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Geneticist develops tool to identify genes important in disease and for tailoring individual treatment

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Though the human genome has been sequenced, scientists are still trying to figure out how the accomplishment can help people, for example, how it can be used to treat disease. As University of Massachusetts Amherst geneticist Jacob Mayfield notes, "It was easy to think of the human genome as the big prize, but what we realize now is, it's just a foot in the door."

"What we're beginning to understand is that the information we're interested in knowing lies in comparisons between genomes," he adds. As society moves to personalized, genome-based medicine, "much work remains for us to grasp what it is that we know. As we uncover new variations at the DNA level, we have to address their consequences. To learn what genetic differences mean to the whole organism, we have to find ways of asking about function. Because the way genes interact to cause traits like red hair isn't understood, let alone how they cause disease!"

To tackle this and make a difference in a human disease, he and colleagues at the University of California Berkeley devised a technique for testing the consequence of variant human gene alleles (alternative sequences of a single gene) by moving them into yeast cells. Once swapped into yeast, colony growth can be compared to reveal functional differences. The technique is reported in the current issue of Genetics.

For this study Mayfield's team first used DNA sequence databases to select variant gene sequences that tested positive for the metabolic disease homocystinuria, which can cause a range of mild to severe symptoms such as blood clots, mental retardation and other problems. Deficiencies in a gene called Cystathionine-beta-synthase (CBS) are often the cause. Because early intervention for homocystinuria can eliminate severe symptoms, newborn screening began in the 1970s.

(Medical Xpress) -- The researchers selected CBS gene sequences from 84 patients and made a piece of recombinant DNA that matched the patient's sequence using a process called site-directed mutagenesis. Each gene differed from the others at a single DNA position. "So in each case among the 84, we know there is a patient who had this exact change, accompanied by mild to severe disease," Mayfield explains.

Next, the researchers transferred each of the variants into a yeast cell, yielding 84 yeast strains that differed at only a single place in the CBS gene. A control strain containing the fully functional major allele of the CBS gene was constructed for comparison.

"Once we put an allele into yeast we can conduct experiments to ask whether it's functional or not by growing the colonies, both in dishes and in liquid culture," the geneticist explains. A mildly mutated allele may allow yeast cells to grow normally, but severely mutated cells fail to grow at all. Many variants function somewhere in between. "When you know where two sequences differ, you can start to identify changes that affect biological function from those that don't," he says.

But "the really interesting and exciting part of this project is what we learned about treating the disease," Mayfield adds. Some homocystinuria patients respond to simple vitamin B6 supplementation, while others require severe dietary restriction to avoid disease symptoms. In a series of experiments with yeast in liquid culture, the researchers added

different levels of supplemental vitamin B6 to the 84 disease alleles and found that 37 percent of the alleles were functional or could be rescued by vitamin B6. "In the clinic, then, you can expect the patients with these alleles to respond to B6 treatment," the geneticist explains.

"This method gives us information about allele function and is applicable to other disease genes. Metabolic diseases and proteins that need vitamin co-factors are amenable to similar studies. Our findings indicate clearly that sequences alone can't tell the full story. This is a first step in gaining information from the genome to move toward a treatment. We can't claim to cure the disease, but it is good news."

This study was funded by the Howard Hughes Medical Institute as part of a larger project at UC Berkeley to design an entry-level biology curriculum that included discovery-based learning. It was performed in part by undergraduates mentored by Mayfield, who feels it is extremely important that university-level science courses begin to teach the next generation techniques they will need to make sense of natural variation in gene sequences and the difference between natural variation and disease.

"Right now, our ability to generate DNA sequences is far ahead of our ability to understand it," he notes. "We're going to see in our personal genome sequences that each of us has variations in disease genes. We must not panic. In this study, even in the extreme case of sequences from patients with a disease, we saw that some variants were coincidental, not causal, while others were treatable. We need to develop a suite of tools like this to help us investigate which alleles are functional and which are not, before we can comprehend the human genome."

Provided by University of Massachusetts Amherst