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Researchers Discover New Route to High Blood Pressure

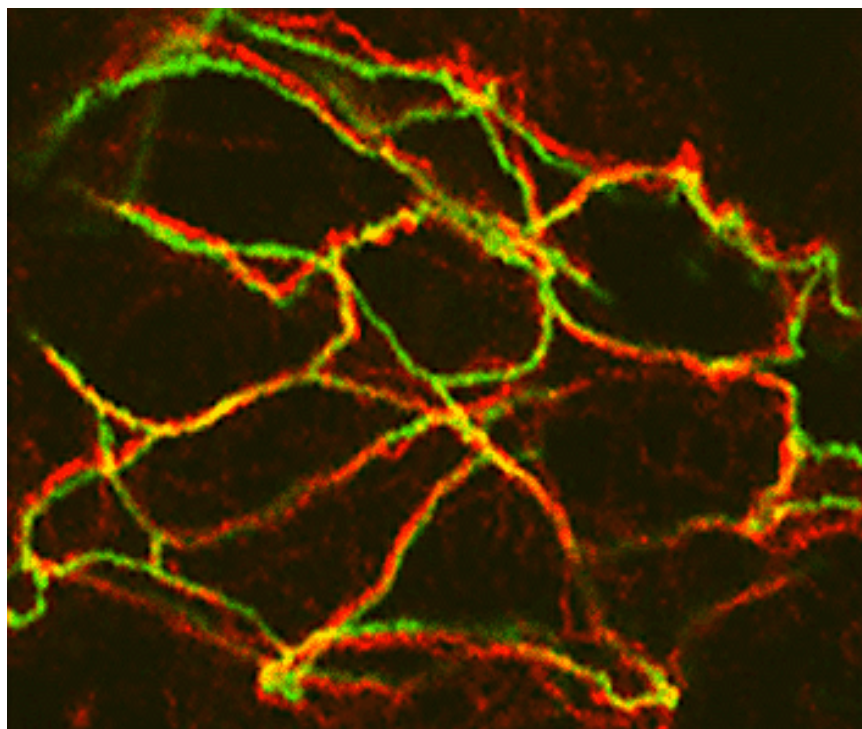


Image Title: A composite confocal image of cells of the distal convoluted tubule of the kidney is shown stained with antibodies to WNK4 (red) and the tight junction protein ZO-1 (green). The two proteins co-localize, establishing WNK4 as a component of the tight junction. - Keith Choate and Richard P. Lifton

After years of detailed genetic analysis, researchers have discovered two genes that underlie a new metabolic pathway that governs blood pressure in humans. These findings could offer novel molecular targets for new blood pressure medicines.

High blood pressure affects about one-quarter of all adults worldwide and is an important risk factor for death from stroke, heart disease, and congestive heart and kidney failure. In an article published in the August 10, 2001, issue

of the journal *Science*, an international research team led by Howard Hughes Medical Institute investigator [Richard P. Lifton](#) at Yale University School of Medicine reported identifying two genes that cause pseudohypoaldosteronism type II (PHAII). This disorder leads to hypertension by causing increased reabsorption of salt by the kidneys and impaired secretion of potassium and hydrogen ions.

Although the disorder seemed to point to an unknown cause of hypertension, said Lifton, tracing its genetic roots in affected families proved difficult. "In contrast to the other single-gene forms of high blood pressure we have studied, PHAII was complicated," he said. "Patients with the disorder get hypertension as adults -- rather than as children -- like the majority of people with hypertension, and the abnormal potassium and acidity levels are variable. This complicated unraveling the genetics."

After attempting to trace the genetics of the disease in numerous affected families, Lifton and his colleagues identified two types of families -- one with a gene mutation on chromosome 12, and the other with a mutation on chromosome 17. This provided researchers with the information they needed to begin to zero in on the genomic location of the mutated genes.

In analyzing genetic data from a family with a mutation on chromosome 12, the researchers determined that the disorder appeared to be due to a deletion of a segment of DNA in a large region of the chromosome. Fortunately, said Lifton, analysis by Frederick H. Wilson at Yale, the lead author of the *Science* article, yielded a critical clue that helped the researchers pinpoint the gene.

"One of the genetic markers he was using was completely absent in chromosome 12 of the disease gene, but present in normal genes," said Lifton. "That was an incredibly fortunate stroke of luck, without which we would still be searching."

A search of a human genome database revealed that the disease-causing deletion lay within a gene called *WNK1*, which is expressed at high levels in the kidney, heart and skeletal muscle. The *WNK1* gene codes for a type of enzyme, called a serine-threonine kinase, that often acts as a metabolic activating switch in cells.

WNK1's role in PHAII was confirmed when the researchers discovered an overlapping but different *WNK1* deletion in members of another family with chromosome 12-related PHAII. To understand how the deletion might cause PHAII, the scientists next studied the function of the mutated *WNK1* gene in affected family members. They found that the mutated gene was expressed at a five-fold higher level in affected family members.

"Thus, we believe that PHAII in these families is caused by an overexpression of *WNK1*," said Lifton. The discovery of the *WNK1* gene

defect gave the scientists a vital clue to the possible identity of the disease-causing gene in families whose affected members had a gene mutation on chromosome 17.

"Although we had mapped the gene for PHAII to chromosome 17 in those families, we had not been able to narrow down the segment containing the gene," said Lifton. "So we were still swimming in a very large sea of about fifteen million base pairs of genomic DNA."

By searching the human genome database for *Wnk1*-related genes on chromosome 17, the scientists found one called *Wnk4* that was right in the middle of the region containing the PHAII gene. After screening affected families for mutations in *Wnk4*, the scientists found four families whose affected members showed different but closely related "missense" mutations in *Wnk4*. The scientists theorized that the *Wnk4* mutations also increased the activity of the gene or its enzyme, said Lifton.

Using antibody markers, the researchers traced the localization of both the WNK1 and WNK4 enzymes in the kidney. They found that both enzymes appeared in regions of the kidney involved in regulating the reabsorption or secretion of salt, potassium and hydrogen ions. While the WNK1 enzyme appeared in the cytoplasm within kidney cells, WNK4 appeared in "tight junctions" -- interfaces between cells that are thought to be important in regulating the passage of ions, such as chloride, in the kidney and other tissues. According to Lifton, the localization studies suggest that the enzymes might be important in a regulatory pathway by which the kidney "decides" about reabsorption of sodium, chloride, potassium and hydrogen ions. Overactivity of the enzymes could increase reabsorption, expand blood volume and raise blood pressure.

Further exploration of the new blood-pressure-regulating pathway revealed by the *Wnk1* and *Wnk4* mutations could lead to new anti-hypertensive drugs, said Lifton. Intriguingly, he said, the *Wnk4* gene maps to the same region as the gene linked to blood pressure regulation in the long-term Framingham Heart Study, which has followed the health of a large group of people over many decades.

"Since this is a new pathway proven to affect blood pressure, antagonists of this pathway might prove to be useful new medications," Lifton said.