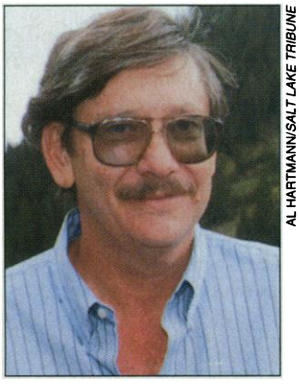
Breast Cancer Gene Offers Surprises

Now that an elusive breast cancer susceptibility gene has finally been tracked down, researchers are trying to figure out what it does and why it doesn't seem to be involved in nonhereditary cancer

"By Christmas, by Christmas." For the past 4 years, that had been cancer researchers' standard prediction of when they would capture the elusive breast cancer susceptibility gene. They knew that the gene, dubbed *BRCA1*, was sitting in a well-defined region on the long arm of chromosome 17, so, they reasoned, it couldn't take long for one of the dozen or so groups that were hot on the trail to track it down. But by the end of 1993, *BRCA1* had proved so difficult to spot that while the gene jockeys hadn't exactly lost hope, they had gotten to the point that, when someone repeated the mantra in public, they'd roll their eyes and say: "Which Christmas?"



AL HARTMANN/SALT LAKE TRIBUNE

Leader of the hunt. Mark Skolnick led winning team.

They got their answer on 14 September, when *Science* announced that it had accepted for publication a report detailing the cloning of *BRCA1*. The report links specific mutations in the gene with breast and ovarian cancer in a handful of families with multiple cases of these diseases. The paper is scheduled for publication in the 7 October issue; *Science* took the unusual step of releasing it to the press after NBC News reported on 13 September that the gene had finally been found. That announcement triggered a media blitz that made a fitting finale to one of the most riveting of the fierce and grueling gene hunts that have come to epitomize life in the fast lane of genetics research. (In the last year alone, media fanfares have greeted the isolation of two colon cancer susceptibility genes.)

But even as the flag fell on the race for *BRCA1*, dedicated gene hunters were already in pursuit of a second major quarry: A paper to be published in the 30 September issue of *Science* reports that a second breast cancer susceptibility gene, *BRCA2*, resides somewhere on a stretch of the long arm of chromosome 13 (see box, p. 1798). (The embargo on this paper has also been lifted.) Between them, *BRCA1* and *BRCA2* may be responsible for most hereditary breast cancers, which account for 5% to 10% of all breast cancers.

BRCA1's capture is already launching a fresh era of investigation, as researchers rush to explore the gene's modus operandi, hoping to gain new insights into the biochemical underpinnings of some types of cancer. On first perusal, *BRCA1* resembles genes that code DNA binding proteins. "There's going to be a flood of new information," predicts molecular geneticist Andrew Futreal of the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina, a member of the winning team that was led by Mark Skolnick of the University of Utah Medical Center in Salt Lake City. The team included more than 40 researchers from Utah's Medical Center; NIEHS; Myriad Genetics Inc., a Salt Lake City biotechnology company; Eli Lilly and Co. of Indianapolis; and McGill University in Montreal.

The unveiling of *BRCA1* has also caused some disquiet, however. Breast cancer activists fear that a test to identify the estimated one in 200 women who carry defective *BRCA1* genes will be rushed to the market before the ramifications of widespread testing have been fully explored. And scientists are disturbed by a surprise finding that *BRCA1* appears to play no role in common, nonhereditary forms of breast cancer that strike about 173,000 women in the United States each year—a finding that undermines some long-held assumptions about how the gene works.

But those concerns haven't dampened the turbo-charged emotions of the winning team. "I'm overwhelmed," said molecular geneticist Roger Wiseman of NIEHS. He looked it, as he spoke at a hurriedly organized press conference on 14 September at the National Institutes of Health (NIH). Biochemist Yoshio Miki of the University of Utah Medical Center, the first author on the report, professes himself "really happy." Skolnick, however, is more sanguine. "I'm relieved," he says. "But I feel I'll barely get to touch my feet down before the next race begins."

The runners-up—who included such gene-hunting luminaries as Mary-Claire King of the University of California, Berkeley; Francis Collins, director of the National Center for Human Genome Research (NCHGR) in Bethesda, Maryland; Barbara Weber of the University of Pennsylvania; Bruce Ponder of Cambridge University in England; Ellen Solomon of the Imperial Cancer Research Fund in London; Gilbert Lenoir of the International Agency for Research on Cancer in Lyon, France; and Ray White of the University of Utah—were also emotional. They chose epithets like "beautiful," "outstanding," and "revolutionary" to describe the netting of *BRCA1*, and many admitted disappointment at not having been part of the winning effort.

In the bag

Like their competitors, the Skolnick team had been feverishly sifting through candidate genes on the target region of chromosome 17, looking for giveaway mutations. This July, they hit a bull's eye when they came across a defect called a "frame-shift mutation" in one gene. The mutation was carried by three members of one Utah family who had breast cancer, but not by two healthy members of the same family.

Unlike harmless genetic variants called polymorphisms, a frame-shift mutation is a sure sign of a dangerously crippled gene. As its name implies, it causes the translation of codons (the three-symbol genetic "words" that specify the acids in a gene's protein product) to start in the wrong place, scrambling the gene's message and creating a nonsense protein. With the discovery of that mutation, Skolnick and his colleagues shelved their other candidate genes. By early September, they had collected a total of five potentially cancer-causing mutations in the gene in families with the disease, including a second frame-shift; a "stop codon," which

BREAST CANCER RISKS	
Lifetime risk from all causes	1 in 8 to 1 in 10
Fraction of cases caused by inherited susceptibility	5–10%
Estimated incidence of inherited *BRCA1* mutations	1 in 200 women
Estimated incidence of inherited *BRCA2* mutations	1 in 200 women
Fraction of cases unexplained	90–95%

The Hottest Race in Cancer Genetics

In October 1990, University of California, Berkeley, geneticist Mary-Claire King completed a feat that many had deemed unachievable: She mapped a breast cancer susceptibility gene to a region of the long arm of chromosome 17 (*Science*, 29 January 1993, p. 622). It was only a rough location—the stretch of DNA probably contains well over 1000 genes—but King's work transformed the gene, which she called *BRCA1*, from the genetic equivalent of the unicorn into a potentially trappable trophy.

"It galvanized research," recalls Mark Skolnick of the University of Utah Medical Center in Salt Lake City, who had turned his back on *BRCA1* in 1979 after having made his own "not-too-fruitful" attempts to localize the gene. Following King's discovery, hundreds of researchers—including Skolnick—jumped into the fray, melding and dissolving international alliances according to conflicting desires to pool resources, avoid work-style clashes, and capture the glory of snaring *BRCA1*.

JANE SCHERR

Key finding. Mary-Claire King mapped *BRCA1* to chromosome 17 in 1990.

All the competitors employed the same basic strategy: First, they narrowed the search using linkage analysis, the technique King used to map the gene in the first place. This involves monitoring the inheritance of genetic markers—easily identifiable stretches of genomic DNA—in families with a high incidence of breast cancer. If a marker is consistently inherited along with the disease, but is not inherited by unaffected family members, that indicates the marker lies close to the culprit gene. By studying hundreds of markers, the gene hunters homed in on a smaller and smaller region of chromosome 17. Next, they pulled out candidate genes from that region and searched them for crippling mutations that occur in family members who have the disease. The fact that Skolnick and his many colleagues managed to nab the gene first was due, in part, to their expertise in these latter techniques, Skolnick's competitors say.

Once they had the gene in the bag, the Skolnick team rushed off a paper to *Science*, but they purposely omitted an important piece of information from the draft version of the manuscript: the DNA sequence (or GenBank accession numbers) of the *BRCA1* gene itself, and of the PCR primers used to detect and amplify its segments. The reason, says Skolnick, is that they feared the information would get into the hands of their competitors before the paper had been accepted for publication. "Even though it should never happen, papers circulate even during the review process," he says. The sequences have since been deposited in GenBank, however, and as of 7 October—the paper's publication date—can be obtained using GenBank accession number UI4680.

The unpublished manuscript was indeed faxed to rival teams around world—but only after *Science* lifted the embargo on the manuscript last week. "It's hard not to be disappointed," says Jeff Boyd of the University of Pennsylvania, a member of the *BRCA1* team that includes King and Francis Collins, director of the National Center for Human Genome Research in Bethesda, Maryland. But, he adds, when he met with Collins to discuss *BRCA1*'s discovery, "the bottom line was that we were happy that some progress had been made." Mary-Claire King shared that sentiment. "I keep asking myself am I suddenly going to feel terrible about this. But I don't. I think it's great."

–R.N.

prevents one third of the gene's protein from being synthesized; a "missense substitution" which replaces a small amino acid with a large, highly charged one; and a "regulatory mutation," which appears to prevent the conversion of the DNA into its protein product. They knew for sure that they had *BRCA1* in the bag.

BRCA1's DNA sequence gave some instant information about the probable nature of its protein product. It bears more than a passing resemblance to a family of proteins called transcription factors that interact with DNA to switch other genes on and off, providing the fine control needed to regulate the thousands of genes that keep cells running. Now, research will focus on which genes the putative BRCA1 transcription factor controls. The answer could shed light on how defects in the gene upset normal cell growth and lead to cancer.

Unwelcome surprise

The excitement over the new findings is, however, tempered by a measure of disappointment: Against all expectations, the Skolnick team found no indication that defects in *BRCA1* play a role in the 90% to 95% of "sporadic" breast and ovarian cancers that are not thought to be due to an inherited susceptibility to the disease. Until now, cancer researchers had assumed that once they had identified *BRCA1*, they would also find mutations in the gene in sporadic breast cancers—mutations that, rather than having been inherited, would have been triggered by, for instance, environmental factors. But, in a companion paper also due to be published in the 7 October issue of *Science*, the Skolnick team reports that it detected defects in *BRCA1* in only four of 44 breast and ovarian tumors from patients whose family history was unknown. What's more, in those four cases, they also found the mutations in the patients' healthy cells, which means that they had been inherited; the patients apparently were members of unidentified high-risk families.

The breast cancer research community is struggling to explain why the gene appears to play such a powerful role in inherited cancers but none at all in sporadic cancers. "It's conceptually problematic," says Jeff Boyd of the University of Pennsylvania in Philadelphia, a member of the Collins-King-Weber *BRCA1* consortium. "The most obvious possibility," he says, is that there are *BRCA1* mutations in sporadic cancers, but they haven't been found. For instance, he points out that there could be mutations in the noncoding, or "junk," portion of the gene or in the DNA sequences that regulate *BRCA1*, most of which have yet to be sequenced.

That's possible, agrees Futreal, who played a key role in searching for *BRCA1* mutations in sporadic cancers. But he suggests a more intriguing explanation: "The lack of mutations in sporadic cancers is telling us that it's really bad to have a mutant copy of the gene sometime during growth and development," he says—possibly when the body is experiencing the hormone surges associated with puberty and breast development.

If *BRCA1* really isn't involved in sporadic cancers, a long-held assumption that *BRCA1* is a tumor suppressor gene starts to look slightly shaky. Typical tumor suppressor genes—for example, *APC*, which when defective triggers colon cancer—are involved in both familial and sporadic cancers. As the name implies, tumor suppressors act as "brakes" on the conversion of a normal healthy cell into a cancerous one, and their loss or inactivation leads to cancer. Breast

On the Trail of a Second Susceptibility Gene

Four years ago when cancer geneticists tracked a breast cancer susceptibility gene to a region of chromosome 17, they quickly realized that this rogue gene could only account for about half of all hereditary breast cancers. In many families in which vulnerability to breast cancer appeared to be inherited, the gene, *BRCA1*, was not linked to the disease. This implied that another, quite separate, breast cancer susceptibility gene was at large.

Now, an international consortium headed by molecular biologist Michael Stratton and genetic epidemiologist Doug Easton at the Institute of Cancer Research in Sutton, Surrey, United Kingdom, and genetic epidemiologist David Goldgar at the University of Utah has dramatically narrowed the search for this gene. In next week's issue of *Science*, the team will publish evidence that it resides on a stretch of chromosome 13. Gene hunters are now gearing up to find the gene, dubbed *BRCA2*, which researchers believe could be implicated in as many cancers as *BRCA1* is—perhaps half of hereditary breast cancers.

Homing in on *BRCA2*. Team co-leader Michael Stratton.

To pin down the new gene's location, members of the consortium—mostly groups trying to track down *BRCA1*, which have refrigerators full of tissue and blood samples from families in which breast cancer is rife—pooled their data. They came up with 15 families, each of which had between two and 25 cases of female breast cancer diagnosed before age 50 that didn't show any linkage with *BRCA1*.

Having identified these non–chromosome 17-linked families, the researchers used standard methods of genetic linkage analysis (see box, p. 1797) to try to find a "marker" sequence that was consistently inherited along with breast cancer, an indication that the marker is physically close to the DNA containing the susceptibility gene. Hoping to short cut their way to the right chromosome, "we first looked at parts of the genome which harbor known cancer-susceptibility genes such as *p53*, or which show abnormalities in sporadic breast cancer," explains Goldgar. But when they didn't turn up anything, they began a systematic search of the whole genome. The Sutton and Utah labs "typed over 200 markers before we found one linked to breast cancer," Goldgar says.

Richard Wooster, a postdoc in Stratton's lab, says "The day we found it was very exciting.... I'd seen a result..., and Mike said he thought he'd found something [too]. We all sat down in a huddle and decided we were 90% sure we'd got it." The teams now have a set of markers linked to *BRCA2* that have placed the gene somewhere within a 6-centimorgan stretch on region q12-13 of chromosome 13. Easton estimates that about 100 genes may lie in that region. As for *BRCA2*'s role in causing cancer, Stratton's first guess is that it, like most other cancer-susceptibility genes, is a tumor suppressor. But proving that will require pinning down its identity.

The race is now on to do just that. Geneticist Bruce Ponder of Cambridge University, one of Stratton's collaborators on the *BRCA2* paper, predicts that the groups who worked on *BRCA1* will now be "ransacking their fridges for forgotten families" that might hold the clue to *BRCA2*. "There are one or two possible candidate genes in the region—we know that from human genome maps," says Ponder. One possible candidate is *BRUSH1*, a gene of unknown function that was partially sequenced earlier this year by Helene Smith's group at the Geraldine Brush Cancer Research Institute in California. This gene is expressed in breast epithelium, and Wooster says "we know it maps to the right location."

Mutations in either *BRCA1* or *BRCA2* appear to result in a similarly high risk of female breast cancer—a woman carrying one defective gene has an 80% to 90% chance of developing the cancer. However, the two gene products seem to act by different mechanisms. *BRCA1* defects, for example, are associated with a much higher risk of ovarian cancer than are *BRCA2* mutations. And families with breast cancer due to *BRCA2* often include a case of male breast cancer, which is not true for *BRCA1* families.

Identification of *BRCA2* is unlikely to be the end of the breast cancer susceptibility gene story. In their *Science* paper, Stratton and his colleagues point out that breast cancer in some families in which the disease seems to be hereditary does not appear to be attributable to either *BRCA1* or *BRCA2*, indicating that there may be yet more breast cancer susceptibility genes to be identified.

–Claire O'Brien

Claire O'Brien is a science writer in Cambridge, U.K.

cancer had seemed to follow the classic model of a tumor suppressor at work: In about one half of sporadic, and all familial, cancers, a stretch of chromosome 17 where researchers had been searching for *BRCA1* is lost from tumor cells, suggesting that *BRCA1* mutations play a role in both types of cancer. But the apparent absence of *BRCA1* defects in sporadic tumors suggests otherwise, and could even mean, says Bert Vogelstein of Johns Hopkins University, "that there's another tumor suppressor gene on [the relevant portion of chromosome] 17."

Indeed, genetic epidemiologist Neil Risch of Yale University argues that because the gene fails to show all the predicted features of *BRCA1*, the evidence that it really is the long-sought gene is not completely watertight. Risch calls the work "highly suggestive," but says "the ultimate proof" will require further evidence that the gene is mutated in families with hereditary breast and ovarian cancer, but not in healthy individuals.

Risks and uncertain benefits

Once the gene's sequence is released on 7 October (see box, p. 1797), there will be no shortage of researchers to follow up Risch's suggestion. Assuming they confirm that Skolnick and his collaborators have the correct gene, one of the first tasks will be to get better estimates of the lifetime risk of cancer associated with *BRCA1* defects. The current guesstimate is that a woman who carries a defective copy of *BRCA1* has an 85% chance of developing cancer by age 65. But that could be an overestimate, because it's derived from studies of the families used to track down the gene, which were chosen precisely because so many of their members had been stricken with cancer.

With *BRCA1* in hand, adds King, "we will [also] be able to get some assessment of the risks associated with the different kinds of mutations [in the gene]." Breast cancer experts expect that different mutations will trigger cancer at different ages of onset and will carry different likelihoods of triggering breast or ovarian cancer, or both. However, because of the sheer size of the gene—it's estimated to be a massive 100,000 base pairs

long, 10 times the length of the average gene—researchers may turn up scores of mutations, and the task of cataloging the impact of all of them could take several years.

Despite such hurdles, diagnostic tests for some *BRCA1* defects could be available in 6 months to 2 years, predicts Donna Shattuck-Eidens, who led the *BRCA1* effort at Myriad. Myriad has applied for a patent on the gene and has licensed its use for the development of drugs and diagnostic kits to Eli Lilly, which, besides contributing people power to the winning effort, also funded Myriad to the tune of $1.8 million.

Not everyone is thrilled by the prospect of an imminent *BRCA1* test, however. In a written statement, Fran Visco, president of the National Breast Cancer Coalition, an activist organization of breast cancer patients headquartered in Washington, D.C., expressed concerns that "we may soon have a test that will tell a woman [that] she may have as much as an 85% chance of getting the disease, for which there is no known cure and which she cannot prevent." The test may actually do harm, argues Visco, because women who test positive risk losing their health and life insurance (*Science*, 22 July, p. 464).

And for some, the gap between understanding the genetic basis of cancer and learning how to treat the actual disease needs the most concerted research effort. "We're going to have many examples of people cloning cancer-susceptibility genes, without any idea of how we are going to treat these people differently," says Stephen Friend of Massachusetts General Hospital in Boston. National Cancer Institute director Samuel Broder agrees: At the NIH press conference, he pointed to one finding that has drawn little attention: A small minority of women can carry a mutant version of *BRCA1* and remain cancer-free into their eighties. That suggests that some as-yet-unidentified genetic, environmental, or dietary factors can ameliorate the impact of a rogue copy of *BRCA1*, allowing a woman to dodge her genetic destiny. Discovering those factors, says Broder, should be a "high research priority."

–Rachel Nowak

CANCER TREATMENT

Boron Therapy Gets Early Test

Last week, for the first time in 33 years, U.S. researchers pumped a boron compound into the blood of a patient with incurable brain cancer, wheeled her up to a nuclear reactor, and irradiated her brain with neutrons. In theory, the boron compound will serve to concentrate the deadly effects of the radiation in the tumor, while sparing healthy tissue. But the patient, her family, and the researchers—a team at Brookhaven National Laboratory—can only hope that the theory will prove correct. The last time boron neutron capture therapy (BNCT) was tried in the United States, it failed to kill the tumors and even hastened the deaths of some patients. The researchers think they've now laid the groundwork for BNCT to work. But they never expected to be trying it this soon.

As this article went to press, the patient remained in good condition, according to her doctor, but complications from the therapy could arise anytime within the first several weeks. And while the patient copes with her medical uncertainties, BNCT researchers are facing uncertainties of their own, brought on by their decision to bow to political pressures and treat the woman months before they had planned to begin clinical trials of BNCT.

Just last summer the researchers were publicly voicing fears that premature trials could lead to a spectacular failure and kill research in the whole field (*Science*, 22 July, p. 468). But now, although Darrel Joel, chair of Brookhaven's medical department, admits that he and his colleagues changed their plan under pressure from the patient, her family, and the Department of Energy, he maintains that the treatment was ready for clinical use. "We did not have all the information we might have had prior to treatment, but we were reasonably well prepared," he says.

For BNCT to work, the boron compound has to concentrate selectively in the tumor. There, the boron nuclei are meant to capture neutrons and fission into energetic fragments that kill the cancer cells. In the first trials, boron lingering in the brain capillaries spread the radiation damage beyond the tumor and killed four subjects. But since then researchers have developed better boron compounds and tested their promise by "curing" rats with implanted tumors, says Joel. And preliminary human studies, in which researchers infused the boron into brain cancer patients and tracked its distribution, convinced Joel and his colleagues that the disaster of 30 years ago won't happen again.

They expected to treat their first few patients starting next year. But among the patients who took part in the distribution studies was a Long Island resident named Joann Magnus. Magnus, who has discussed her case freely with reporters, had surgery to remove the bulk of her cancer, an aggressive tumor called glioblastoma, last spring. When it recurred, however, she had a second surgery and decided to seek the full treatment.

When Brookhaven told her that its reactor was not going to be ready to deliver neutrons to experimental patients until 1995, she appealed to energy secretary Hazel O'Leary, whose agency funds Brookhaven. Her letter landed on the desk of Martha Krebs, a physicist who heads energy research and has said publicly that the Department of Energy has gotten a "slow start" in developing BNCT. Krebs says she then leaned on Brookhaven to speed up preparations to treat Magnus and other patients. Collaborating doctors at Beth Israel Hospital applied to the FDA for a special, one-time permit for testing the treatment, known as a single-patient IND (investigational new drug) protocol. The permit was granted, but the agency will not discuss its rationale for the decision.

The result was that Joel and his colleagues were unable to complete the series of distribution studies they had originally planned, and they've had to work faster than intended to ready the neutron beam. But Magnus's doctor, Richard Bergland of Beth Israel Hospital, says Magnus was a good first patient. The site of her tumor made it easy to target with the neutron beam, Bergland says, and the distribution studies showed that the boron had concentrated in her tumor significantly better than it had in five other patients. "She was the perfect patient for this beam," says Bergland.

Medical ethicist Arthur Caplan of the University of Pennsylvania thinks the decision to treat Magnus raises questions, however. A terminal condition doesn't necessarily justify subjecting a patient to a potentially harmful medical experiment. "[Harm] could mean dying sooner, or dying more painfully." Brookhaven's Jeffrey Coderre responds that he and his colleagues were careful to calibrate the dose of boron and neutrons to safe levels.

Then again, if Magnus recovers, it may be difficult to hold back the floodgates of desperate patients. Joel says the treatment won't be available to other patients until the researchers have watched Magnus's progress for 2 or 3 months. But Brookhaven sources say that the publicity surrounding her case has already prompted a flurry of inquiries. And the pressure may jeopardize careful study of the treatment, says Caplan. "Compassion [can] make it impossible for us to learn what works and what doesn't work."

–Faye Flam