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## Rare Mutations Can Significantly Increase Risk Factor for Heart Disease

Certain rare gene mutations can contribute significantly to low levels of a beneficial form of cholesterol in the blood, researchers have found. Low levels of this cholesterol, known as high-density lipoprotein (HDL), are a major risk factor for heart disease.

Gene mutations previously known to affect HDL levels had small effects individually, and it was thought many such mutations needed to accumulate before HDL levels were significantly reduced. The new finding, however, demonstrates that mutations in a few genes can be sufficient to affect blood cholesterol levels. According to the researchers, the strategy used in this study can be generalized to analyze the role of rare variations in candidate genes in other clinically important complex human traits.

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— **Helen H. Hobbs**

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Led by Howard Hughes Medical Institute investigator Helen H. Hobbs, who is at the University of Texas Southwestern Medical Center at Dallas, the researchers published their findings in the August 6, 2004, issue of the journal *Science*. Hobbs' colleagues from the University of Texas Southwestern and the University of Ottawa Heart Institute were coauthors on the paper.

HDL is important for preventing heart disease because it transports cholesterol in the blood back to the liver, leading to its removal from the body and preventing its buildup on artery walls. The level of HDL in the blood is a complex trait, influenced to varying degrees by many genes, as well as environmental and lifestyle factors such as diet and exercise.

Previously, researchers believed that the genetic component of this trait depended primarily on the cumulative effect of many common genetic variations, each of which influenced HDL levels in a small way. However, rare variations with stronger effects are also likely to be involved. "What we wanted to know," Hobbs said, "was how much do single gene defects with

major effects contribute to complex traits?”

Although mutations with such a strong effect on overall HDL levels may be rare individually, the researchers said, collectively, mutations of this type might be common enough to contribute to the variation seen throughout the population.

To determine the influence of rare mutations, the scientists relied on data from individuals enrolled in a study run by Hobbs, known as the Dallas Heart Study. Hobbs and her colleagues designed the study in 1999 to examine the biological and social causes of ethnic disparities in cardiovascular disease. Through this study, they have collected extensive data and samples from approximately 3,000 multiethnic participants through health surveys, blood and urine samples, and imaging studies.

The usual approach to identifying relevant genes, Hobbs said, is for researchers to identify a mutation in individuals with low levels of HDL and look for the frequency of that mutation in the general population. In this study, however, the researchers increased the likelihood of detecting gene mutations that significantly influenced the final trait by focusing on individuals at the extreme ends of the spectrum of HDL levels. Using 128 subjects from the Dallas Heart Study, they compared the genes of those with HDL levels in the lowest five percent of the population to those whose HDL levels fell in the top five percent.

“The value of the approach,” Hobbs said, “is that you not only get the common variations, but you can address whether individual rare genotypes also contribute to the phenotype.”

In their search for genetic differences between the two groups, the researchers focused on three genes that, when defective, had been implicated in rare forms of HDL deficiency. These were genes coding for proteins that play critical roles in cholesterol metabolism and transport, known as apolipoprotein A1 (APOA1), adenosine triphosphate binding cassette transporter A1 (ABCA1), and lecithin cholesterol acyltransferase (LCAT). Individuals with two defective copies of any of these genes have virtually no HDL circulating in their blood, whereas individuals with only one functional copy of the gene have about half the normal plasma level of HDL.

The researchers sequenced the three genes and looked for changes in the DNA that would alter the resulting protein. Such variations were found in 16 percent of the individuals with low HDL levels, whereas they occurred in only two percent of those with high HDL.

To ensure that these differences were specific to mutations that might affect HDL levels and exclude the possibility that mutations in general were occurring more frequently in one group, the researchers also compared the frequency of non-synonymous sequence variations - those that exist only at the DNA level and would not affect the protein. They found these to be similar in the groups with high and low HDL levels.

Additionally, they confirmed their results in an independent population of patients and examined cells from the individuals in that analysis to show that sequence variants in the low-HDL group were functionally important.

According to the researchers, the results of their study provide direct evidence that rare variations in DNA significantly affect levels of HDL in the blood. A better understanding of the DNA sequence variations that contribute to low levels of HDL will not only help researchers understand the disease, but, with further study, may also help in the identification of new treatment targets and diagnostic tests.

This new understanding of the role of rare genes is just one piece of the complex network of factors that shape heart disease risk, but according to Hobbs, the Dallas Heart Study provides an invaluable means to further examine those factors and their interplay. “We've developed a carefully phenotyped population, with information not just on lipoprotein levels, but also drugs, weight, family history, etcetera,” she said, “so we can now go back and look at how those things together may have shaped these individuals' risks.”