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## New Technique Greatly Increases Sensitivity of Genetic Testing

A new laboratory method improves the accuracy of current genetic diagnostic tests for colon cancer by detecting defective genes otherwise "masked" when one copy of the gene in question is normal. The technique may also be used to enhance the accuracy of diagnostic tests for a wide range of inherited diseases, including other forms of cancer and neurological disorders.

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Humans carry two copies of each gene, one of which is inherited from the mother and one from the father. One of the weaknesses of genetic testing is that a normal gene can mask the presence of the defective or missing gene. In instances where masking occurs, the diagnostic tests will either not detect the genetic defect or will prove far less sensitive to it.

"We have overcome the problem of the normal allele masking the mutant allele by simply separating the alleles and analyzing them independently," explained Bert Vogelstein, a Howard Hughes Medical Institute investigator at The Johns Hopkins University Oncology Center. In technical terms, the technique involves conversion of the cells' paired-chromosome state of "diploidy" to a single chromosome state of "haploidy," said Vogelstein.

The genetic testing enhancement was reported in the February 17, 2000, *Nature* by researchers led by Vogelstein and Hopkins colleagues, Hai Yan, an HHMI associate, and Kenneth Kinzler.

The conversion technique involves fusing human cells possibly containing a defective gene with a special strain of mouse cells developed by the researchers. The mouse cells are deficient in the machinery that continually rejects foreign chromosomes and, thus, can be made to harbor human genes that function normally.

Included in the assortment of fused human-mouse cells are some that have come to possess only the single defective copy of the human gene. The scientists can isolate these cells and use them as a basis for a far more sensitive genetic test than using cells containing both normal and defective gene copies.

In the *Nature* paper, the scientists demonstrated the conversion technique's power by showing that they were able to verify the presence of mutant genes in 22 patients with hereditary non-polyposis colorectal cancer. In contrast, conventional genetic testing detected the mutant gene in only 10 of the 22 patients.

Vogelstein and his colleagues predict that the conversion technique will prove widely applicable to enhance tests for many other genetic diseases.

"The sensitivity of the tests for those other diseases will depend on the nature of the mutations," he said. "But certainly for many of them, the sensitivity should be similarly increased substantially. And for certain hereditary colon cancers, virtually all mutations in a gene will be detectable by this approach.

"The technique is simple enough that it should not add much to the costs of testing for certain diseases if it is implemented in clinical laboratories," said Vogelstein. And in fact, in some cases conversion will considerably lower costs by enabling more efficient new tests.

"Once you don't have the complicating diploidy and the other allele, the signal-to-noise ratio is enhanced enormously," said Vogelstein. "Thus, one can imagine applying a whole range of analytical techniques now on the drawing boards that are complicated by diploidy." For example, he said, genes from such haploid cells could be effectively detected using "microarrays" chips containing many thousands of base pairs that can be tested simultaneously. Vogelstein emphasized that the conversion technique must still be refined before it can be introduced into routine genetic testing.

HHMI investigator Sanford Markowitz at Case Western Reserve University, who is also an author of the *Nature* paper, agreed, adding that "there is still some work to be done to make it easy to do in clinical practice. However, the conversion strategy is a real step forward in improving the sensitivity of these tests. The technique will clearly increase the number of individuals for whom we will be able to make a diagnosis." Markowitz and his colleagues are collaborating with the Vogelstein laboratory to improve the ability to distinguish specific cells resulting from the conversion technique.

The conversion technique will also allow basic research advances in understanding the mechanisms of cancers and other diseases, said Vogelstein.

"For example, there are many families with certain inherited colon cancers in which no mutation has been found. And the question is whether they have a mutation that conventional techniques have not detected or whether they have a mutation in an unknown gene. Discovering these genes would not only be important in genetic diagnosis, but could help illuminate the mechanisms underlying the disease," Vogelstein said.