

## **Supplementary Methods**

### **Samples**

We analysed samples from unrelated women with breast cancer, who each had a family history of breast and/or ovarian cancer in relatives or had bilateral breast cancer. We quantified the extent of the family history of breast and/or ovarian cancer using a Family History Score. This was defined as the number of relatives of the index case with breast cancer (or ovarian cancer, for the families with cases of both cancers), weighted by their degree of relatedness to the index case to adjust for the expected allele sharing (score=1 for each affected 1<sup>st</sup> degree relatives, 0.5 for 2<sup>nd</sup> degree relatives and 0.25 for 3<sup>rd</sup> degree relatives; bilateral breast cancers score double). In this study all cases had a minimal Family History Score of 1, which is equivalent to a family with two first degree relatives with breast cancer or an individual with bilateral breast cancer. All samples were from breast cancer families ascertained through cancer genetics clinics in the UK and families from non-UK ethnic groups were excluded. We obtained informed consent from all families and the research was approved by the London Multicentre Research Ethics Committee (MREC/01/2/18).

The control samples were from the 1958 Birth Cohort Collection which is an ongoing follow-up of all persons born in Great Britain during one week in 1958, including a recent biomedical assessment during 2002–2004 at which blood samples and informed consent were obtained for creation of a genetic resource<sup>1</sup>. All cases were white, and at least 97% of the controls were of white ethnicity.

### **Mutation screening of *BRCA1*, *BRCA2* and *BRIP1***

We screened genomic DNA, extracted from peripheral blood samples, for mutations in the full coding sequence and intron/exon boundaries of *BRCA1* and *BRCA2* by Conformation Sensitive Gel Electrophoresis (CSGE)<sup>2</sup>. Primers and conditions are available on request. We also performed Multiplex ligation-dependent Probe Amplification (MLPA) analysis on all samples using the P002 *BRCA1* kit and P045 *BRCA2* kit (MRC Holland) and the manufacturers' protocols. MLPA is a dosage test which measures copy number of all 24 coding exons of the *BRCA1* gene and 23 of the 27 coding exons of the *BRCA2* gene.

We only included samples negative for *BRCA1* or *BRCA2* mutations in the *BRIP1* mutation screen. We screened the coding exons and intron-exons boundaries of *BRIP1* by CSGE in 25 fragments. Primer sequences and conditions are shown in Supplementary Table 1. Genomic DNA from cases showing mobility shifts on CSGE was bidirectionally sequenced on a 3730 automated sequencer (ABI Perkin Elmer). A small (<3%) number of samples failed >3/25 fragments due to poor quality DNA or a robotic tip failure during PCR set-up. Such cases were excluded from further analyses. In our laboratory, CSGE has 95% sensitivity for small insertions / deletions and 85% sensitivity for base substitutions. To ensure that the commonest reported *BRIP1* mutation, R798X, was detected we also analysed all samples for this mutation by Pyrosequencing using the PCR primers 5'-GCTTGAGATCACACAGCTGATTAG-3' (forward) and 5'-biotin-GGCCAGGTAGAAGACCTCTCAATT-3' (reverse) and the sequencing primer 5'-GGTGGTCATTGTATTGTC-3'. We used the manufacturers' reagents, protocols and software to sequence and analyse the data (Biotage AB)<sup>3</sup>. In total, 1212 breast cancer

cases and 2081 controls were successfully screened through *BRIP1*. Over 700 of the breast cancer cases had a Family History Score of at least 2 (i.e. the screened individual with breast cancer had at least two first degree relatives with breast cancer or equivalent).

We evaluated the likely effect of missense *BRIP1* variants identified in cases and/or controls using two *in silico* algorithms, PolyPhen<sup>4</sup> and SIFT<sup>5</sup>. All intronic and coding *BRIP1* variants were also evaluated for possible affects on splicing using the Netgene2 (ref. 6) and MaxEnt<sup>7</sup> *in silico* prediction programs.

### **Statistical Methods**

We compared numbers and likely effect of mutations or variants between cases and controls using a two-sided Fisher exact test. We used the Wilcoxon Rank Sum test to assess the difference in Family History Score between index cases with and without *BRIP1* mutations, and a two-sided Fisher exact test to compare the numbers of bilateral breast cancers in index cases with and without *BRIP1* mutations. The analyses were performed using STATA 8.2 (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

An unadjusted comparison of mutation frequencies in cases and controls would be biased, since cases were ascertained on the basis of a family history of breast cancer. We instead estimated the *BRIP1* combined mutation frequency and the breast cancer risk ratio relative to noncarriers of a *BRIP1* mutation simultaneously in a segregation analysis using the program MENDEL<sup>8</sup>. We conditioned the likelihood on all phenotypes for each pedigree to correct for ascertainment. Since *BRIP1* mutation

screening had been carried out in all cases and controls we were able to incorporate information from all controls and the full pedigrees of all cases (including those without a *BRIP1* mutation) in the likelihood, in addition to the segregation information from the four families in which a *BRIP1* mutation was detected and genotyping was possible in relatives of the index case. We excluded four families which each contained a consanguineous marriage, leaving 1208 families. Pedigrees were pruned to include only women with breast cancer, individuals tested for *BRIP1* mutations and the first degree relatives of members of these groups. The *BRIP1* mutation screening sensitivity for index cases and controls was assumed to be 85% to allow for mutations that were not detectable by our methods (such as large rearrangements) and for insensitivity of the mutation detection. Estimates were robust to some degree of misspecification of this parameter. We computed risks to noncarriers such that the total risk averaged across carriers and non-carriers agreed with the age-specific breast cancer incidence rates in England and Wales for the period 1992-1997 (ref. 9), as described in previous segregation analyses<sup>10</sup>. Because this model does not explicitly incorporate the effects of other susceptibility genes, it assumes implicitly that the effects of *BRIP1* and other potential susceptibility genes can be regarded as independent, as in a multiplicative model.

## Reference and URLs

1. National Child Developmental Study <http://www.cls.ioe.ac.uk/Cohort/Ncds/mainncds.htm>
2. Ganguly, A. *Hum. Mutat.* **19**, 334-342 (2002).
3. Langaee, T. & Ronaghi M. *Mutat. Res.* **573**, 96-102 (2005).
4. Ng, P.C. & Henikoff, S. *Nucleic Acids Res.* **31**, 3812-3814 (2003)  
<http://blocks.fhcrc.org/sift/SIFT.html>.
5. Ramenshy, V., Bork, P. & Sunyaev, S. *Nucleic Acids Res.* **30**, 3894-3814 (2002)  
<http://www.bork.embl-heidelberg.de/PolyPhen/>
6. Hebsgaard, S.M. et al. *Nucleic Acids Res.* **24**, 3439-3452 (1996).  
<http://www.cbs.dtu.dk/services/NetGene2/>
7. Yea, G. & Burge, C.B. *J Comput. Biol.* **11**, 377-394 (2004).  
<http://genes.mit.edu/burgelab/maxent/>
8. Lange, K., Weeks, D. & Boehnke, M. *Genet. Epidemiol.* **5**, 471-472 (1988).
9. Parkin, D.M. et al. Cancer incidence in five continents. Vol VIII. Lyon  
(France): IARC (2002).
10. Antoniou, A.C. & Easton, D.F. *Genet. Epidemiol.* **21**, 1-18 (2001).