M. SMITH

ENVIRONMENT AND GENETICS

Making sense of the noise

The search for the genetic determinants of breast cancer risk is focusing on ever smaller effects, requiring larger groups of subjects.

BY ANNA PETHERICK

Possibly the most famous cancer gene is called *BRCA1*, for breast cancer 1. It is expressed in breast tissue and encodes a protein that fixes double-stranded breaks in DNA. When such breaks cannot be repaired, the same protein triggers cell suicide, protecting the cell from replicating the mutated DNA. Mutations in *BRCA1* that prevent its expression or cripple the protein raise the risk of developing breast cancer. A woman with a faulty *BRCA1* gene has a 65% chance of developing breast cancer by the age of 70 (ref. 1).

No other genes with an equivalent level of breast cancer risk have been found. And as the search for candidate genes considers ever more subtle effects, the study population that is needed to provide the statistical power to spot these effects is growing correspondingly larger. Finding *BRCA1* and *BRCA2* (another tumour-suppressor gene linked to breast cancer) required only a few families with a high incidence of breast cancer and a panel of only a few hundred genetic markers². But now the search for correlations between disease incidence and genetics routinely encompasses tens of thousands of people and scans the full length of their genomes. These genome-wide

association studies (GWAS) have so far identified about 25 genetic loci linked to breast cancer, implicating a variety of cellular mechanisms in the disease.

THE MISSING INHERITANCE

Using GWAS does not definitively answer the question of which genes raise the risk of cancer, however. The 25 or so single nucleotide polymorphisms (SNPs) that have been linked to breast cancer³ label sections of DNA that are typically tens or hundreds of kilobases long, rather than specific genes. These 'haplotype blocks' might each contain several genes, any of which could be causing the association. Only in a few cases have researchers confirmed the specific gene responsible, such as the fibroblast growth-factor receptor 2 gene, which was discovered by a team led by Kerstin Meyer at the Cancer Research UK Cambridge Research Institute.

But GWAS fall short in other ways as well. The loci found so far through genetic screening account for only 9–10% of breast cancers. Many breast cancers are caused by gene variants too rare to show up in these studies, and even after considering genes that have strong effects (such as *BRCA1* and *BRCA2*), 70% of breast cancer cases are still unexplained⁴. Yet having a family

history of breast cancer is a far stronger predictor than the genetics alone imply.

Shared environmental details of family life might play a part, of course, although results from studies on identical twins raised apart suggest that this is unlikely. Calculations based on these studies reveal that having a mother or sister with breast cancer doubles a woman's risk of developing the disease, without the environment playing any role⁵. So where is the missing heritability?

Several large cohort studies are trying to plug this gap in our knowledge. Douglas Easton, head of the Cancer Research UK genetic epidemiology group at the University of Cambridge, was keenly involved in the hunt for *BRCA1* and *BRCA2*. He is now running a study called EMBRACE, which is beginning to make sense of the way the genetic variants interact. Half of the 3,000 women enrolled in EMBRACE have either a faulty *BRCA1* or *BRCA2* gene, and half do not. Using GWAS has helped to clarify the risk conferred by a harmful mutation in these genes. The risk associated with a faulty *BRCA1* gene is modified by the presence of other SNPs that have been independently linked to oestrogen-receptor (ER)-negative breast cancers, so called because these cells underexpress oestrogen receptors. "Meanwhile, *BRCA2* risk is

A TRAUMATIC ENVIRONMENT

Is psychological stress a risk factor?

A lonely rat is an anxious rat. Place a female Norway rat in lifelong solitary confinement, and her odds of developing a malignant breast tumour more than triple⁷. Put a happy rat on her own, and you raise her risk of breast cancer — or, if the animal is already afflicted, you reduce the effectiveness of chemotherapy. There is evidence linking this observation to hormone levels: at least two months before tumour development, the rat's reaction to the stress hormone corticosterone changes.

For decades, epidemiology has suggested that stress can also be a factor in the development of human breast cancer. Lital Keinan-Boker, an epidemiologist at the University of Haifa in Israel, and her colleagues investigated breast cancer rates among more than a quarter of a million people who had all, for a significant period, experienced severe psychological stress. Keinan-Boker's team compared Jews migrating from Europe at different times and found that Holocaust survivors have a higher chance of developing some form of cancer, with breast and colon cancers the most common. Women who were children during the Holocaust have a particularly high risk⁸.

A follow-up study that has yet to be published attempts to filter the confounding effects of physical stresses, such as delayed

menarche and rapid weight gain after the war, from the psychological stress. Keinan-Boker and her team focused on Holocaust survivors who spent much of the war in certain camps for which there are extensive records. They probed the survivors for signs of post-traumatic stress syndrome connected to the Holocaust and created physical stress indices of the survivors' experiences using information from the camps, such as average daily calorie consumption. The results suggest that extreme psychological stress is an independent predictor of breast cancer, she says.

But what does this mean for ordinary life? "It's not clear whether everyday stress levels are a contributing factor," says Christopher Mueller, a molecular oncologist at Queen's University in Kingston, Canada, who was not involved in the Holocaust study. But acute stresses, such as the death of a family member, seem to have a clear effect, he says. In early 2012, Mueller's lab reported⁹ that the human stress hormone cortisol causes a glucocorticoid receptor that normally binds to the *BRCA1* promoter to dissociate from it, lowering *BRCA1* expression levels and increasing breast cancer risk. Mueller thinks this might be one route by which stress alters gene expression in breast tissues, making cancer more likely. **A.P.**

genuine molecular, behavioural and environmental risk factors from factors that merely correlate with them (for a different approach, see 'A traumatic environment'). "Many factors on the list — obesity, exercise, the age at which women go through the menopause — might act via relatively few hormones," he explains.

Swerdlow suspects that lifelong exposure to changing levels of these hormones, in particular during critical periods of female life history (such as menarche and menopause), might amplify the impact of the genes. "The snapshots that one-time questionnaires and blood samples provide are useful, but they don't really get at the subtle shifts over a woman's lifetime that are likely to increase or decrease her chance of developing breast cancer," he adds. The study has already discovered independent yet useful details of life history, including four common genetic loci that help to predict the age of early menopause — and thus when a woman's fertility is likely to start declining, which is typically ten years earlier⁶.

THE HISTORY OF RISK

Breakthrough Generations includes women as young as 16, but determining the role of different aspects of female life history in breast cancer risk requires cohort studies that follow girls while they develop breasts. Ethical considerations make these trials harder to run, but a few groups are trying.

The University of Cincinnati in Ohio, the University of California, San Francisco, and the Fox Chase Cancer Center near Philadelphia, Pennsylvania, have each recruited groups of about 400 girls aged 6 to 8. They plan to follow these cohorts to at least the age of 17, to help understand the environmental and genetic factors that lead to the onset of puberty, because earlier menarche is a risk factor for breast cancer in adults. At least once a year, these 1,239 girls will be assessed for exposure to environmental toxins, including phyto-oestrogens and other chemicals known to disrupt hormone levels, their body mass index will be calculated, and other medical and social details will be recorded. But such a small study has its limits. "We're nowhere near being able to really understand the environmental influence," says Meyer. ■

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Women who survived the Holocaust are more likely to develop breast cancer.

affected by a different set of variants, which makes sense because you tend to get ER-positive disease with *BRCA2*," says Easton.

Such combinatorial data are being added to a computer program called Boadicea (named for a historical British warrior queen), developed at the University of Cambridge, which calculates the odds of breast cancer on an individual basis. With these new data, "instead of only being able to tell a woman with a *BRCA2* mutation that she has a 50% chance of

developing the disease by the time she is 70, we can identify women with a 70% or more risk, and women with a risk below 30%," says Easton.

Another intriguing cohort study is Breakthrough Generations. Anthony Swerdlow, an epidemiologist at the Institute of Cancer Research in London who is joint head of the study, hopes that the repeated, detailed questionnaires and blood samples supplied by the study participants will help distinguish

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