HIS DAUGHTER'S DNA

Despite a training in clinical genetics, Hugh Rienhoff didn't know what was wrong with his daughter. So, as he tells **Brendan Maher**, he set about finding out.

Nearly four years ago, Hugh Rienhoff watched as his baby girl was pulled from a small incision in his wife's belly. It was their third child — the two boys had also been delivered by caesarean — and Rienhoff was there for all three births. But this child seemed different. He remembers her looking a little dark and sort of floppy, possibly attributable to the stress of delivery. Then he caught a glimpse of her feet, which were just a little longer than normal. For an instant, his training as a clinical geneticist kicked in. Could she have Marfan's syndrome?

In the joy of the moment the question vanished as quickly as it arose. "I didn't really think about anything from that point on medically, at least for that day," says Rienhoff. "I did all the usual things you do when you have a baby, which is cry and call my family." When the paediatrician handed his new daughter to Rienhoff, she offered some technical terms — nevus flammeus for a port-wine-stain birthmark down the middle of her face and arthrogryposis for the reluctance of her tiny fingers to extend all the way. Rienhoff had to write them down to remember them.

Although in the weeks and months after his daughter's birth the port-wine stain receded, it soon became clear to Rienhoff that she wasn't developing normally. Her fingers and toes wouldn't uncurl. More worryingly, in spite of ample feeding, the girl just wasn't gaining weight. Fleeting first impressions aside, she didn't have Marfan's, a disorder affecting maybe 1 in 5,000 births that arises from alterations in the gene for a protein called fibrillin-1. Skinny and birdlike with long fingers and flat feet, his daughter fit the physical characteristics of Marfan's fairly well, but she lacked some hallmark clinical criteria. In particular — thankfully — the typical defects in the cardiovascular system seemed to be absent, at least for now.

Rienhoff's daughter is one of the thousands of children born every year who have a congenital defect that resists satisfactory diagnosis. Such cases could be a known disorder that presents in an unusual way, or they could arise from a mutation rare enough not to have made it into textbooks or databases. Detailed genetic analyses are rarely undertaken on such cases unless a group of families with compelling commonalities can be found. Instead, these children are cared for as best can be.

But for Rienhoff that wouldn't do. Although he had largely left his practice, he had trained as a physician under Victor McKusick, the father of clinical genetics. Rienhoff knew genes, and he wanted to know his daughter's. For almost four years he has been trying to understand what makes her different at a molecular level, hoping that such knowledge could inform her care and treatment. He's quizzed experts, gone to meetings, and even set up gene-amplification equipment at home so that he can test his hypotheses with sequence data. He has also begun sharing the information he's found, telling his story on the Internet in the hope of helping others and of learning more. He may even have found a treatment that improves his daughter's condition.

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As a medical student, intern, resident and research fellow, Rienhoff trained and worked at Johns Hopkins Hospital in Baltimore, Maryland, during the late 1970s and most of the 1980s. In 1992 he put clinical medicine and research largely behind him, leaving Johns Hopkins to become a partner with a Baltimore venture-capital firm, New Enterprise Associates. After years of helping biotech companies get off the ground, he decided to start one of his own. In 1998, he and his wife Lisa Hane moved to San Francisco where Rienhoff founded Kiva Genetics, later named DNA Sciences, a company aimed at developing a high-throughput sequencing platform for use in genetic discovery and diagnostics. He left his post there in 2001, and has continued to advise and found biotech ventures.

Confounding some expectations for a corporate type who has danced at the dizzying pace of start-ups for more than a decade, the 54-year-old Rienhoff is patient, thoughtful and soft spoken. He talks in lists as if every thought has been backed up by a careful tabulation of pros and cons. That's certainly how he has managed his daughter's care. With every new doctor she's seen, every test she's been given, there's been a meticulous calibration of the risks and of the benefits that she might receive.

One of his first carefully weighed decisions is one he remains adamant about: "I didn't want to be my daughter's doctor." (And in the con part of the table, he is quick to point out that he's not a paediatrician.) Even though he has begun to practise medicine again, Rienhoff has one relationship with her — as her father — and wants no other. He's happily drawn his own blood, but when he wanted to sequence his daughter's DNA, he took her to a phlebotomist. He couldn't bear to put her through pain.

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Because of her arthrogryposis, the first doctors Rienhoff took his daughter to see were orthopaedists. They saw one ten days after her birth. "He was a thoughtful guy," remembers Rienhoff. "He said: 'This reminds me of

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Beals', but it's not complete'." Beals' syndrome is a congenital disorder largely characterized by contracted joints, like those curling fingers and toes. Aside from that, the symptoms are quite similar to those of Marfan's, from which it was first distinguished some 35 years ago. The cause is similar to Marfan's, too: but in Beals' the mutation is in the gene for fibrillin-2 rather than fibrillin-1.

Being familiar with the genetics community has its perks. Rienhoff read some papers on the disorder and contacted the authors. One put him in touch with the eponymous Rodney Beals at Oregon Health and Science University in Portland. Beals, also an orthopaedist, responded that it didn't look like the syndrome he had described in 1971. Among other things, Beals' patients typically have their 'contractures' in larger joints than those of fingers and toes; but Rienhoff's daughter's knees and elbows were lax — indeed hyperextensible. Beals didn't think that he could help.

That said, Rienhoff knows all too well the difficulty in definitively ruling out disorders such as Marfan's and Beals'. They are genetically dominant, arising from a mutation in just one of the two copies people have for most genes. The mutant gene can be inherited from either parent, but that's not necessarily the case; sometimes a new mutation will crop up in sperm or egg. And because not all mutations in a gene will affect its expression, or the structure of its associated protein, in the same way, the symptoms associated with such a mutation can be quite different from the 'classic' form of the disease. Moreover, they may take years to manifest themselves. So Rienhoff's daughter may have a defect in fibrillin-1 or 2 that no one has ever seen before, and thus be a cryptic case of Marfan's or Beals'. But because there has never been enough evidence that his daughter has either of these diseases, she has not been sequenced for these genes, although she might be in the future.

"I think the most important thing that people could take away from this is that the process is not mysterious."
— Hugh Rienhoff

Rienhoff's first visit with a geneticist didn't provide much more clarity. The doctor suggested amyoplasia congenita, a diagnosis Rienhoff calls a relic, a "dustbin" for kids with various symptoms. And the collection of problems associated with this condition is so heterogeneous as to be useless. Like arthrogryposis, the term was merely a description of his daughter's symptoms. Thousands of children receive diagnoses like these, which don't shed light on what causes the problem or how it might be treated. "I couldn't go very far with that particular diagnosis. It was clear as we went forward that she had a syndrome," Rienhoff says. He believed her symptoms were related to each other and that they were probably caused by something specific in her genes.

Rienhoff's need for clarity was not purely intellectual. About five months after their daughter's birth, Rienhoff and Hane became very concerned about her failure to thrive. Although growing taller, she wasn't putting on weight. "She was just melting away," says Rienhoff. The gastrointestinal specialists they went to see advised them to stuff her with calories, but it didn't do any good. The doctors drew up a list of things that might be causing her problems — disorders of the metabolism, of the way nutrients were absorbed from gut and stomach, of the way that mitochondria in her cells produced energy. One possibility that arose was an unusual form of cystic fibrosis, but her symptoms looked quite unlike this condition.

Rienhoff thought that a mitochondrial disorder was a particularly plausible cause. The typical symptom is muscle weakness, which his daughter clearly had, but making a precise diagnosis is very tricky. Rienhoff dove into the literature and talked with the experts, quickly finding himself in what he calls a very messy field. "That really ate up a lot of time — eight or nine months," says Rienhoff.

As Rienhoff studied the murky world of the mitochondriacs, his daughter had her first birthday and took her first steps. She was developing — and, as a result, so was what could be said about her condition. When she stood up from a squatting position, she needed to brace her hands on her thighs. This behaviour, known as Gowers' sign, is common in children with muscle-wasting diseases such as Duchenne's muscular dystrophy. Sometimes, says Rienhoff, in a hard-to-determine diagnosis, you try to find a guiding principle. The inability to form muscle mass and tone, he says, "became the North Star of the case".

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In the spring of 2005, Rienhoff and his daughter visited family and friends in Baltimore. He made an appointment with David Valle, a paediatric clinical geneticist whom he had met while working under McKusick. Valle, now director of the Institute of Genetic Medicine at Johns Hopkins, knows the limitations of his field as well as anybody. "Although there's been great progress in recent years, it still comes down to a careful [case] history, family history and physical exam, looking at the standard laboratory data and trying to put all these clinical features together to come up with some sort of diagnostic probability," he says. And even after a battery of standard genetic screening, "we're still left with maybe a third of patients who come in with morphological abnormalities for whom we're unable to make a diagnosis".

But when Valle was looking at Rienhoff's daughter with a couple of colleagues, something clicked. Her widely spaced eyes and marfanoid features, which are admittedly common in genetic disorders, looked strikingly similar to a syndrome that had just been defined. They asked the girl to open her mouth wide, and when they looked down her throat, they thought they'd cracked the case.

In January of that year Hal Dietz and Bart Loeys, both at Johns Hopkins at the time, had published a paper defining another condition similar to, and previously confounded with, Marfan's — Loeys–Dietz syndrome, to which they ascribed a related but distinct cause[1].

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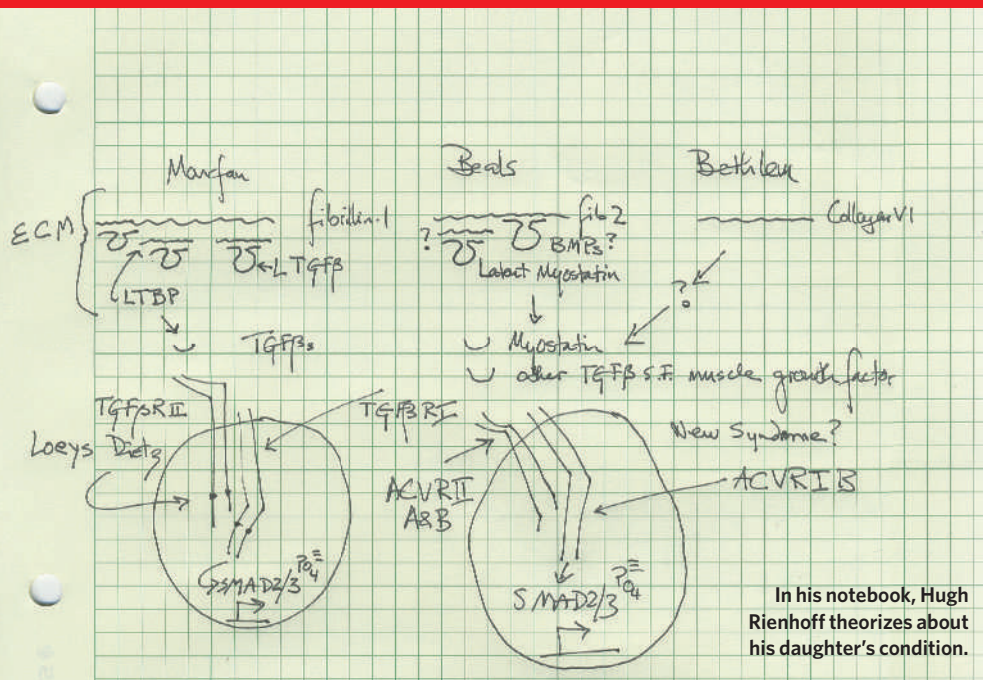
Hugh Rienhoff: father and basement DNA sequencer.



In his notebook, Hugh Rienhoff theorizes about his daughter's condition.

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Fibrillin, the protein affected by mutations in Marfan's, is a structural component of the extracellular matrix, the protein netting that holds cells together. As a result it had long been assumed that the long, thin physical features and cardiovascular problems found in Marfan's were a result of the extracellular glue being structurally unsound. More recently, various lines of evidence, including research on Marfan's by Dietz and others, have suggested that the extracellular matrix does more than passively hold cells together; it mediates communication between them[2]. Fibrillin binds and sequesters the intercellular signalling molecules in the transforming growth factor-β (TGF-β) superfamily, which plays an important role in development. Fibrillin defects, it seems, free up the TGF-β signalling system with a range of effects: bones may grow extra long; vascular tissue may degrade.

Loeys and Dietz found that some people who seemed to have Marfan's harboured mutations not in the gene for fibrillin-1, but in the genes for two TGF-β receptors. Exactly how the mutations, which seem to disable the TGF-β receptors, have an activating effect on the pathway is an ongoing puzzle. But the result is a syndrome that, because it disrupts the same bodily system, is quite similar to that caused by the fibrillin-1 defects in Marfan's.

In addition to the molecular details, Loeys and Dietz had found three obvious bodily symptoms for their syndrome: the widely spaced eyes; a cleft in the palate and/or the uvula (the soft tissue that hangs down at the back of the throat); and severe structural defects in the arteries.

Strikingly — although it had never been noticed before, despite a great deal of medical and parental inspection — Rienhoff's daughter had a forked uvula. Valle and Loeys took blood samples to sequence the TGF-β receptor genes and suggested that the girl be given an echocardiogram as soon as possible. By chance, Rienhoff had scheduled one months earlier at the suggestion of David Clapham at the Children's Hospital in Boston, who suspected that her failure to thrive might be related to a heart defect. On the plane trip back Rienhoff read the Dietz and Loeys paper, which showed detailed pictures of the patients and their devastating aortic defects. His own heart sank.

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"The problem is," Rienhoff said to me with a levelling look the first day I met him at a café in San Francisco, "she's amazingly cute." He smiled with the look of someone who knows that a desperate search for a diagnosis can sometimes end with a bad diagnosis. The average age of death for someone with Loeys–Dietz is 26. Over the year and a half of draining doctors' visits, Rienhoff and his daughter had developed a special bond. He has almost filled an entire notebook documenting his research on her case. But he has two more that are personal logs of his experiences with her, and casual observations of her and the funny things she says and does — he has similar notebooks for her brothers. One time, she asked him about a benign growth in the corner of his right eye. She calls him "Poppy", so his growth became a "poppy-oma".

On the Thursday after their return to the West Coast they went in for the heart exam. Rienhoff watched every moment of the echo, and her aorta came back "clean as a whistle" — a huge relief. The sequence data on the TGF-β receptors arrived a few weeks later; they showed none of the mutations Loeys and Dietz had identified for the syndrome. Dietz, whom Rienhoff went to visit the next year, says he wasn't completely surprised that the genetic testing came back negative. Rienhoff's daughter didn't have all the 'classic' symptoms associated with the syndrome.

It was reassuring that one dreadful diagnosis could largely be ruled out. But there was still the matter of what was actually going on. Inspired by Loeys, Dietz and Valle, Rienhoff found himself newly focused on the TGF-β signalling pathway. Maybe his daughter's disorder looked like Marfan's and Loeys–Dietz because related molecules were damaged. Rienhoff threw himself into the literature on TGF-β activation, once again guided by his North Star, his daughter's inability to build muscle.

There are dozens of different growth factors in the TGF-β superfamily and one of them, myostatin, is predominantly expressed in skeletal muscle. Defects in the gene for myostatin can result in overly muscled animals — notably the extraordinarily chunky Belgian blue cattle. In 2004, researchers in Germany and the United States described a young boy born to a former professional athlete[3]. He was remarkably muscular; at four-and-a-half he could hold two 3-kilogram weights at shoulder height with arms fully extended. Both his copies of the gene for myostatin were defective; his skeletal muscle was out of control.

Myostatin works through three activin receptors: ACVR1B, ACVR2A and ACVR2B. These look similar in sequence to the TGF-β receptors mutated in Loeys–Dietz. Rienhoff thought that a mutation in one of these specific receptors might explain why his daughter's skeletal muscle was so dramatically affected while her blood vessels were not. But as far as he knew no one had ever looked at them in relation to a disease. So he bought a used PCR machine, a microcentrifuge, some small-volume pipettes and a brand new gel box. All told, the equipment cost him about $2,000. With these simple tools and some sequence-specific DNA primers of his own design, he could pick the relevant genes out of his daughter's genome and amplify them enough for sequencing. Freezing the samples and packing the tiny tubes on ice, Rienhoff sent them off for sequencing at about $3.50 a pop. He prepared upwards of 200. If he was right, the data he got back would show a mutation in one of the genes for the activin receptors analogous to the mutations seen in Loeys–Dietz.

When he got the sequences, Rienhoff compared them to the human reference sequences in GenBank. In the gene encoding the ACVR1B receptor he found a variant. But it was a long way upstream of where he would have expected it to be, far from the active domain where many of the Loeys–Dietz mutations are found on the TGF-β receptor genes.

An obvious way to clear the mutation of any blame is for Rienhoff to sequence the copies of the gene in both his genome and his wife's. If one of them has the mutation too it is probably irrelevant — a harmless change, not one that explains the syndrome, because if it did the parent

Rienhoff, Hane and the children at home.

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with the faulty copy would share the symptoms. Rienhoff says that he plans to sequence his and Hane's genes when he gets the time.

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His notebooks are not the only record of Rienhoff's journey into his daughter's DNA. There's also mydaughtersdna.org, where he presents the clinical facts of his daughter's case both in layman's terms and also with the details required by a more medically astute audience. Although in some ways the website is a conduit for bloggish catharsis, Rienhoff sees it as an attempt to serve others with unidentified genetic disorders who are looking for answers. He hopes it will give others a chance to share their experiences, bring together parents and patient advocates, and perhaps even identify other patients with symptoms similar to his daughter's. The site got off to a slow start, but a few people have now begun posting their stories, including Rienhoff's colleague Clapham, who recounts the heartbreaking loss of his son, Ben, to an inexplicable neurological disorder.

With help from George Church, a Harvard Medical School professor with an extensive track record in new technologies for sequencing and synthesizing DNA, Rienhoff developed a sort of 'phenotype spreadsheet' on which to record his daughter's clinical history. The idea is that such data representations might someday allow a computer to search through his daughter's symptoms along with those of others with unidentified genetic disorders looking for clinical commonalities. Church plays down his contribution as just a seed of an idea that he thought worth testing, but says he is intrigued by Rienhoff's gumption: "I'm interested in cases of altruists who, rather than hiding from genetics, are using the opportunity to be sort of social activists, working to raise consciousness and maybe raise money for diseases affecting their family and friends."

Such activism is not new. Parents quite frequently become advocates for research on behalf of their children — when they are rich, famous, persistent, lucky or very well placed, they can make a difference. But with a sequencer and a website, Rienhoff has stepped over the threshold of personal genomics in a way set to catch the imagination. As sequencing gets ever easier and knowledge bases ever larger, it may not be fanciful to imagine more and more people following him, developing theories about abnormalities and testing them through sequencing. Such attempts will often fail, and in some cases lead to frustration and heartache. But some may make significant contributions to our understanding of the function of various human genes.

"I'm really being given an opportunity at this time in the history of genetics."
— Hugh Rienhoff

Rienhoff recognizes that he has benefited from his training and connections. But he told me part of his mission is to empower others. "I think probably the most important thing that people could take away from this is that the process is not mysterious," he says.

His enthusiasm is not universal. In the course of my reporting, Rienhoff gave his daughter's doctors permission to speak to me, and not all of them agreed that he was doing the right thing. Dietz says he worries that Rienhoff's example may lead some parents down the wrong path, searching for answers in the genes and diverting resources from the important goal of making sure their children are receiving proper care.

Rienhoff has heard these criticisms, and understands the discomfort. "There is a certain sense that all of this will unravel, meaning all of this will become driven by the people," he says. In deference to Dietz he has removed from his website a folder called 'How to sequence DNA' that he had never filled. "The purpose of the website is not about teaching people how to sequence DNA, at least not now," he says. But he still believes that patients and patient advocates can usher in what he calls a golden age in genetic research. It won't be for everyone. Rienhoff's search has been slow and methodical, and as yet inconclusive. Still, it has been fulfilling. "I'm really being given an opportunity, if you will, with this site and at this time in the history of genetics."

And the journey continues. Despite his daughter's DNA revealing not quite what he had expected, Rienhoff is still hopeful about his myostatin hypothesis, and it has led him to the most difficult decision he's ever made about his daughter. In May, based on the hypothesis that errantly activated signals might account for her inability to build muscles, Rienhoff, Hane and their daughter's cardiologist decided to put her on losartan, a drug for treating high blood pressure. Recent evidence suggests that it reduces the activity of secondary messengers triggered by TGF-β receptors[4], and that marfanoid mice are helped by the drug.

Rienhoff knows it is a controversial move, but there are two quite powerful factors on his pro list. First, if his daughter does have some aberrant form of Loeys–Dietz or Marfan's, the drug could forestall the vascular disease associated with the condition. Although he may eventually sequence her fibrillin and other genes for these disorders, the most definitive answers will come from regular cardiograms. The side effects of losartan are minor and reversible, he says, but vascular disease isn't. Second, her muscles might get a little better. "I tortured myself over that one," he says. "I took a Hippocratic oath — but I also took a parental oath — to do no harm."

Meanwhile he devours any literature on TGF-β signalling he can find. He has begun looking for scientists with whom he might collaborate on related projects, such as finding other patients with similar symptoms who might have mutations in the genes he's been looking at, or creating knock-out mice. He's waiting for more people to start using his website. He keeps a close eye on his daughter's progress, where he sees grounds for hope. "Her more proximal muscles seem to be growing," he says. "She can walk upstairs with a little assistance."

But he's cautious not to overinterpret. "I can't ascribe it to anything. I just keep my fingers crossed that she doesn't have a vascular disease. It's a quiet vigil." ■

Brendan Maher is a features editor at *Nature*.

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