

Perspective

Heterozygous Mutations in DNA Repair Genes and Hereditary Breast Cancer: A Question of Power

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The emerging technology of massively parallel DNA sequencing has had a major impact on progress in genomics and personalized medicine [1]. Most recently, DNA sequencing of whole exomes (complete coding regions of the human genome) has revealed the genetic basis of many previously-not-localized Mendelian traits [2]. In diseases where the underlying genetic basis is more dilute and complex, old challenges reappear in new clothes [1,3].

Both the promise and the limitations of these new technologies have been evident in the untangling of the polygenic basis of susceptibility to human breast cancer. The identification of single-gene defects in cancer susceptibility syndromes in the 1990s provided a deterministic model of genetic susceptibility to cancer. Discovery and genetic analysis of the hereditary breast and ovarian cancer genes *BRCA1* and *BRCA2* offered a preview of personalized genomics, improving medical management of a common form of inherited human neoplasia [4,5]. Supporting the idea that new breast cancer genes (often referred to collectively as *BRCA3*) could be identified, analysis of the genetic variance remaining after *BRCA1* and *BRCA2* mutations had been excluded suggested that most of the excess genetic risk was concentrated in a small percentage of persons [6]. Yet, genetic linkage studies provided little encouragement for the existence of *BRCA3* [7]. While genome-wide association studies (GWAS) uncovered new pathways in cancer biology, the GWAS results identified markers of very modest effect size [8]. The realization that a small proportion of excess genetic risk can be accounted for by common variants has resulted in a return to the study of multiplex breast cancer kindreds and the utilization of massively parallel DNA sequencing to uncover rare, disease-causing mutations in hereditary breast cancer.

The article by Thompson et al. published in this issue of *PLOS Genetics* [9], along with a similar article published recently in the *American Journal of Human Genetics* [10], provide a glimpse of the early

applications of new sequencing technologies to the search for the “missing heritability” in hereditary breast cancer. In Thompson et al., the authors performed exome sequencing of multiple breast cancer cases from a small number of families (33 persons in 15 families) in whom *BRCA1* and *BRCA2* mutations had been excluded, and they focused on mutations that are predicted to ablate the function of the gene product, namely, mutations that cause premature termination of translation or that destroy splice-sites. After filtering out the overtly deleterious mutations that are polymorphic in the human population (under the assumption that the risk-causing variation in these breast cancer families should be rare), each sequenced individual harbored on average 35 overtly deleterious mutations. Thus, additional filtering criteria were needed to narrow the field: various strategies are possible, and here the authors focused on genes both hit by mutation in multiple individuals and participating in DNA repair through the homologous recombination pathway, a pathway that repairs double-strand breaks with high fidelity. Previous candidate-gene DNA sequencing studies have implicated homologous recombination in breast cancer susceptibility [11–16], and *BRCA1* and *BRCA2* themselves are players in this pathway [17]. Remarkably, two families carried overtly deleterious mutations in the Fanconi anemia (FA) gene *FANCC* and one family carried an overtly deleterious mutation in the Bloom’s syndrome (BS) gene *BLM*.

FA and BS are rare, autosomal recessive conditions that are characterized by

multiple developmental abnormalities (small size and congenital defects of the dermal, immune, skeletal, and reproductive systems), striking DNA repair defects and genomic instability in the somatic cells, and enormous predisposition to the development of various cancers (Figure 1) [18,19]. Bi-allelic mutations in *FANCC* and *BLM* result in FA and BS, respectively, whereas in Thompson et al. heterozygous mutations in *FANCC* and *BLM* were identified in a few breast cancer families studied. The notion that heterozygous mutations in DNA repair genes might predispose carriers to incremental increases in cancer susceptibility is a long-standing and sometimes controversial hypothesis in cancer genetics that has increasingly gained traction. As noted above, heterozygous mutations in FA genes have been associated previously with increased breast cancer risk, and, conversely, bi-allelic mutations in breast cancer-associated genes *BRCA2*, *BRIP1*, *PALB2*, and *RAD51C* have been identified in persons with FA or FA-like syndromes [20–23]. Although the concept of increased cancer risk conferred by heterozygous mutations now seems unassailable, the evidence for specific associations between *FANCC* and *BLM* and breast cancer risk is not yet convincing [24–28]. The challenge of the “heterozygous-mutation” hypothesis is generally one of power. Because the allele frequency of *FANCC* and *BLM* mutations in most populations is very low (<0.001), large numbers of individuals are needed to test for differences in the allele frequency between cases and controls. Moreover, heterogeneity in the frequency of mutations across different populations could

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| | Bloom's syndrome | Fanconi anemia |
|-----------------------|--|---|
| Dermal | Sun-induced rash Pigmentation lesions | Pigmentation lesions |
| Immune | Moderate deficiency | Bone marrow failure |
| Reproductive | Reduced fertility | Reduced fertility |
| Skeletal | Short stature | Short stature Skeletal abnormalities |
| Genomic instability | Increased homologous recombination | Defect in homologous recombination repair |
| Cancer predisposition | All types and sites | Leukemia Squamous cell carcinoma Liver cancer |

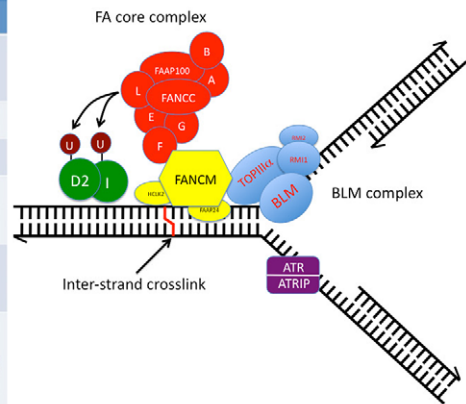


Figure 1. Fanconi anemia (FA) and Bloom's syndrome (BS) overlap at the clinical and molecular levels. Left panel: Comparison of the clinical and cellular features present in FA and BS. FA and BS have features that are distinct to each syndrome; however, there are broad similarities. Some individuals with mutations in *BLM* have been diagnosed with FA, exhibiting classical FA features. Right panel: Depiction of a super-complex that is formed at sites of repair of replication forks that have been impeded by an inter-strand crosslink. The super-complex consists of two complexes that form independently in the nucleus. One complex consists of the interacting proteins identified through FA mutations and the other complex consists of proteins that interact with *BLM*, the gene mutated in BS [33]. These two complexes are brought together by mutual interactions with the FA gene product FANCM [34]. Molecular interactions between FANCI and BLM at stalled forks have also been described [35]. Downstream signaling effects (FANCD2 ubiquitylation and ATR activation) are also depicted. Figure was redrawn from Deans and West [34]. doi:10.1371/journal.pgen.1003008.g001

complicate interpretation of associations when populations are admixed and as investigators combine results from different populations to increase power. As an example of frequency heterogeneity, *FANCC* and *BLM* mutations are more frequent in Ashkenazi Jews (~0.008), where a specific allele is present in most cases of FA and BS [29,30].

Selecting cases with strong family history of cancer is an enrichment strategy that can reduce the numbers of cases needed to find associations [31]. In Thompson et al., the authors sequenced *FANCC* and *BLM*, and in total they found *FANCC* mutations in four probands out of 1,395 *BRCA*-negative hereditary breast cancer families from Australia and *BLM* mutations in two probands out of 438 such families. No mutation carriers were found in either gene in 464 controls. No overtly deleterious *FANCC* or *BLM* mutations have been reported in 1,192 completely sequenced

persons in the 1000 genomes data [32]. On the other hand, in the NHLBI GO Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), three *FANCC* and four *BLM* mutation carriers had been identified in 3,510 exomes. Persons from the 1000 genomes and EVS do not constitute a good control group; thus the authors refrained from calculating *p*-values, 95% confidence intervals, and effect sizes. Going forward it will be important to compare consecutive breast cancer cases and matched controls drawn from the same population to provide robust data to calculate these important parameters; it may take tens of thousands of cases and controls to quantify them! In addition, we will need to combine studies of hereditary breast cancer cases to increase power and drive segregation analysis, to conduct larger case-control studies in the Ashkenazi Jewish population where the increased frequency of specific alleles

increases the power of the study group, and to continue to quantify cancer risk in the relatives of persons with FA and BS.

Thus, rare alleles identified by sequencing of multiplex kindreds pose significant challenges for the estimation of effect sizes in cancer susceptibility. In the end, the critical questions that grip rare alleles are how much increased risk do they confer and do they account for the missing heritability? The resolution of these questions is of paramount importance to genetic epidemiologists studying human populations as well as to clinicians caring for families at risk for hereditary breast cancer.

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