Bora Baysal and Joan Willett-Brozick found a mutation that causes paraganglioma tumors. That's causing a stir because it looks as if the same defect may encourage the growth of common cancers.

WITH HELP FROM ANDEAN MOUNTAIN DWELLERS,

AN OXYGEN-SENSING GENE IS LINKED TO TUMOR GROWTH

BY ROBERT MENDELSON

UP FOR AIR

According to the clock on the laboratory wall, it's 11 p.m. Besides that clock, there is only one other hint that the workday has long ago ended for most people. It's the lone window across the room. From this vantage point on the 14th floor of the University of Pittsburgh's Western Psychiatric Institute and Clinic, Fifth Avenue looms in the distance. The thoroughfare's perpetual traffic has dwindled to an occasional PAT bus and nocturnal automobile.

But nobody bothers to peer out the lab window tonight. Nobody glances at the clock, either. Bora Baysal and Joan E. Willett-Brozick focus only on their data. They don't like what they see.

They are searching for a genetic mutation within one single gene from a pool of what has been estimated to be 100,000 genes in the human genome. The search had been progressing well. Until now.

For Baysal, who today is an assistant professor of psychiatry and human genetics at Pitt, the search began in 1994, shortly after he left Ankara, Turkey, for Pittsburgh. His departure was the culmination of a career-altering decision. In 1988, he graduated from Gulhane Medical School in Ankara, and practiced medicine for two years as a family physician; but he found himself drawn to the "unknowns" of genetics, fascinated by the idea that answers to what makes one susceptible to certain conditions and diseases could be hidden away in the human genome. He began predoctoral training and received an international fellowship, which stipulated that he pursue his graduate education abroad. He decided on the University of Pittsburgh because, in large part, he had great respect for Robert E. Ferrell, who at the time chaired the Department of Human Genetics at the Graduate School of Public Health.

After settling in at the University, Baysal needed to choose a dissertation topic, although in his field, he points out, "choose" isn't a totally appropriate word for selecting a project:

"It's not something you can entirely determine yourself. It depends on patient material, so availability of family material is one of the most important factors directing your research. You find a very interesting genetic family and start your career."

That is how hereditary paraganglioma entered his life. His PhD advisor, Charles W. Richard III, had contact with several families located in Pennsylvania with this rare hereditary disorder.

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Researchers found that the frequency of the tumors increased for inhabitants of higher elevations.

For those with hereditary paraganglioma, tumors generally start appearing after the age of 30, sometimes on the head, but usually along the carotid bodies (the tiny neurovascular workers located on the carotid artery that help the body respire). By the time the patient is 60 years old, some sort of tumor presence is highly probable. It's not a life-threatening disorder, although it may compress critical nerves and arteries in the neck, which can lead to health problems. Surgery in an advanced state is risky because of potential nerve damage and significant disfigurement of the head and neck. Tumors can grow as large as half a skull. Richard's access to several local families with the mutation was a boon to Baysal. "These genetic families," he says, "are precious resources for gene mapping."

He had his project.

Baysal wasn't alone in his search. This genetic condition is most prevalent in the Netherlands, so Dutch researchers had been searching for the genetic mutation, too. Through Richard, Baysal established a collaboration with a Dutch group.

"The Dutch had crudely identified the gene location," Baysal recalls.

On the basis of their research, he focused on approximately 500 genes.

"We elaborated on this mapping process, and for years we and the Dutch tried to narrow down the critical region further and further and further."

The mapping process won't be the pilot for a new television drama anytime soon. "This process is extremely boring," he says. "All you do is obtain new families and analyze chromosomes of affected individuals to see if you can narrow down the location."

He and Willett-Brozick, a laboratory researcher, systematically did this day after day, week after week, year after year. Usually 10 to 12 hours daily, Monday through Friday; more hours on Saturday and Sunday. No vacations. Few distractions for the bachelor, other than a two-mile, straight-line jogging course from his Shadyside home to his Oakland laboratory.

"Bora lives this stuff; it's his life," says Willett-Brozick, who conducted the gene-mapping experiments.

The hard work paid off. By the beginning of 1997, the search had been narrowed by Baysal to approximately 175 genes. More good news. The Dutch had identified a specific region where they believed the mutated gene would be found; that region was located among the 175 genes still under Baysal's scrutiny. It seemed they were on the right track. Seemed.

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Baysal found a mutation that causes mitochondria to act as though they're in an oxygen-deprived environment. As a result, the tissue works overtime producing cells.

"Bora looked at the mutation not just mechanistically, not just technically; he thought, 'What would make this tumor happen at this very point with this type of tissue?'"

Baysal and Willett-Brozick continue to stare at the data. At each other. At the data again. It doesn't make sense.

Clearly, something is wrong. There can be only two explanations, reasons Willett-Brozick. It is a technical problem on Baysal's end, or else the Dutch haven't interpreted their data correctly.

Baysal finds no discrepancies in his data. At last they go home, the search narrowed no further. Tomorrow is no different. Or the next day. Or the next.

It's time for a lab meeting. Willett-Brozick, Baysal, and Richard talk. And, in the case of Baysal and Richard, they talk loudly. Very loudly. Willett-Brozick isn't surprised. In fact, she is used to it. "Charlie and Bora have really big hearts for science," she says. "They just love it; they get very excited, tense about it, not at each other, but excited about their ideas." At this particular meeting Baysal is adamant. It's clear to him they are conducting their search in the wrong region. Richard, the principal investigator, is not convinced. The search goes on. More data. No progress. More frustration. More meetings.

At last, Richard needs no more convincing. Baysal's comprehensive collection of data speaks volumes. There is no sign of the mutation in the region identified by the Dutch. They redirect the search.

However, more roadblocks surface. It's now the summer of 1997, and just as Baysal is about to receive his PhD, a job offer from a pharmaceutical company lures Richard away from Pitt. Meanwhile, the ultimate results from the project are far from a sure thing. Amid all the uncertainty, Ferrell—the man most responsible for Baysal's leaving Turkey for Pittsburgh—reassures Baysal and for good reason: "It was clear Bora was exceptional and Pitt would be foolish to let him founder in any way."

Pitt wasn't foolish.

David Kupfer, chair of psychiatry, supports Baysal's lab operations, and Bernie Devlin comes on board as the new principal investigator. (Devlin, a statistical geneticist, consults as necessary, oversees the Dutch collaboration, and makes sure the project has needed resources.) Baysal and Willett-Brozick end up discounting the region identified by the Dutch and continue systematically to weed out possible genetic contenders.

In the next two years, Baysal narrowed the search for the gene mutation to approximately 10 genes. Systematic research of those remaining genes—by Willett-Brozick's estimate—should have taken another five years, but Baysal came up with a theory.

He had come across some obscure autopsy reports from South America written nearly 30 years ago. In those reports he noted an interesting parallel between those case studies and his families with hereditary paraganglioma.

The reports detailed how indigenous Peruvians and Bolivians—who lived among the peaks of the Andes—frequently acquired paraganglioma. Yet, these were not instances of hereditary paraganglioma; they were isolated cases. Moreover, researchers found that the frequency of these tumors increased for inhabitants of higher elevations. Their interpretations, naturally, focused on decreased oxygen levels at higher altitudes. They noted that the most common tumor site was located along the carotid bodies.

"This is the most oxygen-sensitive tissue in the whole body," says Baysal. "When it detects a decrease in oxygen, it stimulates the heart and lungs to work harder so the body can adapt to lower oxygen levels. If you are in a continuously low oxygen environment, like the Indians living in high altitudes, this tissue keeps working and working and working and the number of cells increases and leads, eventually, to the Indians' tumors.

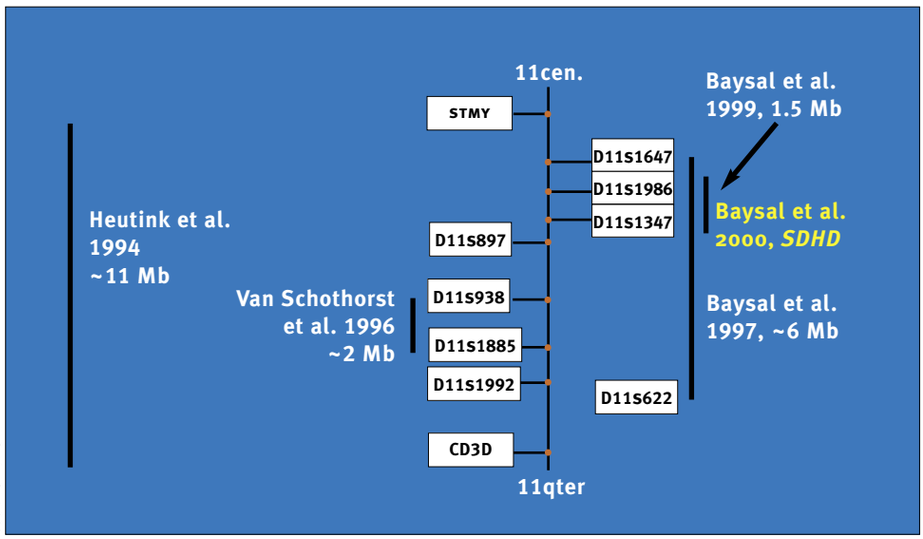
"While there are hundreds of papers about carotid bodies and paragangliomas," says Baysal, "I couldn't see anyone emphasizing the similarities: people with this genetic defect who lived in low altitudes experiencing the same kind of tumor that normally occurs in people living in high altitudes."

From this "coincidence" springs Baysal's theory: The problem for hereditary paraganglioma might be a defect in oxygen sensing.

Immediately, Baysal and Willett-Brozick scan those genes in the remaining .4 megabase target region (i.e., approximately 10 genes) to find out whether they might be involved in oxygen sensing. They find one. Next, they need to run what's known as a single stranded conformational polymorphism test on one of their hereditary paraganglioma families to find out if Baysal's hypothesis is correct.

The test, which finds mutations in small DNA fragments, normally takes a week to run, according to Willett-Brozick. She runs the test on Labor Day, 1999. "We seem to work a lot of holidays," she says. The plans are to develop the film a week later on the following Monday. Baysal can't wait. He goes to the lab on Saturday while Willett-Brozick attends to a previously booked weekend party at her home. Amid the Saturday afternoon revelry, her telephone rings. It's Baysal.

"Joan! We found it!"



COURTESY BAYSAL

The boxes portray polymorphic markers used to guide the search for the mutated gene causing hereditary paraganglioma. Black lines show how published works narrowed the search. Note that the 1996 paper was misleading, as evidenced by Baysal's 1999 findings that pointed to other polymorphic markers.

What Baysal found was a mutation in the gene, named SDHD, that makes a protein eventually transported to the mitochondria—"the powerhouse and brains of the carotid bodies," according to Baysal. The mutation causes the mitochondria to react as if they were in an oxygen-deprived environment, even though that is not the case. Consequently—as experienced by Andean Mountain dwellers—this tissue never stops working. The number of cells increases, and the increased mass eventually results in tumors. Baysal also found that genomic imprinting (an unexplained male/female inheritance protocol in the genome) passed on the mutation. In the case of hereditary paraganglioma, the disorder can be passed only from father to child.

"It was a moment of eureka," recalls Willett-Brozick. She marvels at how Baysal came up with his thesis:

"Bora looked at the mutation not just mechanistically, not just technically; he thought, 'What would make this tumor happen at this very point with this type of tissue?' He looked at it as a disease state, through the eyes of a medical doctor. I don't think he could have done it without his medical training. He went from a physiological approach to a biochemical approach, and then we used our genetic techniques to determine that this gene is mutant."

In February of this year, *Science* published the findings. (Authors included Baysal, Ferrell, Elizabeth C. Lawrence, Willett-Brozick, Devlin, Richard, five Dutch collaborators, and three MDs who provided the family materials: David Myssiorek, Wendy S. Rubinstein, and Eugene N. Myers.) The paper drew international attention, and for good reason. Though the mutation is rare, Baysal's identification of the SDHD gene's influence may well have a wide impact.

"Common cancers, not genetic in nature, have abnormalities where this gene is located," he explains. "We are postulating that these alterations may be helping those common cancer developments, through the loss of our gene, because oxygen sensing is a critical function for cells. If you remove oxygen sensing capabilities, it may help cancer cells become more aggressive."

"It will take time to put Bora's research into perspective, but I think what he discovered has the potential to be one of the more important findings to ever come out of the human genetics department," says Ferrell.

A more immediate outcome of Baysal's findings is the ability to test for the SDHD mutation. Persons with the mutation have a 90 percent chance of developing the disorder. For those who test positive, Baysal strongly suggests certain lifestyle precautions to lessen the condition's impact. If patients can avoid putting themselves into situations that make their bodies work harder to obtain enough oxygen, such as smoking or living at high altitudes, he is convinced a paraganglioma's growth could be slowed or even prevented. In addition, he recommends frequent screenings for the tumors. If they are discovered while in their infancy, they can be removed without great risk.

Ferrell points out that Baysal's findings impact not only hereditary paraganglioma and cancer research, but hypoxic conditions as well:

"Patients who are more or less similar concerning a pulmonary insufficiency or some other medical condition that leads to hypoxia will have either a relatively benign clinical course, or, if their bodies don't respond, they will have a very poor outcome. This gene may give doctors a clue as to why some patients do well and other patients don't."

For Baysal, many more late nights lie ahead. "The real issue is just now starting for aspects of oxygen sensing and whether common cancers have anything to do with SDHD or other genes involved in oxygen sensing," says Baysal. "We're trying to attack some of these questions and obtain some funding for the cancer aspect and for the genomic imprinting as well."

Baysal's six-year search may be over, but evidently his project has just begun. ■

MISBEHAVING GENES

Imprinting is a genetic battle of the sexes. In nature, a fetus's genes are derived from paternal and maternal copies of genes. Typically, the inherited parents' genes are equal partners in the creation of the child's genes. However, for unknown reasons, a handful of genes receive instructions exclusively from either the maternal or paternal inheritance. This "imprinting" phenomenon occurs even though the chemical composition of the imprinted gene has the genetic inheritance of both parents.

In the case of hereditary paraganglioma, the father's gene dominates. As a result, when the genetic mutation is passed from a mother to her son, the mutation will be overridden by the father's gene and the son will not be susceptible to the disorder. However, once the son has children, their genetic mutation will prevail over their mother's gene. Consequently, his daughters and sons will have a 90 percent chance of developing the tumors during their lifetime. The lucky 10 percent, according to Bora Baysal, of Pitt's Departments of Psychiatry and Human Genetics, reflect environmental factors that may not be conducive to tumor growth. ***—RM***