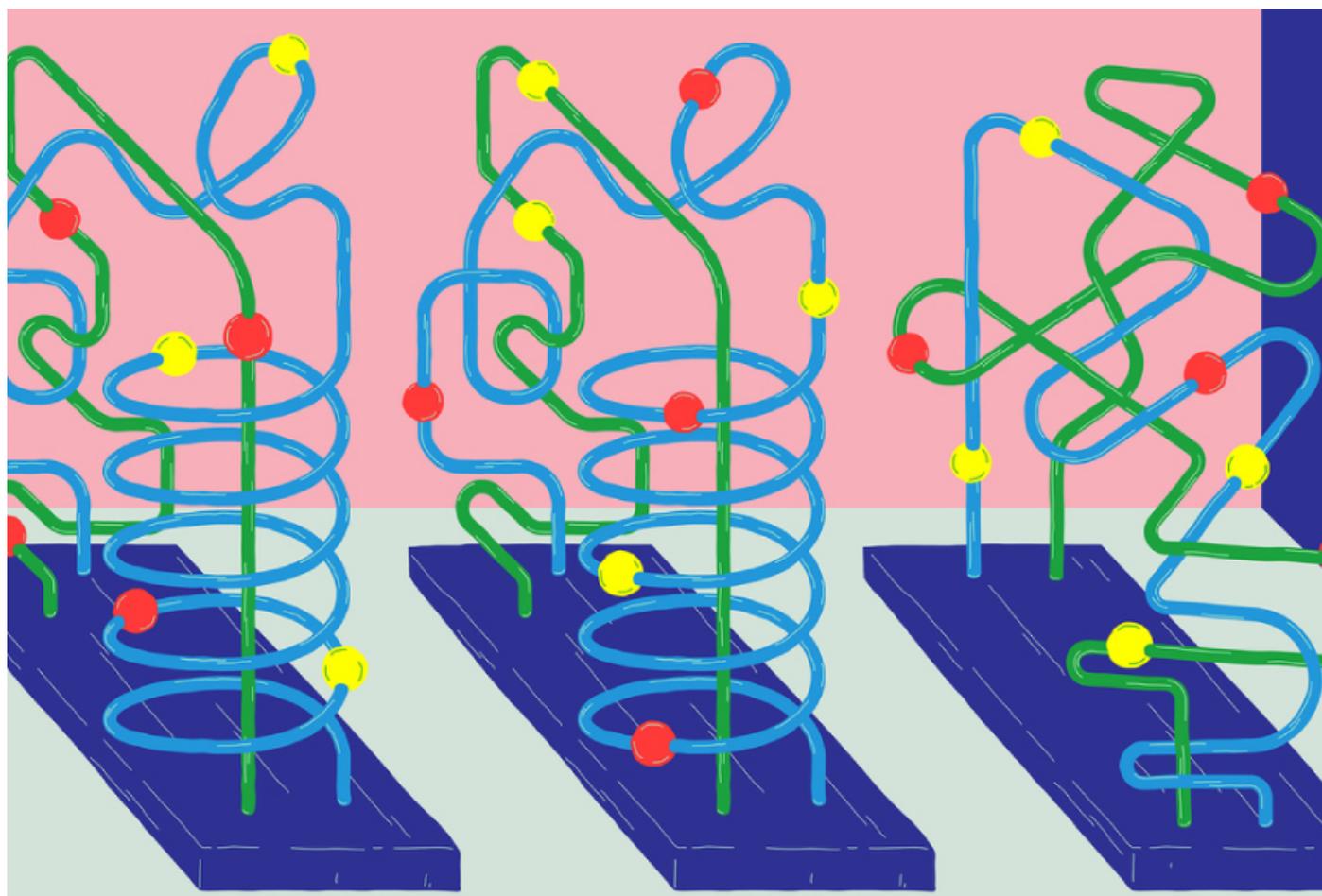


NEWS

How genetics is revealing rare childhood conditions

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Evie Walker sits on Alison's lap, playing a game she never grows tired of: turning her mother's hand over and over, stroking and examining it. When she takes a break and looks around, it is with the open-mouthed look of curiosity and awe that you see in many infants. Evie's vocabulary

currently consists of a repertoire of squawks and “mmm” sounds. In the past few months, she has begun to stand unaided for short periods -- even taking a few steps in her walking frame -- progress that fills her parents with immeasurable pride, not to mention hope for the future.

Despite her baby-like demeanor, Evie is 8 years old. She has PURA syndrome, a vanishingly rare

developmental disorder that didn't officially exist until four years ago. Developmental disorders affect children's mental or physical development. Before she was diagnosed, all Evie's parents knew was that she suffered from 'global developmental delay': a vague umbrella term for a set of symptoms with myriad potential causes -- some, but not all, associated with a heartbreakingly poor prognosis.

Yet thanks to advances in genome sequencing, more and more families like the Walkers are receiving an accurate diagnosis for their child's condition, and with it a clearer picture of what the future holds. In some cases, like Evie's, it reveals they have a completely new disease; in others -- albeit a minority of cases, for now -- it reveals a potential treatment for a condition that had hitherto seemed untreatable.

It's not only children with developmental disorders whose lives are being transformed by this technology. In October 2018, NHS England will launch its Genomic Medicine Service, 13 genomic medicine centres that will provide whole genome sequencing to people with undiagnosed rare diseases and cancer -- another disease of mutated genes.

By uncovering the exact genomic changes driving tumor growth, doctors can choose more effective treatments. In so doing, the U.K. National Health Service (NHS) says England will become one of the first countries in the world to routinely offer "comprehensive and equitable access to the latest in genomic testing and management for the whole country, regardless of condition and where people live."

People with developmental diseases or cancer will be the first to benefit, but as our understanding of genetics grows, the infrastructure will be in place for the testing of other conditions as well, such as early-onset dementia or multiple sclerosis, which may be caused by several gene variants.

Ever since the announcement that a first draft of the human genome had been sequenced, scientists and politicians have been claiming we're on the threshold of a new era of personalized genomic medicine. Has it finally arrived?

Archie Walker was 15 months old when his sister, Evie, came into the world. Alison Walker had sailed through both pregnancies, and when Evie was delivered, a week overdue, by normal vaginal delivery, it appeared the Walkers had been blessed with a second healthy child -- and an excellent sleeper to boot. But this attribute rapidly became a source of concern. Her parents had to wake her to feed her, and then she didn't feed for long. She also felt abnormally cold. Alison raised these concerns with her health visitor when Evie was 5 days old, and she was told to take her to the hospital.

Immediately, the triage nurse grabbed her and raced off down the corridor: Evie spent the following week in intensive care while the medical team worked to keep her warm and fed. "We assumed it was just a little bump in the road, that she'd get better, and then we'd go home with our gorgeous

little girl and all would be well,” Alison says.

But even though Evie returned home, she remained extremely sleepy, and as she turned from infant to toddler, it became clear that she wasn't hitting the usual developmental milestones. She regularly returned to the hospital, undergoing blood tests for various hormonal and nutritional deficiencies, brain scans to detect neurological damage and genetic tests for common inherited diseases.

The Walkers naively assumed that one of these tests would eventually reveal how to fix Evie's issues: “Then we started to realize that not only could we not fix it, we couldn't figure out what the problem was to start with,” Alison says.

Evie was 2 years old when a hospital consultant sat her parents down and broke the devastating news that, not only was it unlikely that Evie would ever catch up with typical children her age, they wondered if she would develop much further at all. Evie still couldn't hold her head up, stand, walk or talk -- and yet the medical tests kept returning normal results.

Diagnostic odyssey:

The Walkers' experience was traumatic but surprisingly common. A rare disease, as defined by the European Union, is one that affects fewer than 5 in 10,000 people. According to **Rare Disease UK**, there are between 6,000 and 8,000 known rare diseases -- and five new ones are described in the medical literature every week.

They range from well-known inherited diseases such as cystic fibrosis and Huntington's disease to childhood cancers and obscure developmental conditions such as PURA syndrome. Around three-quarters of rare diseases affect children, and a third of those born with one of these conditions will not survive to see their 5th birthday.

Parents of affected children often describe the 'diagnostic odyssey' of tests, false leads and medical head-scratching they endure before the source of the problem is identified -- if it ever is. In an estimated 80 percent of rare diseases, the cause is genetic, but despite enormous progress toward understanding the human genome in recent decades, searching for mutations that cause rare diseases in individuals isn't as simple as it might sound.

One thing that has changed is the development of next-generation sequencing technology. DNA-sequencing machines don't sequence the whole genome in one go. Instead, the DNA is cut into short pieces, around 150 'letters' long, and the sequences of these fragments are read and then stitched together by comparing them with a reference sequence.

Next-generation sequencing enables hundreds of thousands to millions or billions of these DNA-sequencing reactions to be performed simultaneously, massively speeding up the process. It's

only in the past few years that researchers have been able to sequence an entire genome in a cost-effective way.

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Helen Firth, a clinical geneticist at Cambridge University Hospitals in the United Kingdom, has

spent her career trying to help those affected by rare diseases.

“Once you’ve got a diagnosis, you can try and learn from other patients with that condition about what the future might hold,” she says. This is so you can try and tailor future medical surveillance and management of the disease, she adds, as well as “provide accurate advice to other family members about the chances of another child or family member being affected by the condition.”

Yet, despite her best efforts, for many years Firth could diagnose only about a quarter to a third of the patients coming through her doors. Traditionally, geneticists like Firth used a process called karyotyping, which involves pairing up and ordering all the chromosomes to see if any are missing, duplicated or contain subtler structural changes.

Karyotyping remains a useful technique for pinpointing large abnormalities, but the level of resolution is limited to around 5 million base pairs. Geneticists’ work became easier with the development of array technology, which enabled smaller abnormalities to be detected, with a resolution of 50,000 to 100,000 base pairs.

However, neither of these techniques can spot tiny (but often significant) changes, such as a single chemical letter being substituted for a different one. For this, you need DNA sequencing, a technique first developed during the 1970s. The initial sequencing of the human genome took around 13 years and cost more than 2 billion pounds. The process has become progressively faster and cheaper.

Finding the gene:

Even so, until recently it was only practical for clinical geneticists to sequence one gene at a time -- limiting what could be achieved in an individual.

However, by 2010, array technology had improved and the cost of genome sequencing fallen to the point where Firth, together with **Matthew Hurles**, who is now head of human genetics at the Wellcome Sanger Institute in the U.K., believed they might be able to diagnose many more individuals if these technologies were systematically applied.

So, in partnership with NHS Genetics Services and several other research groups, they recruited more than 12,000 British children and adults with undiagnosed developmental disorders for the Deciphering Developmental Disorders (DDD) study and sequenced all the gene-coding regions of their DNA. They also sequenced the parents’ DNA, enabling them to identify mutations that had occurred ‘de novo’ -- either during egg or sperm production or when the affected person was still an early embryo.

“The question with these families has always been why so many of them have one very sick child and everyone else is healthy,” says Hurles, who leads the DDD study. “What we’ve found is that

when we can make a diagnosis in these children, it's often because of one of these new mutations."

One of those recruited was Evie Walker. She and her parents each gave a saliva sample, and the genetic sequence of each of their 20,000 genes was compared with a database of 1,450 known developmental disorder genes. Although doing this has enabled many DDD participants to receive a diagnosis, it didn't work for Evie. Instead, for Evie and those like her with no diagnosis in the DDD study, the researchers turned to her other genes -- those with no known link to disease -- looking for ones containing a significant excess of de novo mutations.

This led them to the PURA gene, which encodes a protein that helps regulate the expression of numerous other genes. Evie and two other girls were found to have small deletions or spelling mistakes in different areas of the PURA gene, which the researchers believed accounted for their illness.

Although the girls had similar symptoms, they weren't identical: "We're often finding that these disorders are quite variable from one patient to the next, and this might be one reason why they haven't been recognized before now -- because a clinician couldn't have said, 'These are all one thing,'" Hurles says.

To date, rare variants in nearly 1,500 genes have been shown to cause developmental disorders. So far, the DDD study has identified 30 new genes associated with developmental disorders and has led to the recognition of 14 entirely new disorders -- although the data are still being analyzed and more are likely to emerge.

"It has been wonderful to have families who I struggled for years to get a diagnosis for recruited to the DDD study, and then to have been able to sit in clinic with and actually explain the molecular diagnosis to them," Firth says.

As for Alison Walker, though she wanted a diagnosis for Evie, she underestimated the impact it would have on their lives: "We thought it would just be a name for what we were already living with. We didn't expect it to be life-changing, but then when it came, it really was."

'That's Sarah':

On the other side of the world in Melbourne, Australia, 6-year-old Sarah Anderson was going through a diagnostic odyssey of her own. The first sign that something was wrong was when Sarah, at 36 hours old, couldn't stay warm, was incredibly floppy, wasn't feeding properly and would temporarily stop breathing.

"I remember the geneticist coming in and saying, 'I give her seven days; enjoy her; spend as much time with her as you can,' and thinking, this wasn't supposed to happen," says Sarah's

mother, Mel.

Fortunately, Sarah defied the odds, although as she grew older she failed to meet the normal developmental milestones and struggled to control her movements. At 14 months she developed severe **epilepsy**, suffering up to 20 seizures per day, which left her exhausted and made her other symptoms even worse.

She was misdiagnosed with several conditions before the cost of genome sequencing fell to a point where her parents were able to fundraise the \$8,000 needed to get it done. Sequencing didn't provide an immediate diagnosis, but it did narrow the search to around 20 genes -- one of which was PURA.

The breakthrough came when a doctor from Melbourne who knew about Sarah's case attended a conference where early results of the DDD study were being presented. One slide listed the PURA gene along with other genes of interest. Afterward, the doctor walked up to the presenter and said, "We've got one of those."

Several months of emails and telephone conversations later, Sarah's neurologist was emailed a draft of the research paper the British researchers were putting together on this new condition. It contained a photo of the first child they had diagnosed.

"Suddenly, they were more excited I think than I was," Mel recalls. "Our neurologist couldn't get the words out, but then we looked at this child and ..." Mel takes a sharp intake of breath. The photo showed a pretty 4-year-old girl with a round face, long dark hair cut into blunt bangs, and a slightly open mouth. Mel thought: "That's Sarah." It was Evie Walker.

Even though Sarah's doctors could now give Mel a diagnosis for Sarah, they couldn't tell her any more about what it meant. Mel credits Sarah's neurologist with what happened next. He said, "Find the others; bring the parents together; form a foundation; find researchers -- it's the only way you're going to get the answers that you need."

Things happened quickly. In the autumn of 2014, a paper describing the three British girls with PURA mutations, plus Sarah, was published. At the same time, an American group published a study describing another group of children with similar mutations. Mel arranged to fly out to meet some of them.

On that first visit, she met with seven other families, and they agreed to set up a foundation together. Soon afterward, they launched a website and began to formally register the foundation. They then reached out to any researcher they could find who had worked on the PURA gene or the protein it encodes.

Family meeting:

One of them was **Dierk Niessing** at the Institute of Structural Biology in Munich. Niessing had spent the early part of his career trying to solve the structure of the PURA protein and understand more about what it does. Mice engineered to lack the PURA gene show serious defects in their brains and nervous systems. The mice also tend to struggle with eating, breathing and walking.

“Everything was weaker,” Niessing says. “So, my guess was that it’s an important protein, and perhaps at some point a disease would be found.”

When the 2014 paper was published, he was proved correct.

In 2016, the **PURA Syndrome Foundation** held its first conference near London, and Alison and Evie Walker attended. It was the first time Alison had met Mel face-to-face. “It was like meeting a long-lost sister,” Alison says.

“Initially it made me really sad to think that so many people had been through the same thing we’ve been through,” she says. “But it was nice to know, on the days when you just felt like putting your head in your hands and sobbing, that other people do understand.”

The DDD project isn’t only playing the role of disease detective for people with developmental disorders. One interesting theme that’s emerging from such studies is the overlap between these conditions and cancers -- both childhood and adult -- all of which are driven by mutated genes.

Embryos and cancers have several features in common: Both arise from a single cell and undergo rapid growth. And developmental signalling pathways controlling cell division and migration are often found to have become reactivated and dysregulated in cancer.

Increasingly, some of the same genes -- and even the same mutations -- are being implicated. For instance, mutations in a gene called BRAF can result in a developmental condition called **Costello syndrome**, which is characterized by intellectual disability and delayed development, loose folds of skin, unusually flexible joints and a large mouth. BRAF mutations also drive the growth of cancers, including some childhood brain cancers, as well as the skin cancer melanoma in adults.

What seems to be important is the timing of when mutations occur: Developmental disorders largely seem to arise from mutations in the eggs or sperm that aren’t present in the rest of the parents’ cells -- or from mutations occurring shortly after the egg is fertilized.

Research into adult cancer has led the way in terms of using discoveries about the genetics of the disease to find new treatments for it. Many newer cancer drugs target relatively common genetic mutations that drive tumor growth, and biopsies from affected individuals are tested to predict whether they are likely to respond.

Childhood cancer is more challenging, though. Often, the mutations driving it are extremely rare, so

you don't necessarily know what to look for. If a child fails to respond to chemotherapy, her doctors may be at a loss as to what to try next.

Far better would be to identify the driver mutation at the outset and proceed straight to targeted therapy -- and that's where sequencing comes in.

Cancer cross-over:

Since 2011, **Rajen Mody** at the University of Michigan and his colleagues have been sequencing normal and tumor DNA, as well as tumor RNA (the template that enables DNA to be translated into proteins), from children whose cancer has failed to respond to standard treatment.

Compared with DNA sequencing, sequencing RNA is more effective at detecting gene fusions -- a common type of mutation in childhood cancers.

So far, they have sequenced 375 children's tumors. In a third of cases, they have been successful in starting children on a pre-existing drug targeted to the particular mutation or fusion identified.

"It just so happens that a lot of these mutations in childhood cancer are targetable mutations, and there is a very good drug out there that has been designed for adult cancer," says **Sam Behjati**, a Sanger Institute researcher who is using genome sequencing to better understand the basis of childhood cancer.

Take melanoma. Adults with melanoma often carry a mutation in the BRAF gene, and if they do, a BRAF inhibitor drug is often effective -- at least until the tumor becomes resistant to it. Yet some children with brain cancer also have this or other mutations in BRAF, for which these drugs would also be effective.

"The whole targeted therapy business has a lot more mileage in children than in adults, because it can actually cure someone," Behjati says. "In adults, it usually only keeps the cancer at bay for a little while."

The cross-over between cancer and developmental disorders means that a similar approach could pay dividends there. "If we can piggyback on the work that's going into developing cancer therapies, it could be a way of getting much more investment in the disorders we're trying to solve," Hurles says.

The 100,000 Genomes Project was launched in England in 2012 by the then prime minister David Cameron, whose own son Ivan was born with a rare neurological disorder that baffled doctors and

eventually claimed his life.

The goal was to sequence 100,000 genomes from people with cancer, rare conditions and infectious diseases, both to find diagnoses and potential treatments for them, but also, in so doing, establish the infrastructure to offer routine whole-genome sequencing for rare diseases and cancer within the NHS by October this year.

So far, they have sequenced more than 75,000 genomes -- most of them from families with undiagnosed rare diseases -- and diagnoses have been returned in around a quarter of cases.

One of them was a boy with severe immunodeficiency and neurological disease, who died at the age of 4 months. "His parents were very distressed; they wanted to try and have other children, but they were very fearful of having another child like him -- so they asked for themselves and the child to be enrolled in the programme," says **Mark Caulfield** of Genomics England, which is charged with the delivery of the 100,000 Genomes Project. The boy's DNA revealed a mutation in a protein that transports vitamin B12 into cells.

Shortly afterward, the mother became pregnant again, and just before the baby was born they discovered he was carrying the same mutation. However, case reports of other children with this mutation suggested that giving high doses of vitamin B12 could overcome the problem, so this is what they did.

"So far, he is developing according to the normal milestones and has not had the experience of the first child," Caulfield says. "We begin to see how, not for all -- and maybe not even for many -- but for some, genome sequencing can lead to treatments."

Looking for answers:

There are potential benefits for the health system too. Caulfield cites another example: a 4-year-old girl with developmental delay, who had visited the hospital 151 times for various consultations and tests -- at a cost to the taxpayer of around 35,000 pounds (\$45,745) -- before entering the 100,000 Genomes Project, which identified the genetic cause of her problem.

"At least 20 percent of those hospital contacts could have been avoided if we had been able to do whole genome sequencing at an earlier point in her care," Caulfield says. For a 600-pound (\$784) test, you could potentially save 7,000 pounds (\$9,149).

Yet, despite the optimism, genome sequencing doesn't provide answers for everyone.

Paul Arvidson's 9-year-old daughter, Nenna, is enrolled in both the DDD study and the 100,000 Genomes Project, yet neither has come up with any answers for why she is the way she is -- at

least not yet.

For the family, the hardest thing is being unable to make long-term plans: “Because we haven’t got a diagnosis, we haven’t got a prognosis. We don’t know if her condition means she isn’t going to make it to adulthood, or to her teens. We want to be able to prepare for that -- to know if there are things we need to think about sooner rather than later,” Arvidson says.

So far, the DDD study has managed to obtain answers for around 35 to 40 percent of the families enrolled, but what about the remaining families who are still in diagnostic limbo: Why is it so hard to diagnose them?

It may be because the DDD project looks only at the protein-coding regions of the genome and conserved regulatory sequences. In the hope of finding diagnoses for more people, the DDD project has applied for access to the 100,000 Genomes Project’s data.

But large projects that are doing whole-genome sequencing -- including 100,000 Genomes -- are also encountering limitations.

One issue is that, although it’s called whole-genome sequencing, there are regions of DNA that are unreadable. “I can read 97.3 percent of the genome with good quality, but there are bits of the genome which are very hard to read because there are some bits that don’t stick back on when you try and reassemble them,” Caulfield says.

Also, whereas the current DNA-sequencing technology is good at identifying single letter changes in the DNA sequence, it’s less good at spotting more than just a single letter change, or insertions or deletions.

For extremely rare diseases, it could also be that there aren’t enough other people with the same sorts of mutation for it to be detected.

“So far, only around 30 percent of our genes have a known role in human disease, and there is much more yet to be discovered,” Firth says. “We will keep reanalyzing the sequence data from families recruited to the DDD study until 2021. This will enable us to diagnose more children as the list of genes causing developmental disorders grows through our own research and that of others.”

Risks and benefits:

With the power that sequencing brings, there is also risk. Genome sequencing carries the potential for detecting genetic variants unrelated to the condition being investigated -- but which could have serious health consequences nonetheless.

Some people might want to know if they have a high risk of developing a serious illness down the

line -- particularly if there's a chance of reducing the risk -- others would prefer not to know. The DDD project made a conscious decision not to report such 'secondary findings.'

The 100,000 Genomes Project, however, offers its participants the option of finding out if they carry genetic variants that strongly predispose them to high cholesterol or certain cancers -- such as breast and ovarian cancer, which are linked to variants in the BRCA1 and BRCA2 genes -- or that might cause cystic fibrosis in future children.

The 100,000 Genomes Project **makes a clear distinction** between gene variants that you can take action on (such as BRCA, where you can have enhanced screening or prophylactic surgery if you carry a high-risk variant) and those that you can't. For instance, some variants of the **APOE gene** strongly predispose the development of Alzheimer's disease in later life, but knowing you carry this variant doesn't enable you to prevent the condition.

In the United States, however, many more potentially life-changing results are reported. In 2013, the American College of Medical Genetics and Genomics published a minimum list of genes that it recommended should be communicated as secondary findings during clinical genome sequencing, which it has since updated to include 59 medically actionable genes.

If you're a parent seeking answers about your child's rare condition, you may feel that the benefits of a diagnosis outweigh the downsides of finding out this additional information.

Increasingly, genome sequencing is being offered to babies in neonatal intensive care units, meaning the age at which children are being diagnosed with rare diseases such as PURA syndrome is becoming lower. This may be helpful, as it cuts out those years of uncertainty and enables therapeutic action to be taken in some cases. Even in conditions such as PURA syndrome, where there is no therapy, the fact that there are other people that parents can learn about their child's condition from, and how to navigate some of the problems it creates, is a huge comfort.

"Even for genes we discovered just a few years ago, like PURA, we've now got profiles of what the typical milestones look like for children," Firth says. "We've got leaflets written with the help of the support groups that we can share with families."

Yet for PURA syndrome, there are many more questions that need answering. For one thing, why are the symptoms so variable among different individuals? "We even have 12 patients now with the very same mutation in the very same position, and they have very different symptoms," Niessing says.

For Alison Walker, the biggest question is what the future holds for her daughter. So far, only a handful of adults have been diagnosed with PURA syndrome, but it's unclear whether this is because children with the condition don't tend to survive to adulthood or because few adults have

been tested for it.

At the 2016 conference, Alison met a family whose daughter is younger than Evie but is up and walking, providing a glimpse of what Evie might be able to achieve with the right support.

Mel and Alison both hope that continuing to share information among families and increasing research into PURA syndrome will help find a treatment that will benefit their daughters. To date, some 250 people across the world have been identified with the syndrome, a condition that didn't officially exist four years ago. With every new case, knowledge about it -- and hope for the future -- grows.

The DDD study is closed to recruitment, but further funded work is continuing to search for diagnoses for individuals already recruited. Any new diagnoses will be fed back to recruiting clinicians and then to participants. **Find out more about the study.**

Wellcome, the publisher of Mosaic, founded the Wellcome Sanger Institute in 1993 and has funded it ever since. The Sanger Institute celebrates its 25th anniversary in October 2018.

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