Coming to Grips With Genes and Risk

As experience with the breast cancer susceptibility genes *BRCA1* and *-2* shows, there can be a long road between finding a disease gene and understanding what it means

A little over 2 years ago, cancer geneticists made a long-awaited discovery: After years of struggle, they finally bagged the first gene linked to hereditary breast and ovarian cancers. The discovery of the gene, called *BRCA1*, was followed a mere 15 months later by the identification of a second, unrelated gene (*BRCA2*) linked to these same cancers.

Coming after intense, highly publicized searches, the news was greeted with elation by scientists, doctors, media, and the public. Although hereditary syndromes account for only 5% to 10% of breast cancers—and mutations in *BRCA1* and *-2* together play a role in only about two-thirds of these—the findings sparked high hopes that the new genes would be the keys to better understanding of all forms of these deadly cancers, and to more effective therapies for their victims. At the least, it was expected that these discoveries would make it possible to identify and help those most at risk of developing the cancers.

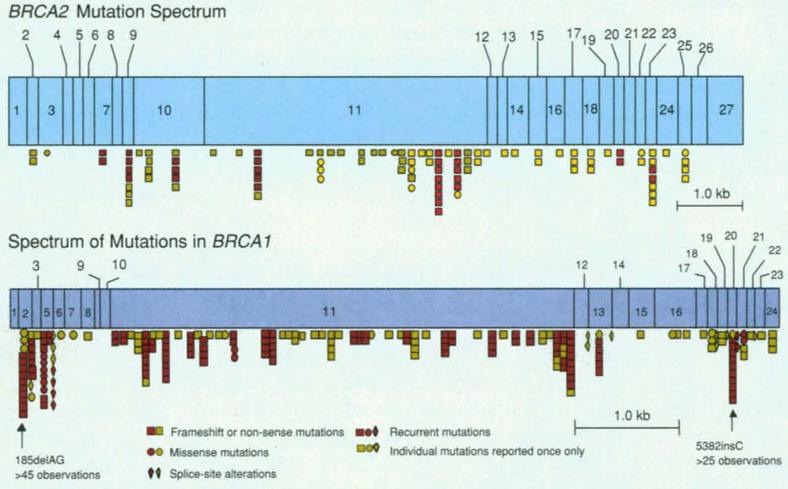
Now, however, high hopes are mixed with sober realities. Within the next week, Myriad Genetic Laboratories, a Salt Lake City, Utah, genomics company, will launch commercial tests for the genes, according to its president, Janet Haskell. And the Genetics and IVF Institute in Fairfax, Virginia, now offers tests for four specific mutations that are particularly common in Ashkenazi Jewish people—glaring exceptions to the relative rarity of *BRCA* mutations in the general population. But even as screening tests hit the market, there are still uncertainties surrounding these potentially lifesaving measures—and clear answers, which require huge studies on large numbers of women and their families, will take time.

The normal functions of *BRCA1* and *-2* are poorly understood (*Science*, 10 May, p. 799), and hundreds of mutations have turned up all through the genes. Worse, too little is known about the mutations to give women carrying them a precise assessment of what they mean: Are these women almost certain to get cancer, or are the odds less grim? "There's a hell of a lot of uncertainty in risk estimates, which very few people appreciate," says epidemiologist John Hopper of the University of Melbourne, who is helping to develop criteria for *BRCA* screening within Australia's public health system.

That wouldn't be so bad if there were routine ways to prevent these cancers, or to detect them very early when treatment is most likely to succeed. But women face a choice either of methods whose efficacy in *BRCA* carriers is completely unknown—such as mammography or chemoprevention—or drastic surgery, according to the report of an expert panel presented to the National Center for Human Genome Research (NCHGR) in Bethesda, Maryland, last May, by Wylie Burke of the University of Washington Medical Center in Seattle. Even with prophylactic removal of breasts or ovaries or both, cancers have been known to recur, the panel found, perhaps because not all tissue at risk can be removed.

As a result, experts are split about the tests going commercial. "I'm quite concerned that we don't yet have enough information ... [and that] private physicians may jump in who have had little exposure to some of these issues," says NCHGR director Francis Collins. Instead, he favors expanding research activities so that concerned women can find their way into strictly monitored testing programs. Hopper agrees: "[A commercial test] will make money out of raising anxiety and exploiting women. We should all take a deep breath and wait until there are decent data on the general population."

Others disagree. Ambiguity is the state of the art, one that patients can't be spared, says Barbara Weber, director of the Breast Cancer Program at the University of Pennsylvania Cancer Center. "I don't have any problem with commercialization of the test—although it's essential to have pretest education and posttest follow-up," she says. "Medical people understand that this is a significant responsibility." Says Mark Skolnick, Myriad's vice president of research, who played a key role in isolating both *BRCA* genes: "First, you save lives, especially with closer breast monitoring and prophylactic removal of the ovaries. ... Then, by testing large numbers of women and collecting information on as many as are willing, we can fine-tune our knowledge."



L. BRODY

A plethora of mutations. Mutations have been found throughout the huge *BRCA1* and *-2* genes, making their detection very difficult. (Numbers indicate the protein-coding exons.)

Mutations galore

One big hurdle in coming to grips with the *BRCA* genes is the sheer number of mutations: So far, researchers have reported over 235 different sequence variations in *BRCA1*, scattered all through the gene—which spans nearly 100,000 bases of the genome and encodes a protein of 1863 amino acids. For *BRCA2*, which is less well studied, about 100 different mutations have been documented in the huge gene, which encodes a protein of 3418 amino acids (see the Breast Cancer Information Core (BIC) database, http://www.nchgr.nih.gov/Intramural_research/Lab_transfer/Bic/). And the list of mutations is still growing, with new reports coming into BIC every week, says Weber.

This high number of mutations means that full sequencing of the genes is the only reliable way to screen them. But that is time-consuming, difficult, and costly—"a huge block" to research in the field, says Weber, with scientists usually falling back on simpler methods that miss some mutations. And it makes commercial testing a major techno-

Susceptibility Genes: Pointing a Way to Prevention?

Human genes are not islands: A host of other genes as well as factors in the outside world can affect their activities. That web of influences can make it hard to know how much risk a disease gene poses on its own (see main text). But there's a bright side to this complexity: If environmental factors shaping the effects of a disease gene can be tracked down, they can provide keys for treatment and prevention.

In 1994, for example, William Cookson and his colleagues at the Wellcome Trust Centre for Human Genetic Diseases in Oxford, U.K., found the first susceptibility gene for asthma and related allergic diseases. This gene turned out to encode part of the receptor for immunoglobulin E, a type of antibody that triggers allergic responses. Now several groups of researchers, including Cookson's, are closing in on some half-dozen additional asthma-susceptibility genes.

Even without knowing precisely how the genes work, Cookson says, once they are in hand researchers can begin linking particular gene variations (polymorphisms) in asthmatic people to the environmental exposures that set off their attacks. "With genetics, we may be able to say that people with this polymorphism have this sensitivity, and others have this one," says Antony Newman Taylor of the Royal Brompton Hospital in London, who coordinates a European study that will follow several thousand newborns for at least 5 years and look at these issues. "It gives us the potential to address issues we can actually do something about."

For some people—for example, those with genes that may make them sensitive to cat hair or tobacco smoke—the best strategy could be avoidance early in life, when a lifelong allergic reaction is probably first triggered. Others may be at risk from allergens such as house dust mites or pollen that are impossible to banish, says William Musk, a respiratory physician who, together with Cookson, is studying allergies in thousands of people in western Australia. Here, he says, "our aim is to figure out how to introduce genetically sensitive people early in life to specific allergens they can't avoid," in a way that could prevent the allergy from ever starting.

Other researchers are hoping to identify the triggers for juvenile diabetes (insulin-dependent diabetes mellitus, or IDDM), which starts when genetically susceptible people encounter agents, probably viruses or dietary factors, that set off an autoimmune reaction which slowly destroys the insulin-producing cells of the pancreas and eventually leads to disease. "We need to prevent autoimmunity from happening in the first place—which means knowing what triggers it," says pediatric endocrinologist Marian Rewers of the University of Colorado, Denver.

Rewers is leading a long-term study that should help find this out, monitoring exposures to risk factors and looking for autoimmune antibodies in thousands of children who carry specific versions of the major histocompatibility genes that increase their IDDM risk about 20-fold. And more help should come as geneticists zero in on up to a dozen other genes that could play a role in IDDM, which in turn will allow a more complete picture of which environmental factors set off IDDM—knowledge that's key to prevention. As Rewers says, "It's easier to change the environment than to change your genes." –P.K.

logical effort: Myriad's *BRCA1* and *-2* screen involves sequence analysis of about 16,500 base pairs and will cost $2400, according to Haskell. Screening for a specific mutation is much simpler and cheaper, however, for it only requires examining one spot in the gene.

For women found negative for specific mutations, such as one already identified in their family or any of those common to the Ashkenazi Jewish population, the test can spare them lifelong anxiety and even major surgery—although it doesn't mean that they will never get cancer for another reason. But not every mutation associated with breast or ovarian cancer can be detected, so negative results without adequate genetic counseling can raise false optimism. A few families have cancers that show genetic linkage to either *BRCA1* or *-2*, but, says Mike Stratton of the Institute of Cancer Research in Sutton, England, "we can't find any mutations." This suggests that the change lies outside the genes' protein-coding regions, perhaps in sequences affecting gene activity or levels of the protein products in the cell. And about one-third of all breast cancers that seem to run in families don't show linkage to either *BRCA1* or *-2*—which has prompted a search for a third *BRCA* gene.

When tests detect a mutation, its meaning must also be looked at carefully. The clearest cases are lesions already found in other family members with cancer and that—like most known *BRCA1* and *-2* mutations—cause the gene to make either a shortened protein product, or none at all. Another class of *BRCA1* mutations that most researchers consider harmful causes a single amino acid change in a region of the protein thought to be crucial for function because it contains a "ring finger" motif, a putative site of protein-DNA or protein-protein interaction.

A third, much smaller group of mutations are those causing amino acid substitutions in other regions of the protein. These can be hard to classify as either harmful or as benign variations (polymorphisms), especially when there are no other relatives with cancer to check whether they, too, carry the mutation. So such test results must be considered uninformative. From the perspective of screening, "these missense mutations will plague us," says Collins.

Are all mutations created equal?

But even women who test positive for a known, harmful mutation face some ambiguity, because there are questions about the degree of risk these lesions carry. Several researchers who spoke with *Science*, especially the epidemiologists, criticized the often-cited figures that *BRCA* mutations carry an 80% to 90% lifetime risk of breast cancer and a 44% risk of ovarian cancer. One problem, says epidemiologist Hopper, is that estimates like these are average probabilities and don't take into account other factors that could strongly affect risk for an individual woman, such as hormonal and childbearing history, and other genes. "One often gets a very deterministic view that you have gene X and this is what it does—finished," he says. "That's nonsense."

Stanford University epidemiologist Alice Whittemore provides some examples. Because *BRCA1* appears to be a tumor-suppressor gene, which tends to protect against cancer, both copies must be lost or inactivated for cancer to develop. The inherited mutation eliminates one copy; the other still must be lost—which is where other risk factors, such as a woman's past exposure to radiation, might be crucial. So could other genes, like the ataxia telangiectasia (*ATM*) gene, whose protein product repairs radiation damage. Mutation of one *ATM* gene raises cancer risk slightly. So a woman with mutations in both *BRCA1* and *ATM*, who also received radiation—perhaps even from mammography—could have a higher cancer risk than another woman with the same *BRCA1* mutation.

In contrast, because prolonged use of oral contraceptives reduces ovarian cancer incidence by half in the general population, *BRCA1* carriers taking birth control pills might also have lower risk—an issue Whittemore calls "one of the most important questions to me" because of its implications for preventive therapy in these women.

But the biggest quarrel with the commonly cited *BRCA1* and *-2* risk estimates is that they are based on atypical, "cancer-dense" families—those with multiple cases of breast and ovarian cancer, especially in members under age 40, and often significant (but smaller) increases in certain other cancers. So these estimates could be biased upward, says Whittemore: "We could be skimming off the top only those families where *BRCA* mutations are especially penetrant [have very strong effects], for whatever reason." An accurate risk assessment requires studying many people outside such families, she says, to see whether *BRCA* mutations are present in breast cancer patients without striking family histories. Because other family members would presumably also be carriers, such families "would lower our risk estimates," she says. Such data will be a while in coming. A few reports in the literature have suggested that these families exist—but numbers are small, and some researchers who spoke with *Science* remain skeptical.

Still, there are other glimmers of lower penetrance *BRCA* mutations. In another recent study looking outside high-risk families, David Barker at the University of Utah found a new missense mutation that may be associated with breast cancer in older women and may therefore be less severe. This work, to be published soon in *Genetic Epidemiology*, raises the crucial issue of penetrance and age: Women carrying *BRCA* mutations that increase their risk for breast cancer at age 65 rather than at 30 or 40 might make very different choices about medical intervention.

But the strongest hint of lower penetrance mutations so far comes from studies on the Ashkenazi Jewish population, the only one known so far where *BRCA* mutations can be called frequent. Two specific mutations—one in *BRCA1* and one in *BRCA2*—are each present in about 1% of this population, "an astonishingly high frequency that flies in the face of any precedent I can think of," says Francis Collins, who collaborated with the lab of NCHGR colleague Lawrence Brody on these mutations.

Because the two mutations are so common, researchers have been able to study much larger numbers of carriers than for any other single *BRCA* lesion. And such studies have now led two groups of researchers to propose that the *BRCA2* mutation is three- to fourfold less penetrant than that in *BRCA1*. (The results appeared in the October 1996 issue of *Nature Genetics*.) The difference is not yet proven, says Ken Offit of the Memorial Sloan-Kettering Cancer Center in New York, an author on one of the papers, but it jibes with clinical experience. "We sometimes get patients with [the *BRCA2* mutation] with no family history," he says. "We never saw that with the [*BRCA1*] mutation."

An ongoing joint study between the National Cancer Institute (NCI) and NCHGR should provide firmer estimates of penetrance for both mutations. The researchers are analyzing the family cancer histories of 5300 Ashkenazi Jewish people who were screened for the two mutations. By looking at cancer incidence in the mothers of the mutation carriers, about half of whom should also be carriers—and are now old enough to have developed many *BRCA*-associated cancers—the researchers expect to make at least an indirect estimate of penetrance, perhaps within the next month, says NCI's Jeff Struewing, who is a study participant.

From the cancer patient's perspective, the fewfold difference in penetrance, assuming it holds up, may not mean much, Offit points out. "Women seem to be just as concerned by a ninefold increase in risk as by a 30-fold increase," he says. But by studying a large group of women with the lower penetrance mutation, researchers hope to get at another key question: What distinguishes those who get cancer from those who don't? Is it some other risk factors, whether genetic, environmental, or lifestyle? The answers may point to ways that *BRCA* mutation carriers can lower their risk—just as researchers on other diseases involving genetic susceptibilities are attempting to do (see box on p. 497).

STUDIES OF *BRCA* GENES AND RISK

Registry	Special Features
Metropolitan New York Registry Cancer Families, Memorial Sloan-Kettering Cancer Center	Wide ethnic representation; of Ashkenazi-Jewish (AJ) study
Philadelphia Familial Breast Cancer Registry, Fox Chase Cancer Center	Informatics; links to NCHGR-ELSI committee AJ study
Utah Cooperative Breast Cancer Registry, Huntsman Cancer Institutes	High-risk families only; some very large
Northern California Cooperative Family Registry, Northern California Cancer Center	Wide ethnic representation; AJ study; Li-Fraumeni families
Australian Breast Cancer Family Study, University of Melbourne; New South Wales Cancer Council	Twins registry; AJ study
Ontario Registry for Studies of Familial Breast Cancer, Ontario Cancer Treatment and Research Foundation	AJ study

Big studies

The umbrella for addressing these questions is an ambitious project—the Cooperative Breast Cancer Registry, organized at NCI—that is now taking off. Over the next 4 years, six participating sites will collect blood cells and tumor specimens from about 6000 women with breast cancer and 20,000 of their relatives. Many women will be chosen based on their degree of familial risk (high, medium, or low), while others will be unselected for family history. The sites will also collect clinical and epidemiological data on everything from age of menarche, contraceptive use, pregnancies, and hormone therapies to smoking histories, environmental exposures, exercise habits, and diet. Records will be updated every year with data on who has developed cancer and the progression and treatment of their disease. The resource will be available for outside researchers, following stringent peer review, to tackle issues such as who is most likely to get breast cancer, why, and how to lower that risk, says project coordinator Daniela Seminara, program director for Genetic Epidemiology in NCI's newly formed Division of Cancer Epidemiology.

Across the Atlantic, the European Breast Cancer Consortium is coordinating prospective studies of known *BRCA1* and *-2* carriers. By following these individuals over the coming years, they hope to get at several issues, says member David Goldgar of the International Agency for Research on Cancer in Lyon, France. One is risk, not only for breast and ovarian cancer, but also for other types that are somewhat more frequent in carriers. Another is whether mutations in different regions of each gene cause a different spectrum of cancers, as first suggested for *BRCA1* by consortium members Stratton and Simon Gayther, Doug Easton, and Bruce Ponder of the University of Cambridge, U.K. The consortium will also collect clinical data for tackling the various medical issues about screening and treatment for mutation carriers.

And Myriad itself is planning a registry to follow up on women who test positive for *BRCA* mutations, provided they consent, says Skolnick. The project, to be carried out at an academic center not yet named, will collect clinical information similar to that of the others.

Eventually, the results of such studies should help women with *BRCA1* or *-2* mutations make informed decisions about how to protect themselves from breast and ovarian cancers. For now, these women face wrenching, potentially life-and-death decisions—and too little information to go on.

–Patricia Kahn