PERSPECTIVE

"The Race" to Clone *BRCA1*

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The existence of *BRCA1* was proven in 1990 by mapping predisposition to young-onset breast cancer in families to chromosome 17q21. Knowing that such a gene existed and approximately where it lay triggered efforts by public and private groups to clone and sequence it. The press baptized the competition "the race" and reported on it in detail for the next 4 years. *BRCA1* was positionally cloned in September 1994. Twenty years later, I reflect on "the race" and its consequences for breast cancer prevention and treatment.

"An important part of the last few weeks has been … to distinguish reality from fantasy. Fantasy has been 'the race'… Reality is having the gene, not knowing what it does, and the realization that in the 20 years that we have been working on this project, more than 1 million women have died of breast cancer. We very much hope that something we do in the next 20 years will preclude another million women dying of the disease."

This comment is mine, from a late-breaking session of the American Society of Human Genetics (ASHG) in Montreal in October 1994. The session was devoted to *BRCA1*, whose sequence had been published in the 7 October issue of *Science* by a group led by Mark Skolnick, Sasha Kamb, and David Goldgar of Myriad Genetics (*1*). Their proof was the identification of mutations in their candidate gene for *BRCA1* that cosegregated with breast and ovarian cancer in five different families. As confirmation, our Berkeley group presented mutations in *BRCA1* cosegregating with breast and ovarian cancer in 10 additional families (*2*). From both groups, nearly all mutations led to predicted truncation of the protein. The function of the gene was completely unknown, but with 15 informative kindreds carrying mutations cosegregating with disease, the genetic evidence was indisputable.

That I was speaking in this session was thanks to Hunt Willard, chair of the program committee of the ASHG that year, who had made time on the program for a loser as well as for the winners. Now 20 years later, I am again grateful, this time to *Science* for highlighting the anniversary of the cloning of *BRCA1* and for inviting me to give a Perspective on "the race" to find the gene and the consequences of its discovery. Demonstrating the existence of *BRCA1* by linkage mapping had been the work of my small group at the University of California, Berkeley, from 1974 to 1990; "the race" to positionally clone the gene consumed the professional lives of more than 100 researchers

in at least a dozen labs for the next 4 years. Success required the exploitation of new genomic tools whose development advanced gene discovery for all illnesses and became the building blocks of modern genomics.

"The Race"

In 1990, after 17 years of work, my group mapped a hypothetical gene for inherited predisposition to early-onset breast cancer to chromosome 17q21 (*3*). The result was immediately confirmed by Gilbert Lenoir, Steven Narod, and their colleagues, who mapped predisposition to both breast and ovarian cancer to the same map location, with the same markers, in different families (*4*). The existence of a gene for predisposition to breast cancer, and the possibility of isolating it by positional cloning, triggered enormous interest in big labs in government, universities, and the private sector.

Linkage Analysis: The Back Story

I had come to the problem of inherited breast cancer by a circuitous route. After completing my dissertation at Berkeley, I moved to Santiago, Chile, to teach in the University of California–Universidad de Chile exchange program and then found myself jobless after the Chilean coup of 11 September 1973 led to the program's termination. My career in genetics, not to mention my mental health, was saved by Nicholas Petrakis of the University of California, San Francisco, who in January 1974 offered me a research position to study genetics of breast cancer, in whatever way I thought best. I started reading.

Descriptions of families severely affected with breast cancer date to ancient Greek physicians. In the mid-19th century, the early years of modern medicine, Paul Broca reported in detail on families with breast cancer in multiple generations. He postulated that breast cancer in these families was hereditary, present in a "latent state" until later in life when it presented and progressed in a malignant fashion (*5*). In the 1920s, Janet Elizabeth Lane-Claypon, a founder of modern epidemiology, demonstrated significantly greater mortality from breast cancer among women whose mothers had died of the disease compared with women whose

mothers had died of other causes (*6*). By the 1970s, multiple epidemiological studies demonstrated that the risk of breast cancer was increased in the daughters and sisters of affected women, particularly those with premenopausal or bilateral disease [e.g., (*7*)]. The absence of environmental exposures or lifestyle risk factors shared exclusively by affected women in these families was a consistent theme. Apart from their cancer predisposition, women in these families were remarkably healthy and productive. Such families seemed the perfect foundation for research.

Breast cancer is a common complex disease. Proving the existence of a causal gene required addressing these complexities, the most daunting of which was causal heterogeneity at several levels. Most cases of breast cancer have no inherited component, but given its high incidence, breast cancer with no inherited component will appear both sporadically and in multiply affected families. Conversely, multiply affected families may include both inherited and noninherited cases of the disease. Other complexities included penetrance of the hypothetical gene dependent on gender, on age, and on unknown nongenetic factors; the possibility of different genes responsible for predisposition in different families (locus heterogeneity); and the possibility of multiple different alleles at each of the hypothetical genes (allelic heterogeneity).

As a recent student of Allan Wilson, I thought about the problem in an evolutionary way. The impact of genes on any trait, including estimates of allele frequencies and effect sizes, could be modeled with the tools of population and evolutionary genetics. In principle, multiple different mechanisms for familial clustering of breast cancer were plausible, including influences of dominant or recessive major genes, polygenic effects, and/or shared environmental factors. Using complex segregation analyses to evaluate a sample of 1579 families ascertained through the population-based Surveillance, Epidemiology, and End Results Program of the National Cancer Institute, we demonstrated that familial clustering of breast cancer was fully explained by an autosomal dominant, highly penetrant susceptibility gene(s) (*8*). The maximum-likelihood model yielded estimates of the critical parameters: that 4% of families developed breast cancer due to a susceptibility gene; that among women carrying mutations in the gene, the risk of breast cancer by age 70 was 82%; and that among women without a susceptibility allele, risk of breast cancer by age 70 was 8%. The model was purely mathematical, and the gene was, of course, hypothetical.

The best way to demonstrate the existence of such a gene was to find it. Given the technology of the 1980s, "find" meant mapping a gene to a physical chromosomal locale using linkage analysis. Defining the breast cancer phenotype was most important. I was enormously lucky to have advice early on from Bernard Fisher, the father of

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minimal surgical treatment for breast cancer. "You're looking for the cause of invasive breast cancer," he said. "Don't get distracted." In taking his advice, we sought families including multiple relatives with invasive ductal carcinoma of the breast. In many of these families, breast cancer was early onset and often bilateral, and occasionally also appeared in men. We did not broaden the phenotype to include the far more common atypical hyperplasia, despite its being an established risk factor for breast cancer. Focusing on a narrow disease definition limited the number of persons in each family defined as "affected" and thus reduced the sample size for linkage, but far more importantly, it eliminated false positives. Keeping our eyes on the prize proved critical to successful mapping.

A rigorous definition of the phenotype did not solve other complexities, including causal heterogeneity and dependence of breast cancer expression on gender and age. To address these complexities, my colleague Ming Lee adapted the elegant linkage methods developed by Newton Morton, Robert Elston, and Jurg Ott (9) to incorporate the parameters of our previous population-based model into our calculations of the likelihoods of linkage of breast cancer to each of our genetic markers. To take an extreme example, an unaffected male or an unaffected female in her 20s did not contribute any information against linkage, whereas an affected male or an affected female in her 20s was highly informative.

Through the 1980s, with the help of oncologists, their patients, and the patients' families, we enrolled 23 extended kindreds severely affected with breast cancer. No detailed human genetic map yet existed, so gene hunters worldwide developed the genetic map collaboratively, in parallel with linkage analysis for each group's own projects. The first linkage markers were protein polymorphisms (10), then restriction fragment length polymorphisms (11), then variable number of tandem repeat (VNTR) markers (12), and—after the discovery of the polymerase chain reaction (13)—short sequence repeat markers (14). The development of the human genetic map had organizational homes at the Centre d'Etude du Polymorphisme Humain in Paris (15), led by Jean Dausset, Jean Weissenbach, Jean Marc Lalouel, and Mark Leppert; and with the beginning of the Human Genome Project, at the National Institutes of Health (NIH), led by Jim Watson. The human gene mapping period was characterized by open sharing of probes, DNA samples, and data, as well as by humor and good fellowship.

In our 23 extended families, Jeff Hall and I genotyped new markers as quickly as we learned of them or could develop them ourselves. Each marker was genotyped individually, the vast majority by Southern blot, in all informative persons.

The 173rd marker that we tested was *D17S74*, a highly polymorphic VNTR on chromosome 17q21. Linkage of breast cancer to this marker, using our model-based linkage parameters, yielded odds of 10^6 to 1 in favor of linkage for the seven families in our series with an average age of breast cancer onset ≤45 years and evidence against linkage for the 16 families with average age of breast cancer onset >45 years. We published the results in 1990 (3).

> **"A path that began nicely enough with two or three adjacent cosmids would soon turn back on itself, yielding more a meander through a swamp than a walk from one signpost to another."**

Positional Cloning

The Human Genome project was also born in 1990, so "the race" to find "the breast cancer gene," began with no genome sequence, no integrated physical maps, no awareness of genomic architecture, and certainly no genome browser. The mainstream estimate of the number of human genes was 100,000, based on inaccurate estimates of gene size and density. Very few genes were characterized and even fewer mapped. Sequencing was done by hand. There was no e-mail or Internet. The revolutionary advance in data sharing was the fax, with curly paper that found comfort hiding beneath your desk.

Following successful mapping, the process to gene discovery was positional cloning, which was experimentally challenging but fun. For *BRCA1* (which in 1991 I was allowed to name, because its existence was now widely accepted), we knew the chromosomal locale, defined by linkage and bounded by recombination at genetic markers in informative members of our families. The corresponding physical region was completely unknown. My group at Berkeley developed a collaboration with Francis Collins, then at the University of Michigan; with Anne Bowcock, then at the University of Texas Southwestern Medical Center; and later with Jeff Boyd, then at the National Institute of Environmental Health Sciences. Our joint strategy was to carry out four activities in parallel: continue genetic mapping with new markers to narrow the linkage region; generate a complete physical map of the linked region via a path of overlapping DNA-containing clones; hybridize the clones representing the region to a

cDNA library of genes expressed in our tissue of interest; and isolate and sequence these revealed genes from the cDNA library, then sequence each gene in DNA from affected members of our families to discover mutations disabling the gene.

Twenty years later, with the complete human genome sequence, it is now clear that the physical size of the region linked to *BRCA1* in our families was 22 Mb in 1990, 4.5 Mb by late 1992, and 1.0 Mb by early 1994, when all groups had resolved the critical recombination events in all families (16–18). Given the difficulty of physically mapping and cloning, the most productive period, by far, was after the region was reduced to a manageable size by linkage. Constructing the physical map was a tremendous challenge. At the time, DNA had been cloned into 40-kb cosmids by NIH and U.S. Department of Energy labs, and into much larger yeast artificial chromosomes (YACs) by Maynard Olson's lab (19). From their previous gene hunts, Francis Collins' lab was adept with chromosome walking with cosmids, but it was soon clear that the 17q21 region had many messy features that made these walks more difficult than previous ones. A path that began nicely enough with two or three adjacent cosmids would soon turn back on itself, yielding more a meander through a swamp than a walk from one signpost to another. In retrospect, the problems were the high density of Alu sequences, of segmental duplications, and of pseudogenes in the *BRCA1* region. It is not surprising that a chromosomal region harboring a high-penetrance cancer susceptibility gene has so many bizarre architectural features. Odd genomic architecture predisposes to errors at mitosis and therefore to the somatic mutations that are the second hits of tumor suppressor genes.

For physical cloning, YACs were a cause for celebration because they captured much more DNA than did cosmids, so each tile (clone) was larger and the number of tiles needed to span a region was smaller. The corresponding challenges of YACs were that the longer the cloned insert, the more likely it was to be chimeric; that is, to include pieces of multiple chromosomes and to have internal deletion of elements of the chromosome of interest. The secret was not to be greedy, to work with YACs that were 100 to 200 kb—so longer than cosmids, but not grandiose. Bacterial artificial chromosomes (BACs) (20) had been developed at about the same time as YACs, but were not reduced to practice for human gene mapping for several years. BACs were intermediate in size between cosmids and YACs, so they are more stable than YACs and more informative than cosmids. BACs became a critical backbone of the Human Genome Project and, indeed, the Myriad group identified *BRCA1* from a BAC (1).

To keep the activities in our Berkeley, Ann Arbor, Dallas, and Research Triangle Park labs

straight, every day I wrote an "Order of the Day" (OOTD) that included progress with every cosmid, YAC, probe, library, genetic marker, and family. The OOTD was sent around by fax, then various small groups of people would chat by phone to plan the next experiments. This continued, if not 24/7, at least 14/6. The OOTD and the organization connected to it became even more important after Francis Collins moved from Michigan to the NIH in 1993 to take over direction of the Human Genome project from Jim Watson. With Francis' lab now in two places, we were four groups in five towns in three time zones, keeping in contact without Internet or cell phones. It seemed normal at the time.

The collaboration painstakingly identified hundreds of cosmids and YACs that mapped to our linked region. To maximize our success in capturing complete and critical genes, each group probed our shared clones to different cDNA libraries. In my lab, Eric Lynch created a beautiful cDNA library from the surface epithelial cells of an ovary donated by one of our participants at the time of her prophylactic surgery. We decided to create a cDNA library from ovarian epithelium rather than from breast epithelium, because breast epithelial cells are very difficult to dissect from other interspersed cell types. In contrast, ovarian epithelium covers the ovarian surface like a delicate blanket, so epithelial cells could be gently removed, the RNA isolated, and a library made of the expressed genes.

By hybridizing the cosmid and YAC clones from the linked region to this library, we iden-tified more than 400 different cDNA clones and ultimately cloned and sequenced 15 genes from the 1-Mb region, as well as several others nearby (21, 22). Of these 15 genes, 5 were known but not previously mapped, and 10 were previously unknown. None carried loss-of-function mutations in our breast cancer families, because none was BRCA1 (Fig. 1).

When the BRCA1 sequence was published, how close were we to finding it? In retrospect, we had a marker inside BRCA1 and did not know it. Our most informative linkage marker was AFM248yg9, in intron 20 of BRCA1, within a few kilobases of mutations in several of our families. Of course, we did not know this marker was inside BRCA1; we knew only that we could not identify a cosmid or YAC that carried it. In retrospect, cosmids carrying BRCA1 were particularly difficult to identify because most of the intronic sequence of BRCA1 is Alu. By masking Alu sequences to hybridize to the cosmid library, we masked the marker in BRCA1 as well. Our physical map of the 1-Mb region had only one gap, exactly at BRCA1 (Fig. 1). Of course, at the time, we had no idea of the size of the gap. Part of the plan was to create probes with single-copy DNA flanking each genetic marker and use them to find more clones. This would have revealed a cosmid containing BRCA1, then BRCA1 itself. All groups in "the race" used essentially the same positional cloning strategy. The limiting factors were time and resources. As Maynard Olson remarked after visiting our lab, we were looking for a pot of gold at the end of a rainbow in the backyard with a hoe, when what we needed was a backhoe.

The Gene

Even after it was cloned, BRCA1 continued to confound its pursuers. For example, there is no exon 4 in BRCA1, because in the cDNA sequence published by the Myriad group, exon 4 was an Alu sequence with stops in all reading frames (1). The faux exon was soon gone from the sequence, but exon numbering did not change. More substantively, the genomic structure of BRCA1 is distinctive, with a long central exon that encodes ~60% of the protein and is remarkably tolerant of amino acid substitutions. The 21 small flanking coding exons encode virtually all the functionally important regions of the protein. Likely for reasons of their parallel evolutionary histories, the genomic structure of BRCA2 is almost identical to that of BRCA1, despite the two genes sharing no similarity in primary sequence (23).

At the time of its discovery, the biological function of BRCA1 was completely unknown. There were no homologous genes and no recognizable motifs other than the RING domain (24), which was an acronym for "really interesting new gene," not a description of function. BRCA1 could only have been found by a genetic approach. Because its function was unknown, it would not have been selected as a candidate gene based on a biological role. A genetic approach, however, is blissfully tolerant of total functional ignorance.
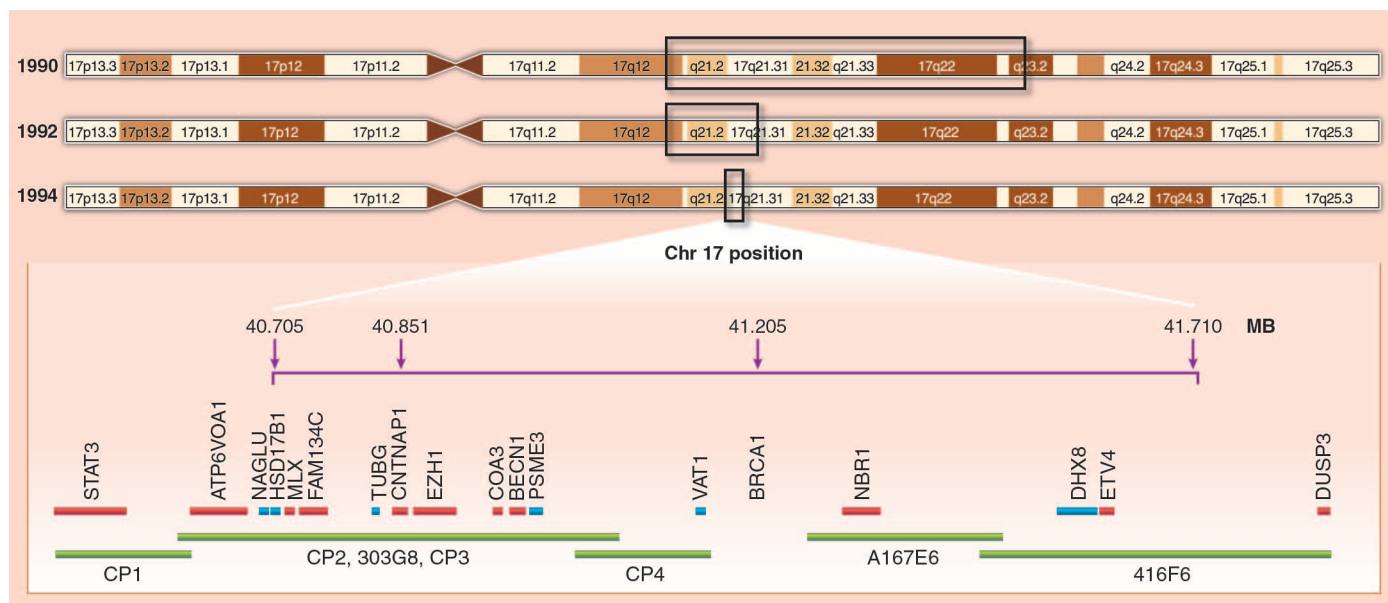

Fig. 1. Linkage and physical mapping of the BRCA1 region as of early September 1994. Drawings of chromosome 17 at the top of the figure indicate refinement of the BRCA1 region (black boxes) by linkage in 1990, 1992, and 1994. The final region defined by recombination in families was bounded by genetic markers (purple arrows), in retrospect known to lie at 40.705 and 41.710 Mb on 17q21.31, so yielding a linked region 1 Mb in length. This region was captured in YACs, P1 clones, and cosmid pools (CPs) and assembled in physical contigs (green bars). In this 1-Mb region, we identified 15 genes, 5 previously known but not mapped (blue bars) and 10 previously not known (red bars). BRCA1 was not captured because it lay in a 100-kb gap in the physical contig that was only fully sequenced 2 years later (37). In retrospect, the very dense packing of Alus in BRCA1 led to it being refractory to clone capture. Thanks to the completion of the Human Genome Project, no gene hunter ever need face this problem again.

Twenty years after its discovery, the biological roles and evolutionary origins of *BRCA1* are still being elucidated. Genetics revealed that *BRCA1* is a tumor suppressor gene following the two-hit model (*25*): Cancer develops as the result of one inherited loss-of-function mutation followed by a somatic mutation causing loss of the remaining wild-type allele in a vulnerable cell type. The central puzzle is why complete loss of function of *BRCA1* leads to cancer. Solving this puzzle has been especially challenging, because the BRCA1 protein is involved in multiple essential biological functions (*26*).

As part of a multiprotein complex, BRCA1 repairs double-strand DNA breaks via the homologous recombination repair pathway. The C-terminal BRCT domain interacts with histone deacetylase complexes and is involved in transcriptional regulation. The N-terminal RING domain heterodimerizes with a sister domain of BARD1 and acts as a ubiquitin ligase of the estrogen receptor (*27*). Missense mutations that abrogate the function of the RING domain lead to breast cancer. Virtually all other cancer-causing mutations of *BRCA1* are truncations: nonsense mutations, frameshifts, or large genomic deletions or duplications leading to stops and loss of the C-terminal domain.

BRCA1 is ubiquitously expressed, so it has been a mystery why *BRCA1* mutations lead specifically to breast and ovarian cancer and, to a lesser degree, to pancreatic and prostate cancer. The estrogen receptor is a substrate of the ubiquitin ligase activity of the BRCA1 RING domain, and missense mutations in critical residues of this domain lead to breast cancer predisposition (*23*). Very recent work indicates that estrogen controls the survival of *BRCA1*-deficient cells via a PI3K/NRF2-regulated pathway (*28*). *BRCA1* has revealed other breast cancer genes by virtue of the functional relationships of their encoded proteins. In particular, other genes critical for DNA repair—including *TP53*, *PALB2*, *CHEK2*, *BARD1*, *BRIP1*, *ATM*, *RAD51C*, and *RAD51D*—harbor mutations leading to inherited breast and ovarian cancer (*29*). Thousands of different disease-causing mutations have been detected in *BRCA1* and *BRCA2*. Each loss-of-function mutation is individually rare, and each independently confers very high risk for breast and ovarian cancer. The other breast and ovarian cancer genes also harbor many different rare, recent damaging mutations with effect sizes ranging from twofold increased risk for *CHEK2* to 10-fold for *TP53*.

Of the seven families in our 1990 linkage analysis with young-onset breast cancer (*3*), six families harbor mutations in *BRCA1*, and one harbors a mutation in *BRCA2*. Of the 16 families in that analysis that we predicted would not carry mutations in *BRCA1*, six are explained by *BRCA2*; one each is explained by *PALB2*, *CDH1*, and *SLX4*; and seven remain unsolved. There are more breast cancer genes to be found.

Genetic heterogeneity of inherited predisposition to breast cancer serves as a model for other complex illnesses. The disorder results from any one of thousands of different mutations in any one of multiple genes. The critical genes known thus far encode proteins in the same and related pathways. The discovery of *BRCA1* and its sister genes illustrates that the degree of biological complexity underlying a phenotype is an excellent predictor of its genetic heterogeneity (*30*).

Our Genomes, Ourselves

In June 2013, the U.S. Supreme Court ruled unanimously that genes are products of nature and therefore cannot be patented (*31*), nullifying the Myriad patents on *BRCA1* and *BRCA2*. The ruling was a victory for science and for patients and led immediately to broader availability of clinical genetic testing.

For nearly 20 years, while Myriad was the only commercial source in the United States for genetic testing of *BRCA1* and *BRCA2*, cost was a major deterrent to widespread screening. The cost to women of *BRCA1* and *BRCA2* testing is now dropping, due both to the end of the monopoly and to two scientific developments that have changed the landscape. First, there are now enough genes identified with mutations predisposing to breast and ovarian cancer that multigene screening panels can be developed and effectively implemented. Second, genomic technology now offers the opportunity to sequence at costs orders of magnitude lower than the cost of Sanger sequencing (*32*). Previously, clinical genetic testing was carried out gene by gene, based on specific clinical indications and family histories, with each test costing thousands of dollars. With the advent of massively parallel sequencing, large panels of genes are now screened simultaneously at far lower cost (*33*).

There was another barrier to genetic testing for inherited breast and ovarian cancer. Some patients and physicians worried that a positive finding would lead to loss of health care coverage. In consequence, mutations were not identified in some women who could have been saved by risk-reducing surgery. Clinical guidelines have been established for women harboring damaging mutations in *BRCA1* and *BRCA2*, including increased surveillance, surgical removal of ovaries and fallopian tubes (salpingo-oophorectomy) by age 40 years or younger, and the possibility of risk-reducing mastectomy (*34*, *35*). The Genetic Information Nondiscrimination Act of 2008 (Public Law 110–233), which protects mutation carriers against loss of health care coverage, should have removed fear as a barrier to testing, so that women with mutations in *BRCA1* and *BRCA2* can be identified without economic reprisal.

So what next? Given that 50% of *BRCA1* and *BRCA2* mutations are inherited from unaffected fathers, and given the small size of modern families, almost 50% of women with *BRCA1* and *BRCA2* mutations have little or no family history of breast or ovarian cancer. Yet, cancer risks to mutation carriers with no cancer family history are as high as risks to mutation carriers from severely affected families (*36*). Identification of cancer-causing mutations in *BRCA1* and *BRCA2* has clear and actionable implications for prevention. *BRCA1* and *BRCA2* screening as part of routine health care for young adult women is sensible and feasible. As in any population-screening program, genetic or otherwise, few participants will prove positive, but for women who learn that they carry mutations in *BRCA1* or *BRCA2*, the consequences are enormous, addressable, and life-saving.

Until there are no more breast or ovarian cancers among women with *BRCA1* or *BRCA2* mutations, the real race is not over.

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