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Diagnosis Emerges from Complete Sequencing of Patient's Genes

For the first time, scientists have diagnosed a genetic disease by completely sequencing all of a patient's genes. Using high-throughput DNA sequencing technology, Howard Hughes Medical Institute (HHMI) researchers successfully identified a gene mutation that was responsible for the patient's disease, but had not been suspected based on clinical observations.

Starting with DNA from a blood sample from the patient -- an infant in Turkey who was persistently dehydrated and failing to gain weight -- the team found in 10 days a gene mutation known to affect electrolyte transport in the intestines and cause a condition called congenital chloride diarrhea. Doctors in Turkey confirmed the diagnosis clinically and were able to provide a treatment tailored to the disease.

Instead of searching the patient's complete 3-billion-basepair genome for the disease-causing mutation, the team, led by HHMI investigator Richard Lifton at the Yale School of Medicine, focused only on the small fraction of DNA that encodes proteins. The success demonstrates that this strategy is a viable and efficient means of diagnosing genetic disease.

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- Richard P. Lifton

"I think in the coming years we're going to see a dramatic increase in the use of this kind of technology," Lifton says. "This is going to be a very powerful technology for disease-gene discovery and clinical application." Lifton and his colleagues reported their method and diagnosis in an advanced online publication of the *Proceedings of the National Academy of Sciences* on October 19, 2009.

“This is the first volley in what I think is going to be an important era in molecular diagnostics,” he notes. “Up to now we’ve been guessing what genes might be mutated and selectively sequencing a handful of genes to try to find a mutation. This paper demonstrates a new capability to capture, ostensibly, all of the genes. Instead of sequencing ten or a hundred or a thousand genes to try to find a mutation, we are now capable of sequencing all of the genes and making clinical sense of the resulting data.”

Only about one percent of the human genome serves as blueprints for protein production. Mutations in these protein-coding regions can alter a protein’s function or prevent it from being made at all – often with significant effects on health. For this reason, 85 percent of mutations known to have major clinical effects lurk in this small portion of the genome. The remaining 99 percent of the human genome contains valuable information -- such as when and where genes should be turned on – and errors in “non-coding” regions can also be problematic. But when searching for an unknown disease-causing mutation, Lifton and his colleagues reasoned, it made the most sense to start with the one percent of DNA where those mutations most commonly occur.

That protein-coding portion of the genome is also known as the “exome,” because segments of protein-coding DNA are called exons. For the past six months, Lifton and Murim Choi, a postdoctoral fellow in his lab, have been developing a method for completely and accurately sequencing the exome.

The first step of their approach is to separate the exome from the vast amounts of DNA that do not encode protein. To do this, the team employs a microarray chip that has on its surface 180,000 short pieces of DNA – bits of the coding-regions of 18,673 human genes. When a sample of DNA is applied to the chip, protein-coding regions pair with their matching segments and stick. Researchers can wash away the unstuck DNA, then shear the coding-regions away from the chip and collect them for further analysis. With the protein-coding DNA collected, the next step is to sequence it. Today’s sequencing technology makes it possible to do this rapidly and efficiently, Lifton says.

Once Lifton and his colleagues were satisfied that their method was accurate and sensitive enough to find mutations hidden in the 34 million base pairs that make up a complete exome, they turned their attention to the patient referred to the lab with an undiagnosed condition. Doctors suspected a rare disease called Bartter syndrome, which affects the kidney – but wanted Lifton’s team to determine whether genetics confirmed their suspicion. “This was an opportunity to try our technique in a case where we didn’t know what the answer was,” Lifton says.

Using a sample of the patient’s blood, Lifton and Choi’s colleague at Yale, Shrikant Mane, sequenced the exome. Within a few hours of receiving the sequencing data, Choi found the mutations that caused congenital chloride diarrhea and were responsible for the patient’s symptoms.

“There has been concern that [this sequencing strategy] would be so involved and generate data that would be so difficult to handle that you couldn’t do this without a team of a dozen informatics people. This is really not the case,” Lifton observes. “This is really quite straightforward to perform and interpret.”

Additionally, he notes, because the cost of DNA sequencing has come down dramatically in the last ten years, exome sequencing is currently very affordable in a research setting. Lifton says it will be important to continue to drive the cost down so that the method can see wide clinical use. “We would like to see the cost come down rapidly to where you wouldn’t blink an eye to get the test,” he says. “The cost to do a test today would be a couple thousand dollars, but we think that cost is going to drop precipitously in the next five years, and will come into the realm where it will be considered to be a routine clinical test.”

Outside of clinical use, Lifton says exome sequencing offers a means to speed the discovery of disease genes, particularly in cases where gene mutations are not passed on from the parents. “In genetics now we’re quite good at finding mutations where we are able to pinpoint the location of a disease-causing gene because it is present in many family members, but we haven’t had a good method to find mutations that arise anew in an affected child of unaffected parents,” he says. “The ability to sequence all the genes will enable one to find these new mutations and link them to disease. We anticipate this will be relevant to a range of diseases such as autism and congenital heart disease.”