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## Researchers Pinpoint Cause of Deadly Blood-Clotting Disorder

Researchers have determined the cause of a potentially deadly inherited blood-clotting disorder that can lead to kidney failure or stroke.

The researchers found that the disorder, thrombotic thrombocytopenic purpura (TTP), is caused by mutations in a gene that render the ADAMTS13 enzyme ineffective. The research suggests that it might be possible to treat TTP by administering an active form of the enzyme, in much the same way that people with hemophilia receive clotting factor.

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In an article published in the October 4, 2001, issue of *Nature*, a research team that included Howard Hughes Medical Institute investigator David Ginsburg at the University of Michigan reported that mutations in the *ADAMTS13* gene were responsible for causing TTP in nearly all of the affected families studied.

"Until about four or five years ago, the cause of the disease was a mystery," Ginsburg said. "It was known as a dangerous disorder, in which patients suddenly became very ill and could be treated only by replacing their blood plasma. No one was really sure, in fact, whether such treatment was removing something bad or adding something good that was missing."

Several earlier studies had implicated a clotting-related protein known as von Willebrand factor (VWF) in the disorder. These studies found that the blood of patients with TTP showed an abnormally large form of the VWF protein that had not been cleaved into two smaller sizes, as is normally the case.

Thus, said Ginsburg, many scientists believed that a defect in a protein-clipping enzyme known as a protease might be responsible for the disorder.

One of the keys to identifying the gene mutations that underlie TTP was the development of a precise assay for detecting VWF protease activity. Han-Mou Tsai, a senior author of the *Nature* paper, and colleagues at Montefiore Medical Center and Albert Einstein College of Medicine developed the assay and applied it to blood samples that were provided by members of four families that had an inherited form of TTP. The assays clearly revealed that within these families, those who had TTP showed low VWF protease activity, while carriers of the disease showed medium levels of protease activity, and unaffected individuals showed normal levels.

"The findings seemed almost too good to be true," said Ginsburg. "They clearly showed the presence of a recessive gene in which all the carriers, who had one good copy and one bad copy of the gene, had about half the level of protease activity."

Using results from the assay as a guide, Gallia G. Levy, lead author of the *Nature* article, performed linkage analyses of the family members and determined which of known genomic markers were inherited with the disease gene. These studies enabled her to narrow down the region containing the disease gene to a specific region of chromosome 9.

A search of the human genome database found several fragments of genes resembling proteases that were attractive candidates, said Ginsburg, but they could not be sure because the database was incomplete for that region of chromosome 9. When Levy studied the TTP patients for mutations in the target region, she found mutations in a gene that coded for a protease that showed DNA sequence similarity to members of a family of zinc metalloproteinases, called ADAMTS.

Levy then obtained the full gene sequence and proceeded to test the other patients for mutations in the gene, which they named *ADAMTS13*. Levy subsequently identified a dozen mutations in the gene among the patients, accounting for nearly all the cases of TTP. According to Ginsburg, Levys findings open the way to understanding how and why the *ADAMTS13* protease cleaves VWF and how the failure to cleave the protein causes disease.

"The current hypothesis in the field is that the large form of VWF that is initially made is too sticky. Unless it is cleaved, it spontaneously sticks to blood platelets and clogs vessels," said Ginsburg.

The discovery of the role of the ADAMTS13 enzyme also suggests a relatively straightforward therapy for TTP, said Ginsburg. "It doesnt appear to take much of this protease to treat this disease, and it lasts for a while in the blood" he said. "So, it might be possible to give people with TTP a periodic injection of the enzyme to maintain their protease activity. Such treatment would work better and be safer than plasma exchange because of

the risk of complications from transfusions."

Ginsburg also noted that a form of acquired TTP clotting disorder can be a complication in patients receiving bone marrow transplants and those with lupus or AIDS. "While the carriers seem normal, it could be that they are more susceptible to acquired TTP in such cases," he said.

In other independent studies, the structure of the ADAMTS13 protease and identification of its gene have also been deduced by a collaboration of HHMI investigator Evan Sadler and researchers Dominic Chung and Kazuo Fujikawa.

In that work, Fujikawa and Chung, who are at the University of Washington in Seattle, purified the ADAMTS13 protein, obtained a partial amino acid sequence, and identified the gene based on data in the Human Genome Project database. Starting with that partial sequence, Sadler and his colleagues at Washington University School of Medicine in St. Louis collaborated with the Seattle group to determine the complete cDNA and protein sequence. Their results have been published online by the *Journal of Biological Chemistry*.

"Our data are entirely in agreement with David Ginsburgs," said Sadler of the findings by Ginsburg and his colleagues. "But Gallia Levy and David have made a further, major advance in the field by identifying mutations in this gene that actually cause a serious and sometimes fatal human disease."