Zeroing In on a Breast Cancer Susceptibility Gene

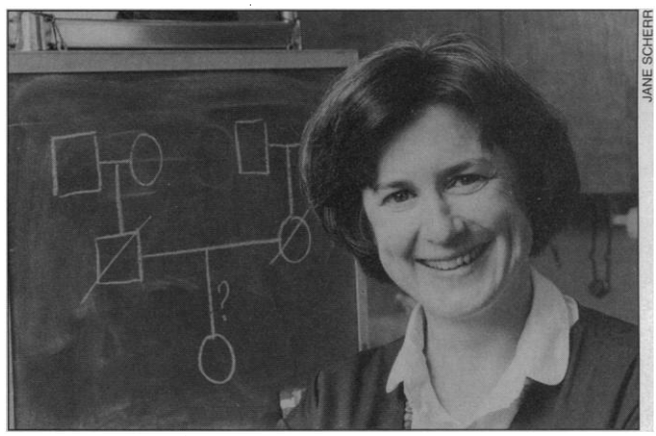
It was 10:30 p.m. and a grueling session at a genetics meeting in Cincinnati in October 1990 was drawing to a close when Berkeley geneticist Mary-Claire King stepped up to the podium to give an unscheduled talk. Weary eyes popped open when King announced that she had found the rough location of a gene that predisposes women to breast cancer. "It electrified the community," recalls medical geneticist Francis Collins of the University of Michigan. But the excitement was tinged with a good deal of skepticism. Many geneticists simply didn't believe a defect in a single gene could cause a substantial portion of breast cancer. And even if there were such a gene, the odds of finding it through conventional genetic linkage studies, such as King had performed, were vanishingly small—so small, in fact, that Oxford University mathematician John Edwards had just written a proof demonstrating that it would be virtually impossible to find a gene for susceptibility to such a complex disease. After he saw her analysis, Edwards sent the proof anyway—with an "oops" scrawled across the top.

Two years later, geneticists are no longer skeptical. Results from labs around the world have shown that the gene is directly responsible for about 5% of all breast cancers—specifically, the rare inherited form that can devastate families, striking women while they are in their thirties or forties. One woman in 200 inherits this defective gene, and those who do so face an 80% to 90% risk of developing the disease. If the gene can be identified, the ramifications could extend well beyond the relatively few families in which breast cancer is rife, for the gene may also play a role in the common noninherited, or sporadic, form of the disease that now strikes 176,000 women in the United States each year. Moreover, investigators like King believe that once the gene is identified, it could lead to new methods to detect breast cancer far earlier than is now possible and perhaps point to therapies targeted to malignant cells.

A slew of big-name gene hunters, attracted by this major prize, are now in hot pursuit of the gene. They have narrowed its location to a region 2 million or 3 million base pairs long on chromosome 17, and with the best of luck—which any one of them will tell you rarely materializes—they may find it within a few months or, more likely, a year or two. But even before the gene itself has been tracked down, researchers have already taken the first agonizing steps toward counseling women from families with the extraordinarily high-risk, inherited breast cancer (see box). Few but King believed that the quest would be even this successful when she set out to find this killer nearly 20 years ago.

Against all odds

What impelled King on this quest that no one else believed could succeed? She was driven by a conviction that breast cancer is an affliction of an affluent society that, unlike lung cancer, cannot be prevented. "If there were something we could do to prevent breast cancer, I would not be doing genetics, I would be focusing on that," says King. "If we can't eliminate the disease," she says, "then we should be able to eliminate mortality from it."



JANE SCHERR

Twenty-year quest. Mary-Claire King persevered although the odds were formidable.

When she began in 1974 as a postdoc in Nick Petrakis' lab at the University of California, San Francisco, numerous epidemiological studies had already pointed to an inherited factor in breast cancer. They had shown that a woman's risk was greatly increased if she had a mother or sister who had died of the disease before age 50, or if more than one sister was affected, or if a family member had bilateral breast cancer. King first did some additional epidemiological studies, which confirmed the earlier ones, then looked at how breast cancer was distributed in some 1500 families of breast cancer patients for whom the National Cancer Institute had compiled careful family histories. In most of these families, just one woman was affected, but in 15%, several family members had the disease. The mathematical model that fit this pattern best was that a rare mutation in a dominant gene on a nonsex chromosome, which could be transmitted by either the father or the mother, caused about 5% of breast cancers in these 1500 families.

Developing that model was a big step forward, but it also suggested just how hard finding the gene would be, for it presented what King calls an "epidemiological nightmare." The model predicted that two-thirds of the families that appeared to have inherited disease—the 15% hit with multiple cases—were in fact victims of phenomenally bad luck, not genetics. What's worse, says King, was "that we weren't going to be able to tell, statistically or clinically, which cases were which." How, then, would they interpret a negative result? Was it because the family being tested for the gene did not carry it or because that gene did not cause breast cancer?

King decided to push ahead anyway, though she now says, "I can't believe I was so naive." She moved across the Bay to Berkeley, where she became an assistant professor in the epidemiology department, and with a 1-year-old daughter at home, launched into years of grueling work. What got her through it, admits King, was her heavy streak of stubbornness. She and her graduate student Ruth Ottman, now an epidemiologist at Columbia University, set out to collect blood and family histories from huge extended families that had multiple cases of breast cancer over several generations. The key person in this endeavor, which ultimately spanned nearly 20 years and identified about 100 families, was Sarah Rowell, a young epidemiologist who had worked with King since she was 18.

The plan was to look for patterns of inheritance of "markers" on the chromosomes of individuals from these families, which included many women who were diagnosed while in their twenties or thirties, as well as some with late-onset disease. The idea in genetic linkage analysis is to see if any particular marker, which serves as a signpost for a specific region of a chromosome, is consistently inherited along with the disease, or "linked" to it. If so, then the gene must lie somewhere in that region. At the time, however, linkage studies were relatively rudimentary: King was working with only about 30 protein markers scattered around the chromosomes. Several years of effort yielded a few intriguing but ultimately false leads.

By the early 1980s, however, King and other gene mappers had a powerful new tool: DNA markers—unique, easily detectable, pieces of DNA that transformed gene mapping. Instead of the 30 protein markers, King suddenly had 100 or more of these new markers to probe the genetics of her breast cancer families. But there was a problem. King's group had preserved blood samples, but the

techniques available at the time were not capable of pulling enough DNA from these old specimens to work with. The material they had painstakingly collected in the 1970s was "almost completely useless," says King.

King and her group, which then consisted of epidemiology graduate student Beth Newman, now at the University of North Carolina, and biochemistry postdoc Jeff Hall, now at CellPro in Seattle, started over. They identified additional families, collected blood, and established permanent cell lines to preserve the DNA. The group began looking at "any gene we could imagine that might make sense," says King—known oncogenes, growth factor genes, and genes involved in breast development. "All of the results were negative," she says.

Then in 1985 came two major advances: the polymerase chain reaction (PCR), a technique for amplifying small pieces of DNA, and a new type of DNA marker that was fast and easy to work with—short repeated sequences, like CACACA, that vary in length from one person to another and can be easily detected with PCR. The combination "broke this whole field open," recalls King. With PCR, investigators could work with a tiny sample of DNA—even a "dirty" one like DNA from those old blood samples King's group collected in the 1970s. Indeed, data from those families were now even more valuable because another generation had been added. King teamed up with Anne Bowcock, then a postdoc and PCR expert in Luca Cavalli-Sforza's lab at Stanford, to "reap the harvest of that earlier work."

By that time a few other brave souls were gearing up to hunt for the gene. They included Bruce Ponder at Cambridge University and Gilbert Lenoir and Steve Narod at the International Agency for Research in Cancer (IARC) in Lyon, France, who were studying high-risk women with both breast and ovarian cancer. They and other European scientists soon formed a consortium to share markers and pool results. "But it was Mary-Claire who ran the most markers and who put the most heart in it," recalls Utah geneticist Mark Skolnick.

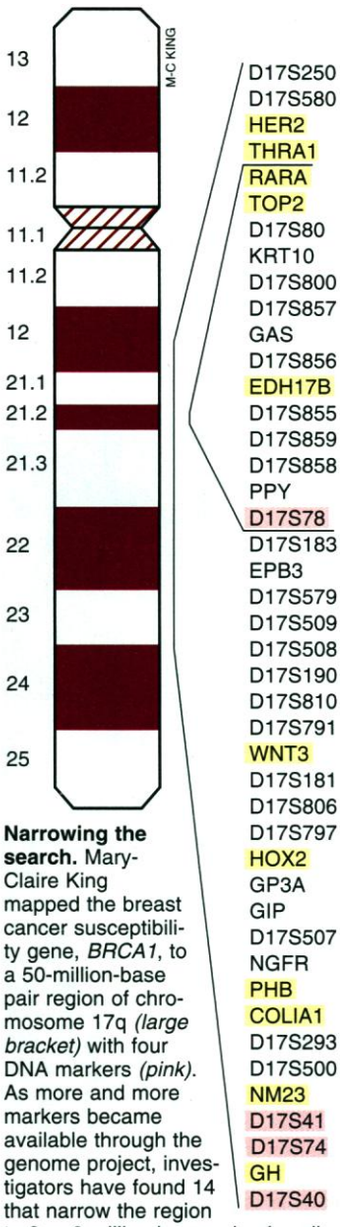
Success—or was it?

With the powerful new molecular tools in hand, King and Hall essentially tried one marker after another to see if it was linked to the breast cancer gene. In August 1990, King's group tried the 183rd marker, which was from the long arm of chromosome 17—an intriguing region because other genes that are altered as breast cancer progresses, such as *HER2*, also reside there. After testing the marker in 23 extended families, King thought she had finally struck gold, as some of them seemed to show clear linkage. But she was confronted with the epidemiological nightmare her original mathematical model had predicted. Instead of one clear positive signal, King kept getting "a smattering of small positive results, some really negative results, then suddenly some very positive results. I couldn't make any sense of it."

Then Newman suggested that they line up the 23 families by age of diagnosis, reasoning that the families in which breast cancer struck at an early age were more likely to have the inherited form of the disease. "Everything fell into place," says King. In the families with early-onset breast cancer, the gene was clearly linked to the marker on chromosome 17. Among these families the odds that the result was correct, known as the lod score, was 5.98—a score of 3 was then considered convincing. But for women diagnosed after age 46, the score dropped off precipitously. The most likely explanation, King reasoned, was that the older-onset families did not have inherited disease but had multiple cases of breast cancer by chance. The alternative, which she did not believe, was that the gene was not really linked to the marker, D17S74.

The skepticism these results generated when King presented them several months later at the American Society of Human Genetics meeting in Cincinnati was understandable. "There had been false linkages reported with even higher lod scores, for diseases such as schizophrenia, so there was well-founded scientific skepticism," explains Skolnick, one of the nonbelievers. Moreover, King reported that just seven of the 23 families were linked. Nevertheless, King says her colleagues were "incredibly enthusiastic and pleased. It is really very nice when people you have gone to meetings with since you were all kids say, 'I don't know if you are right but that is really great.'"

As soon as King announced linkage, both Ponder at Cambridge and Lenoir and Narod at IARC immediately tested the same marker. In Ponder's families, "it was only slightly positive. We might not have detected it," he says. Lenoir, however, provided confirmation within just 3 weeks. What's more, his analysis showed that this locus on chromosome 17 was linked not just to breast but to ovarian cancer as well—so the same gene caused both diseases. Some skeptics still remained, but not many. Says Ellen Solomon of the Imperial Cancer Research Fund in London, "The Narod paper was very important."



Narrowing the search. Mary-Claire King mapped the breast cancer susceptibility gene, *BRCA1*, to a 50-million-base pair region of chromosome 17q *(large bracket)* with four DNA markers *(pink)*. As more and more markers became available through the genome project, investigators have found 14 that narrow the region to 2 or 3 million base pairs *(small bracket)*. Candidate genes, most of which have now been excluded, are in yellow.

Lenoir and Narod's confirmation touched off an international race to track down the gene. Solomon and Utah's Skolnick, who had tried earlier but abandoned the quest, joined the field, as did Ray White, also at Utah. King teamed up with Bowcock, by then at the Southwestern Medical Center in Dallas, to zero in on the gene. Several months later, Francis Collins, who had already bagged the cystic fibrosis gene and the neurofibromatosis gene (which White cloned independently), asked King if she wanted to collaborate. She bit.

Even molecular geneticists, who are legendary for their competitiveness, are happy to collaborate when the task is daunting—and they all needed help because the region the gene could reside in was a whopping 50 million bases long. So the European linkage consortium, joined by King, Bowcock, and Skolnick, pooled all of its resources—DNA from 214 families, with either breast or breast and ovarian cancer, and a common set of markers. This larger sample confirmed that this susceptibility gene, now dubbed *BRCA1*, is indeed on chromosome 17, and narrowed the region to roughly 4 million or 5 million base pairs. But again, the results were a mixed bag. The data showed that the gene seems to cause most of inherited breast and ovarian cancer in the 57 families with both. But of the 153 families with breast cancer alone, just 45% appeared to be linked to this gene. High cancer rates in families not linked are probably caused by other susceptibility genes or chance. Even so, in a study to be published in the *American Journal of Human Genetics*, the consortium reports that the combined lod score from the families studied is 26, which indicates odds in favor of linkage of 10^{26} to 1. Says Collins: "No one can argue with that."

Genetic Counseling: A Preview of What's in Store

As researchers close in on a susceptibility gene that gives rise to inherited breast cancer—and very likely plays a role in the more common noninherited cancers as well—they are opening up a Pandora's box of ethical issues. Already, investigators can tell which women in a few cancer-prone families carry the gene defect and therefore face an 85% risk of developing breast cancer. And once the gene is identified, a diagnostic test will soon follow, and also the ability to screen the general population. Such a test might seem like nothing but good news. But ask geneticist Francis Collins of the University of Michigan about the implications and he will tell you: "This represents every reason I went into this field, every reason I'm glad I'm a physician and a scientist. But at times I'm terrified."

What terrifies Collins now is that he and a few other researchers are grappling with how to counsel the high-risk women participating in their studies—with few rules to guide them. Thousands of other physicians could soon face a similar challenge, for this is one of the most common disease genes yet known—it is believed to be carried by one in 200 women—and the choices faced by a woman with a positive test are dire. They boil down to living with an 85% risk of breast cancer, but with careful surveillance in the hope of early detection, or undergoing a bilateral mastectomy and, since the gene is also implicated in ovarian cancer, removal of the ovaries as well.

PAULINE LUBENS

Making up the rules. Barbara Weber and Francis Collins.

Collins was abruptly brought face-to-face with these issues last September. He has been collaborating with Berkeley geneticist Mary-Claire King in the hunt for the susceptibility gene (see main story), screening members of large families rife with breast cancer with a genetic marker that is inherited along with the susceptibility gene. As *The Wall Street Journal* recently reported, a young woman Collins had tested walked into the cancer clinic at the University of Michigan Medical Center and told Collins' colleague, oncologist-turned-molecular-geneticist Barbara Weber, that she could no longer tolerate the dread. Her mother and sister had already died and another sister had just been diagnosed. She had scheduled a prophylactic bilateral mastectomy in a week's time. Collins and Weber knew from their research who in her family carried the gene defect but had agreed that such information was too preliminary to disclose. After a quick consultation, however, Collins and Weber decided they had no option but to offer to tell her that there was a 98% chance that she did not carry the defect and so faced just the standard 10% risk of developing the disease. She canceled her surgery.

And so began a fledgling genetic screening and counseling program for breast cancer that Collins and Weber now run at Michigan—one of the first of its kind in the world and a test bed of sorts for widespread screening once the gene is identified. So far the Michigan group, which includes genetic counselor Barbara Biesecker and oncology nurse Kathy Calzone, has counseled about 50 members of this one family in what Collins calls one of the most "fascinating and disturbing" experiences of his career. "We are making up the rules as we go," says Collins, and "agonizing" over each one. Over the past year gene hunters King and Bruce Ponder of Cambridge University have also begun counseling the few women for whom they have compelling genetic evidence, and Ponder is about to begin a pilot study in England.

Collins and Weber have assembled a team of 12 professionals, who divide up into groups of three—with a geneticist, oncologist, and genetic counselor in each—to talk to each family member after genetic screening. The first issue they encountered was how solid the data had to be—in other words, what margin of error was acceptable? And then there was how to deal with the very young women at risk. Although they met violent disagreement from some parents, the group has decided that teenagers should not be told their status until they are 18 and can give informed consent. "Informing minors seems to be breaking the rules," says Collins, though he admits there are none.

The kinds of reactions that can be expected once screening becomes widespread are illustrated by what happened when Weber told the good news to the woman who catapulted the team into this brave new world. She and her family danced around Weber's office laughing and crying. A few days later, however, she was devastated by survivor guilt, similar to that experienced by Nazi concentration camp survivors. Collins says that is one of the toughest counseling issues they now face. Another woman in that family had a prophylactic bilateral mastectomy 5 years earlier and was uncertain whether she wanted to learn her status. She decided she did, and when the team told her she did not carry the gene defect, she was not devastated but relieved. She felt her decision had bought her 5 years free of the dread she had lived with all her adult life.

So far, virtually all the family members have wanted to know their status. And, the six or seven women who learned they carry the gene defect are leaning toward surgery. "At first it seemed such a horribly aggressive approach," says Collins. But the trauma of surgery pales in comparison to living with the risk.

As genetic counseling progresses—the Michigan team is ready to begin on two more families—the questions will only mount. The group is now grappling with how to safeguard medical records. Concerned that insurance companies might get hold of the records and deny the patients coverage, the team feels it has no choice but to keep these records in a private file until the issue—applicable to all genetic screening—is sorted out.

Amid all the agonizing and self-doubts, Collins and Weber have seen the rewards of their work as well. In early December the team saw the 40-year-old cousin of the first woman who came into the clinic. She did not consider herself at high risk because her immediate family had so far been spared. When the team told her that she did in fact carry the mutation, transmitted by her father, she panicked because she had not had a mammogram in 2 years. They scheduled one that day, which identified a tiny but malignant tumor, just 6 millimeters in size, which might otherwise have gone undetected for years. She had surgery in December. Because the lesion was caught so early, says Weber, she has a 90% chance of being cured of this particular cancer. Says Collins: "That may be one of the first successes of genetic analysis for breast cancer."

–L.R.

Cutting to the chase

To close in on the gene, all the groups have embarked on a two-pronged strategy of genetic and physical mapping. First, they are trying to narrow the search by looking for more DNA markers closer to the gene, which are increasingly difficult to find, and for "recombination events" that occur during meiosis when parts of the chromosomes are shuffled around. These events are the gene mapper's Holy Grail because, given enough of them, they can pinpoint the gene in a tiny stretch of DNA. It works this way: A grandmother with inherited breast cancer passes on the entire region believed to contain the gene to her daughter. The daughter then passes on only part of that region to her daughter. If the third-generation daughter develops breast cancer, then the gene must reside in the smaller region inherited from her grandmother.

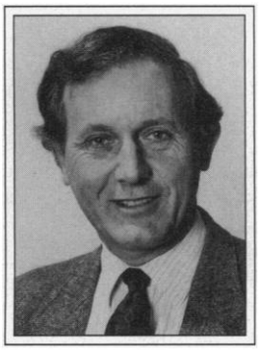
The number of these recombinations is limited, however, and at this point the investigators have probably already found all they will. Even so, several independent efforts have narrowed the region to 3, or perhaps 2, million base pairs. And that, King and others suspect, may be as far as they can get with genetics.

Luckily, the investigators do have some other hints to guide them. Most are convinced that they are looking for a tumor suppressor gene, much like the retinoblastoma gene—and that model points to an ingenious strategy for narrowing the region, one that has already proved invaluable in tracking down the gene involved in familial adenomatous polyposis coli, an inherited form of colon cancer. The hypothesis is that a mutation in one gene is responsible for breast cancer, but both copies of the gene have to be knocked out by mutations for cancer to develop. In familial breast and ovarian cancer, a woman inherits a germline mutation and then acquires a somatic mutation that knocks out the complementary gene. Sporadic cancer requires two such somatic "hits." So another way to find the gene—or at least narrow the region —is to look at the DNA in tumors themselves for evidence of the second "hit," which would show up as a loss of DNA from the relevant region.

King, Collins, and others have turned to an unusual source: Tissue removed during surgery from women who died 40 or even 50 years ago that is embedded in paraffin and stored in hospitals—"linkage among the dead," King calls it. As predicted, various groups have found that a chunk of DNA in the relevant region on chromosome 17 has been lost in breast tumors, providing strong evidence that *BRCA1* is indeed a tumor suppressor gene. The trick now is to find the smallest region that is deleted in all the cells—for that's where the gene must reside.

At the same time, all the groups are making physical maps, essentially trying to clone the entire region in known segments of DNA to facilitate the search. The Michigan group has cloned and reassembled almost the entire region, and they and others have begun pulling out expressed genes and testing each one to see if it might be *BRCA1*. It could be a long haul, as at least 100 genes may reside in the region.

Other groups are assumed to be making similar progress, but as the prize looms ever larger, the open collaboration of last year is falling off. Indeed, at a meeting King organized at Cold Spring Harbor Laboratory in September and at the meeting of the American Society of Human Genetics the following month, many of the groups held back their latest data—or so their competitors believe. "People clam up when they are near the gene," admits Ponder. "At Cold Spring Harbor we were all frustrated and we were all grumbling, but no one's record is pure."



"We are looking for a rare gene with a striking effect. The question is, How much [breast cancer] is like that?"

–Bruce Ponder

Unanswered questions

Collaboration or not, King predicts one of the groups will find the gene within a year, provided the mutation is fairly obvious. If it is subtle, however, "it could take a long time." Once researchers have the gene in hand, they can begin to sort out its role in inherited and sporadic cancers. A big question is just how much breast cancer can be pinned on this gene—is it just 5%, the rare, high-risk cancers? Skolnick, for one, suspects there are several mutations in the same gene—a major one that confers 90% risk and milder ones that confer perhaps 20% or 30% risk. "I think there will be an increasing awareness of the importance of genetic predisposition to breast cancer," he says. The gene now appears to be involved in late- as well as early-onset cancer—all of the groups have now found linkage in some of the families with later onset disease—but in what proportion? And in the common sporadic cancers, Collins and others predict that "this gene mutation will be found in most." But first they have to find the gene and its mutations.

Doing so should make possible the genetic screening, early diagnosis, and therapeutic advances that impelled King on her 20-year quest. Screening is just now beginning on a tiny subset of the women with extraordinarily high risk of breast cancer, those for whom data are good enough to clearly show that they carry the defective gene even before it is cloned. Once the gene is sequenced, Collins says, genetic screening could, in principle, be possible for all women to detect the one in 200 at extremely high risk. "I think it will be the first gene for which widespread presymptomatic testing will be appropriate," says Collins, who points out that while familial breast cancer is a rare cancer, it is one of the most common inherited diseases. Women who carry a mutation at this locus are also at increased risk of ovarian cancer, so finding it might enable the first early screening for that disease for the entire population, says Lenoir.

For any woman with inherited cancer or not, the key to eliminating mortality is early detection. Genetic screening would not be able to predict sporadic breast cancer, but if the gene is involved, it might provide an early warning. King envisions a "molecular mammogram," to detect altered cells years before they would be palpable or visible on a mammogram. At this point, King says, she doesn't know exactly what this diagnostic technique will be, "but I am confident the technology will come along very fast" once the gene and its alterations are identified. If caught early enough, the tumor could be removed with very limited surgery. If cancer cells have already spread, says King, it should be possible to design therapies targeted just at those, unlike current chemotherapy, which kills normal as well as malignant cells.

When the *BRCA1* gene is finally tracked down, however, King's quest will be far from over. Although it appears to be the major gene that predisposes women to breast cancer, most researchers believe that several others also play that role. How many, where they reside, and the risk each confers is unknown, however. In the end, predicts Ponder, "we will be left with families with very complex inherited cancer. "What we are looking for [in *BRCA1*] is a rare gene with a striking effect. The question is, how much is like that and how much is from the interaction of four or five more common genes," as is the case in coronary heart disease. And that, says Ponder,"brings us back to square one." The only way to track down the other susceptibility genes is to take those families that are *not* linked to the gene on chromosome 17 and repeat the same exercise—"do a Mary-Claire all over again," he says—in other words, conduct a whole genome search for each one. With luck and technology, those won't take 20 years a pop.

–Leslie Roberts