How One Child Turned a Sequencing Skeptic into a Believer

Nearly two years after next-generation sequencing solved a child's mystery illness, the physician who doubted the technology now sends his samples to Illumina.

Introduction

Dr. James Verbsky is the first to admit that he chuckled skeptically when a colleague suggested sequencing a child with an unsolved illness.

The young boy suffered from a severe intestinal disease that had baffled the experts for years. Dr. Verbsky, a seasoned immune system specialist and researcher, is used to seeing unusual cases. He treats children with serious autoimmunity and immune deficiency disorders, and is involved in screening Wisconsin newborns for severe combined immunodeficiency (SCID). SCID is a group of inherited disorders that leaves infants extremely vulnerable to infectious diseases, and can be fatal within the first year of life.

Not once had Dr. Verbsky seen a case like this. Though he had never tried sequencing, his experience told him that sifting through thousands of mutations to find an answer could be a long and futile effort. Still, at a virtual dead-end in the search to find a lasting solution for the boy, he figured that sequencing was worth a try. The outcome was nothing like he expected.

Q. Tell us about your experience with next-generation sequencing. Why did the results surprise you?

James Verbsky (JV): This child had a very aggressive inflammation of his colon that kept getting worse. We spent years evaluating his immune system—not just us, but several centers and dozens of specialists. We were at a loss as to what to do, because even the most aggressive immunosuppressive therapies were failing. The easy solution would have been to perform a bone marrow transplant, but we didn't have a diagnosis. If it turned out that he had a defect in the cells of the intestines, a bone marrow transplant could actually make things worse. So, one of my colleagues suggested sequencing.

I initially thought it was a silly idea, because we needed to confirm that a mutation in a protein results in loss of function. I figured we'd find around 20,000 mutations, which means we'd spend the rest of our lives trying to prove it. Studying just one mutation takes months.

But, sequencing worked. We got very lucky—it only took three or four months to identify and confirm the gene with the mutation. This allowed us to determine that a bone marrow transplant was the right solution. There might be complications from the transplant, but the inflammation in his intestines is gone now. The experience turned me from a sequencing skeptic into a believer pretty quickly.

Dr. James Verbsky, MD, Ph.D., is a physician at Children's Hospital of Wisconsin, specializing in autoimmune and immune disorders. He also runs a research lab at the Medical College of Wisconsin.

Q. How do you currently screen newborns for SCID, and how can sequencing help in those cases?

JV: We use a PCR-based assay from blood spots taken during the screening. It will pick up any type of SCID because it just measures T-cell numbers. A normal newborn should have 2,000 T-cells in a microliter of blood, so we look for low to zero T-cells to indicate a problem. In general, if they have a thymus, the area where T-cells develop, the treatment will be a bone marrow transplant. But, some kids don't have a thymus, so they won't have any T-cells. That's where sequencing comes in.

If we can sequence, quickly find a gene that's involved in T-cell development, and confirm it is a T-cell intrinsic defect, we'll know that the child can be cured with a bone marrow transplant. Otherwise, there are many reasons why there could be low T-cell numbers. We're hoping genome sequencing will lead us to those answers as well.

Q. How many children have you sequenced since your first case?

JV: We've sequenced two more children who had SCID and no obvious mutation. We have a small window of time for the most effective treatment—about three months—so the speed that sequencing offers in these cases is essential. The faster we find the mutation, the faster we can do a bone marrow transplant.

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We already know a number of diseases that cause this immune deficiency, and which 30 or so genes to look at. Sequencing is more efficient and cost-effective than looking at one gene at a time.

In both cases, we were able to quickly rule out the known genes, which was very helpful. Now, we're left with a new disease, which means we have to keep testing. Unfortunately, the capability for a full genome analysis in three months just isn't here yet. We can get the data quickly—we got the last case within two weeks from Illumina—but we're limited when it comes to analysis.

> "We have a small window of time for the most effective treatment, so the speed that sequencing offers is essential."

Q. Once you know you're looking at a new disease, how do you confirm the mutation that's causing it?

JV: It's a challenge, because it takes years to gather enough knowledge about a protein to be able to test it. We got lucky with the first case because there were known functions of the mutation. It was an antiapoptotic protein, so we just tested the cells to see if they died easily, and they did. There had also been a recent publication that helped to confirm that this was the mutation we were looking for. It all made sense.

Our analysis of that case went really smoothly, so our goal with the two recent cases was to do something similar; just sequence and say "ah-ha, here's the mutation." But, they're proving to be more difficult. We have to get even more creative with our tests.

Q. You're calling yourself a believer in sequencing now. When do you think the rest of the medical community will see it as a valid test as well?

JV: In the next couple of years, especially with rare diseases. I know of a case where our geneticist had the insurance company pay for the sequencing, because it was cheaper to sequence everything than to guess which genes could be defective. As the price comes down and analysis improves, it will become increasingly accepted.

For us, when it comes to screening newborns, we're hoping it turns into a model where every child without an obvious mutation is sequenced. We just need to prove that it works each time.

Q. Since your first sequencing case, have you heard from other doctors who have also sequenced patients with a rare disease?

JV: Absolutely. There were already several publications where sequencing was used for rare diseases. I can think of two recent cases off the top of my head. For rare, severe, early onset diseases, I think sequencing will prove to be very effective.

Q. Are you considering bringing a sequencer into your lab?

JV: Our institution will do more sequencing—we see at least five rare disease cases each year where sequencing could be beneficial. But I'm leaving that to our researchers at the genomic center. They send our samples to Illumina, get the data, and do the analysis. My lab does the functional testing.

Q. What do you enjoy the most about the work you do?

JV: It's great to be able to give a child or family an answer, and to treat them, especially if they've been sick for a long time. It's also fascinating to discover new diseases, as we did with our first sequencing case. I'm still doing research on that disease, to try to determine why he got sick and the mechanism of inflammation. I keep learning more and more about human biology. It's all very exciting.

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