



Polygenic Predisposition to Breast Cancer

www.cambridgecancer.org.uk/bruceponder

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Our group is aiming to understand genetic susceptibility to common cancers, in particular breast cancer:

The chance of an individual to develop breast cancer is increased approximately two-fold if that individual has a close relative affected by breast cancer. Twin studies indicate that the familial risk is genetic rather than environmental. The work in our laboratory focusses on the following questions:

- (1) Can we develop new strategies to identify additional risk loci?
- (2) How do the already identified risk loci exert their function?
- (3) Do common variants at these loci exert a combined effect by perturbing complex systems within the cell?

The genes that predispose to breast cancer are mostly still to be identified. There are two genes, BRCA1 and BRCA2, where a number of different uncommon mutations confer a large increase in risk. In 2007 we, together with Doug Easton, Alison Dunning and Paul Pharoah, published five new risk loci, identified in the first whole genome scan for breast cancer (Easton et al., *Nature* 2007; 447:1087). The scan showed that for breast cancer the number of variants which confer a large risk is very limited, but instead there are many common variants that individually carry only a small risk.

Our initial scan, based on a total of 22,000 cases and 22,000 controls, found five loci that were significantly associated with increased risk for breast cancer. We are now investigating strategies to identify further variants. One way of increasing the power of genetic association studies is to increase the study size. This approach is driven forward by Doug Easton and colleagues at the Strangeways Research Laboratory. As an alternative approach we are working to develop phenotypic assays that may provide a surrogate read-out of cancer risk. For example, an individual's ability to repair DNA damage may be related to that person's risk of developing cancer. Similarly the elasticity of a cell may be a marker of the

likelihood of a tumour cell from that individual to metastasise. We are currently developing high-throughput assays for these two characteristics to evaluate their use either as prognostic markers or to stratify the population in future whole genome association studies.

We are also interested in understanding how risk genes exert their function. For the FGFR2 locus, the top hit of the whole genome scan, we were able to show that the risk allele correlates with increased FGFR2 gene expression in normal breast tissue. This is consistent with recent studies that have reported that the FGFR2 tyrosine kinase functions in the control of proliferation of the adult breast epithelium. Genetic mapping will usually identify a risk interval without pinpointing the causal variant. We therefore carried out an analysis of protein-DNA interactions of the candidate causal variants in the region. This identified two potential sites of control: a C/EBP β binding site and an Oct-1/Runx2 site. The latter was shown to increase transcription in transfection assays and, furthermore, we were able to show that the rare allele of the Oct-1/Runx2 is occupied *in vivo*.

More recently we have used DNase hypersensitivity mapping to analyse these regions. For the FGFR2 gene this indicated that only two out of the initial set of potential causal variants lies in accessible chromatin, which includes the Oct-1/Runx2 site. In addition, a fine-mapping approach of the FGFR2 gene in multiple populations, including African-Americans, has found that this site was most highly associated with the risk of developing breast cancer. Our results demonstrate that multiple approaches, such as fine-mapping, the analysis of chromatin, and functional studies will need to be combined to identify causal variants. We are employing similar strategies to identify causal variants in the TNRC9 risk locus and in the chromosomal region 8q24 which is known to carry risk loci for breast, ovarian, colorectal, prostate and bladder cancer.

As an alternative approach to identifying risk genes, Ana-Teresa Maia is studying differential expression of the two

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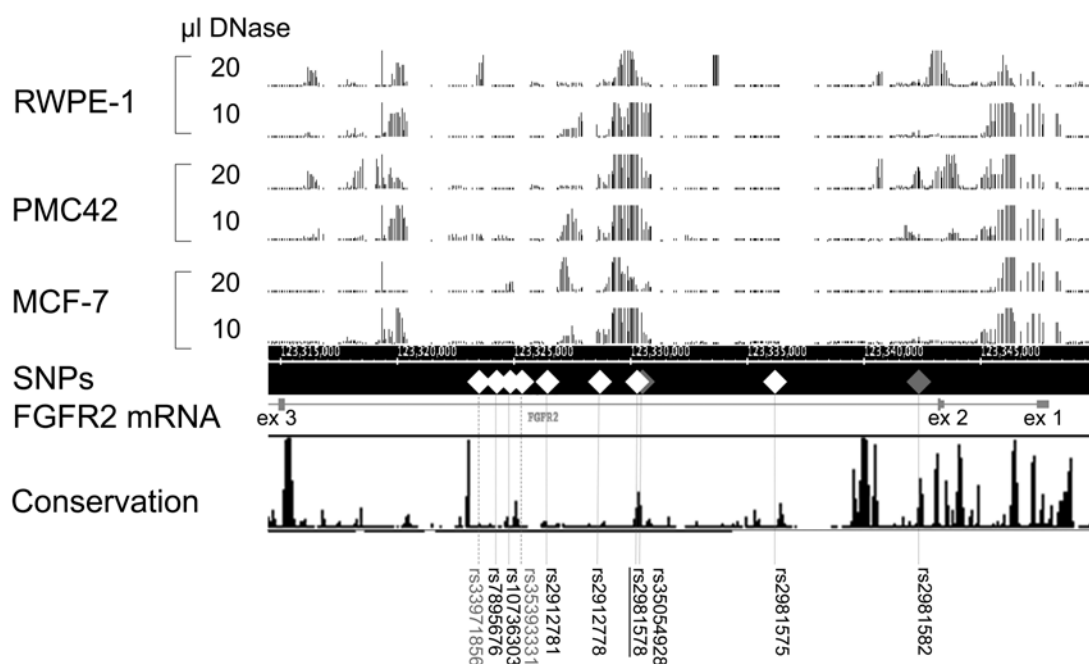


Figure 1. DNase I hypersensitivity profiles of the genomic region of *FGFR2* covering exons 1-3. Profiles for two breast (PMC42 and MCF-7) and one prostate (RWPE-1) cell line are shown. Open diamonds indicate candidate breast cancer susceptibility SNPs as identified by genetic mapping studies. Only two of these lie in open chromatin, making these SNPs more likely to play a role in causing breast cancer.

alleles of a gene. Using Taqman probes she has observed that differential expression of alleles is common in a set of candidate genes known to contribute to breast cancer. In collaboration with the Tavaré laboratory, she is now using bead technology to develop a high-throughput assay for allelic expression, which will allow an analysis of differential allelic expression in larger patient and control cohorts.

We have recently shown that the risk loci identified so far are already useful in stratifying the population into high and low risk groups, for which different screening strategies might be used. However, as yet very little is known about how

the different risk loci interact to have functional effects that increase susceptibility. We will take a network approach to this problem. The concept is of many polygenes feeding into networks of genes that are co-ordinately expressed in such a way that the network can be perturbed by variants in the genes that feed into it. The perturbations in turn alter cellular behaviours, and underlie susceptibility. In the future we will use bioinformatic approaches to predict networks that can be verified in the laboratory. By identifying nodes in the network, it may be possible to identify new therapeutic targets or risk predictors.

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