GENETICS RESEARCH

Discovery of AT Gene Sparks Biomedical Research Bonanza

To some researchers, it's the medical equivalent of the Rosetta Stone, the 18th century discovery that allowed archaeologists to decipher Egyptian hieroglyphics. On page 1749, a team led by geneticist Yosef Shiloh of Tel Aviv University in Israel reports the discovery of a defective gene that not only causes the rare and devastating hereditary disorder ataxia telangiectasia (AT), but also may be the single largest hereditary cause of breast cancer. And just as the original Rosetta Stone was the key to a better understanding of ancient Egyptian culture, this single gene promises to help solve a host of biochemical and epidemiologic mysteries, says team member Richard Gatti of the University of California, Los Angeles (UCLA).

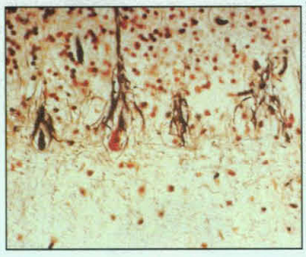
Gatti—along with many other researchers who are not members of the team—expects a lot of answers from the new gene, because it encodes a protein that may be one of the most important cogs in the cell's internal machinery. The protein's sequence suggests it is needed for detecting DNA damage or for blocking cell growth and division until the damage is repaired—or both. The gene "is going to give us insight into what makes cells grow, live, and die. It's involved in all those cellular decisions," says cell cycle expert Michael Kastan of Johns Hopkins University School of Medicine.

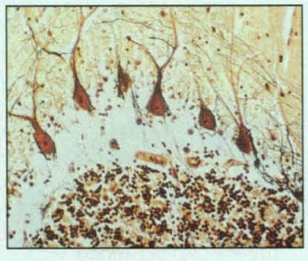
Given the name ATM (for *AT, mutated*) by its discoverers, the healthy gene's key role in the cell's regulatory machinery may also explain how the defective version can cause a plethora of symptoms, including the balance disorder ataxia, a depressed immune system, diabetes (in some patients), a high risk of blood cancers, and most notably an extreme sensitivity to x-rays that leaves great welts and wounds if radiotherapy is used to treat the cancers. For example, the inability to handle DNA damage might underlie both the extreme radiation sensitivity and, by allowing the accumulation of harmful mutations, the increased cancer risk.

The gene's discovery will also help solve a mystery with significant implications for public health and safety. Epidemiologic data suggest that a single copy of the defective gene (AT patients have two copies) gives women an increased risk of breast cancer. And that finding makes intuitive sense, because cultured cells containing one faulty copy of the gene suffer more DNA damage and a higher death rate than do normal cells when exposed to x-rays. Now that the gene is in hand, it should be possible to determine conclusively whether the defect does cause breast cancer. If it does, *ATM* could account for more cancer than *BRCA1*, the breast cancer gene that was cloned last September (*Science*, 23 September 1994, p. 1796).

What's more, because of the link between the gene and radiation sensitivity, it may be that carriers should avoid routine diagnostic x-rays, perhaps even mammograms. For risk assessment and public health alone, *ATM* "is extremely important," says molecular oncologist Bert Vogelstein, who is also at the Johns Hopkins medical school.

ISRAEL HADARI





VINTERS, GATTI, AND RAKIC

Team Tel Aviv. Above are some members of the Israeli-led team that tracked down the AT gene; the micrographs show that Purkinje cells *(dark triangular cells)* are present in normal cerebellum *(bottom)*, but die off in AT patients *(top)*.

Shiloh began studying AT in the 1970s when he first saw the many problems AT patients experienced. "It was looking in the patients' eyes," he says. "My interest was: What is it that does all this to these patients?" Ten years of studying the chemistry of AT cells threw up few clues. Then in the 1980s the advent of positional cloning—in which the coinheritance of genetic markers and disease are used to isolate defective genes—provided a fresh opportunity to get that answer. By 1988, UCLA's Gatti had tracked the gene to the bottom third of the long arm of chromosome 11. After that, the Shiloh team joined a large international consortium, including Gatti, Malcolm Taylor of the University of Birmingham, U.K., and Pat Concannon of the Virginia Mason Research Center in Seattle, Washington, to hunt for the gene.

By 1994, the consortium was closing in on its quarry. It narrowed the search to a 1.5-megabase region of the long arm of chromosome 11. At that point, the race for the gene was on in earnest—and competitive juices started to flow. The consortium split up into its member teams, each of which busied itself by pulling out promising-looking genes in the region and scouring them for mutations. Shiloh, grad students Kinneret Savitsky and Shlomit Gilad, research fellow Anat Bar-Shira, and the rest of the Tel Aviv team hit the jackpot this February when they found that the second gene they examined contained mutations that only occur in AT patients.

When the researchers compared half of the gene's sequence with those already in the databases, "the bells began to ring," says Shiloh. A portion of the *ATM* gene's protein sequence bears a striking similarity to the phosphatidylinositol-3 kinases (PI-3 kinases). That discovery suggested the gene might be involved in cellular growth control, because the PI-3 kinases, by adding phosphates to lipid molecules, transmit growth and other signals from the cell membrane to the interior, and possibly between different systems within the cell as well. In addition, the ATM protein is similar to another group of proteins that include the yeast proteins Rad3 and Mec1 (also called Esr1), whose job is to block the cell cycle in cells whose DNA has been damaged by ultraviolet radiation or x-rays. "With so many different manifestations to the disease, practically anything looks good—that's been part of the problem of finding the gene," says Concannon. Nonetheless, he adds, "those are just the characteristics I would have expected of an AT gene."

Now that researchers have the gene in hand, their next goal is to work out ATM's precise role in the cell. There are several possibilities, and, in fact, the protein may have more than one function. If the healthy AT protein is involved in DNA repair, as its resemblance to the yeast proteins suggest, it may help cells recognize damaged DNA so that it can be repaired before the cells divide.

Alternatively, the AT protein may be a link in the signal chain that tells the cell to stop dividing so that the DNA damage can be repaired. At the moment "there's about a 40:60 split in the support for the two possibilities," says cell-cycle expert Antony Carr

of the University of Sussex in Brighton.

Satisfying as it would be to pin the AT gene's role to just the cell cycle or DNA repair, those functions can't explain one of the hallmarks of AT: the death of the Purkinje cells in the cerebellum of the brain that causes the patients' ataxia. The simple reason is that neurons do not go through cycles of growth and division.

But ATM's presumed action as a PI-3 kinase may help solve this mystery, as the PI-3 kinases are also needed to keep brain cells alive. In the 31 March issue of *Science*, Ryoji Yao and Geoffrey Cooper of Harvard Medical School reported evidence that the brain's growth factors, needed to keep rat neurons alive in culture, work through a PI-3 kinase signaling pathway. If that pathway were defective in AT patients, it could explain why "these kids have ataxia that appears to be due to loss of cerebellum Purkinje cells," says director of the National Center for Human Genome Research (NCHGR) Francis Collins, who collaborated with the Shiloh team on the project.

Although the biochemical role of *ATM* is still unclear, discovery of the gene has already resolved one issue in AT research, much to the relief of both researchers and clinicians. In the 1980s, several groups carried out experiments that involved fusing cells from different AT patients. Some of the hybrids didn't show the radiation sensitivity characteristic of AT, indicating that the original cells carried different defective genes. Indeed, those results were interpreted as meaning that AT might be caused by mutations in any one of four different genes.

But the Shiloh team found no evidence that more than one gene causes AT. Their gene was mutated in all the AT patients they examined. The simplest explanation "is that the original experiments are in error," says molecular biologist Martin Lavin, whose team at the Queensland Institute of Medical Research in Herston, Australia, did one of those early experiments.

"What a relief it was to find that we didn't have to hunt for more [genes]!" says Shiloh. More important for the patients and their families, the existence of only one mutated gene means the genetic markers identified during the hunt for *ATM* can be used for screening AT carriers and for prenatal testing within at-risk families without having to worry about missing other genes that cause the disease. Shiloh cautions, however, that this good news is accompanied by some that isn't so cheerful: The coding portion of the gene is very large—12,000 base pairs—and its function can be damaged by at least 40 different mutations.

The gene's size and its abundance of mutational sites is bound to complicate the task of creating a simple *ATM* test for screening the general population, says Shiloh. Nonetheless, many biotech companies have expressed an interest in doing that, because individuals with one copy of the defective gene are believed to be at a heightened risk of cancer, especially breast cancer.

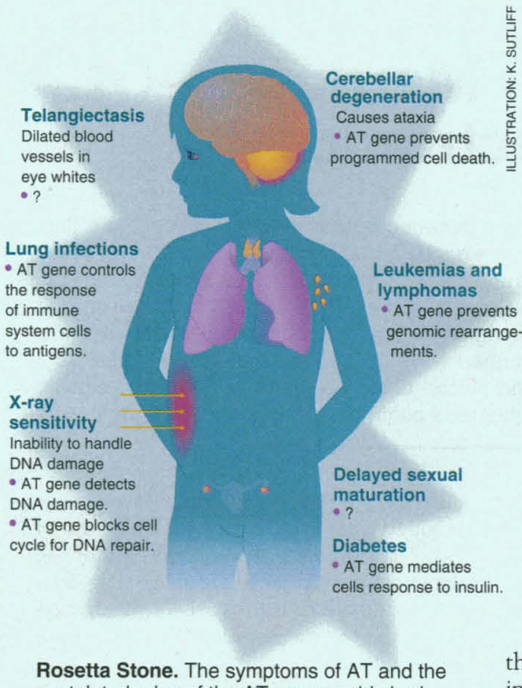


ILLUSTRATION: K. SUTLIFF

Rosetta Stone. The symptoms of AT and the postulated roles of the AT gene could shed light on what makes cells grow, live, and die.

Meanwhile, cancer experts are keen to test that supposition, which is based on epidemiologic studies of female blood relatives of AT patients in the United States, Norway, and the United Kingdom. That work suggests that female carriers of the gene have a fivefold higher incidence of breast cancer than noncarriers. Now, researchers can get direct evidence by looking for *ATM* mutations in women from families with a higher than average incidence of breast cancer whose cancer has not been linked to the previously discovered breast cancer susceptibility genes, *BRCA1*, *BRCA2*, or *p53*. "Now the [*ATM*] gene has been found, the answers will be found too," says medical geneticist Stephen Meyn of Yale University, who had also been chasing the AT gene.

If a defective *ATM* gene does predispose to breast cancer, it could mean that the gene is the most common single cause of the hereditary form of the disease. Although AT itself is rare, approximately 0.5% to 1.4% of the population has one defective AT gene. In fact, the gene could account for up to 8% of all breast cancers.

Finding out just what role *ATM* plays in breast cancer is doubly important because of the need to protect women from getting breast cancer from the very techniques designed to diagnose and treat diseases. In a 1991 analysis, geneticist Michael Swift of New York Medical College in Valhalla reached the controversial conclusion that diagnostic x-rays might cause breast cancer in certain women. Swift's analysis showed that women from AT families who have breast cancer were far more likely to have been exposed to high-dose diagnostic or therapeutic x-rays than family members who did not have breast cancer. And that finding was consistent with earlier findings that cultured cells with one defective AT gene are sensitive to ionizing radiation—although not as sensitive as cells with two defective copies. The idea remains controversial in part because the doses of x-rays received in modern diagnostic procedures are low compared to a person's annual exposure to natural radiation as well as to the doses received by the cultured cells.

But now that the AT gene has been cloned it's possible to test Swift's conclusion. If it turns out to be correct, it would raise the disturbing possibility that women whose risk of breast cancer is increased because they carry the *ATM* gene should avoid certain diagnostic x-ray procedures, perhaps even including the low x-ray doses received in mammography, the technique most likely to find a cancer in its earliest stages. Studies to investigate this disturbing potential are already planned at NCHGR, the National Cancer Institute (NCI), and the Danish Cancer Registry in Copenhagen. Meanwhile, NCI acting Director Edward Sondik advises that there is overwhelming evidence that mammography is a life-saver for most women over 50.

Some of the same qualities that make the defective AT gene so disturbing in relation to cancer causation have something of a positive spin when it comes to new possibilities for cancer therapy. The most obvious is that cancers could be made more sensitive to therapeutic x-rays by disabling the AT gene in the tumor cells. "It's a natural target to think about," Kastan says. The identification of *ATM* also makes gene therapy for AT theoretically possible, although most experts stress that AT is a tough candidate for the procedure because the gene is extremely large and would have to be transported into brain cells—two huge barriers to success.

"Gene therapy is somewhat futuristic," agrees Bradley Margus, father of two AT children and president of the AT Children's Project in Boca Raton, Florida, which in the past 18 months has invested $1.2 million in ATM research in the form of 14 research grants, the largest of which went to Shiloh. But, he adds, "I'm excited about how many good scientists will be interested in the gene, and I hope ... that someone along the way thinks of applying this new information to a [pharmacological] therapy."

–Rachel Nowak