**A network analysis to identify mediators of germline-driven differences in breast cancer prognosis**

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**Abstract**

Identifying the underlying genetic drivers of the heritability of breast cancer prognosis remains elusive. We adapt a network-based approach to handle underpowered complex datasets to provide new insights into the potential function of germline variants in breast cancer prognosis. This network-based analysis studies ~7.3 million variants in 84,457 breast cancer patients in relation to breast cancer survival and confirms the results on 12,381 independent patients. Aggregating the prognostic effects of genetic variants across multiple genes, we identify four gene modules associated with survival in estrogen receptor (ER)-negative and one in ER-positive disease. The modules show biological enrichment for cancer-related processes such as G-alpha signaling, circadian clock, angiogenesis, and Rho-GTPases in apoptosis.

Family-based studies have suggested that breast cancer survival in ﬁrst-degree relatives has a hereditary component1,2. Nevertheless, whereas large scale genome-wide association studies (GWAS) have made considerable progress in identifying germline variants linked to breast cancer risk3,4, the identification of germline variants linked to breast cancer prognosis has proven more challenging5,6. An understanding of how and which germline variants affect breast cancer prognosis could provide novel insights into the etiology of the metastatic process in breast cancer, increase knowledge on the underlying heterogeneity of the disease, and help identify new therapeutic targets or select patients most likely to beneﬁt from existing therapies.

A major limitation of the studies to date is that the sample sizes have been insufficient to detect the small effect sizes of germline variants characteristic for breast cancer risk and survival4,7–9. Even though our previous survival GWAS included over 95,000 patients5,9, the limiting factor was the relatively low number of events (breast cancer-specific deaths) observed. One way to overcome this limited power is to use pathway or network-based approaches10,11. These techniques typically use predeﬁned gene sets, annotated pathways or protein-protein interaction (PPI) networks to detect genetic effects across multiple genes or proteins with similar or related biological functions8,11–13. Using such methods, a biological pathway might emerge as relevant even if none of its individual germline variants reached genome-wide significance. Moreover, assigning the variants to genes reduces dimensionality: considering several pathways as opposed to millions of individual variants leads to a substantial reduction in the number of tests performed14. An additional advantage of performing a pathway analysis is that it naturally suggests which biological processes mediate the genetic association with survival, making the biological interpretation easier10,14–17.

Here we report on a network-based GWAS to identify genetic determinants of breast cancer prognosis in a dataset with a total of 84,457 breast cancer patients of European ancestry. In line with previous studies, we did not find many individual genetic variants with strong effects18–22. However, aggregating the survival estimates of multiple variants across genes and using a network propagation method, we identified several biological processes that may mediate a germline genetic effect on breast cancer prognosis. These include key processes in cancer biology, such as regulation of apoptosis, G-alpha signaling, and the circadian clock mechanism. In our analysis, we show that the identified polygenic effects are associated with survival not only in the discovery set, but also in an independent dataset of 12,381 patients. In addition, we studied the downstream transcriptional changes and their functional consequences due to the prognostic variants. We observed similar biological processes in the enrichment of the downstream and module-level gene analyses suggesting that both levels are perturbed by the identified genetic variants.

**RESULTS**

**Analyses of individual genetic variants and genes reveal few associations with prognosis for women with breast cancer**

We performed an analysis of the association between germline genetic variants and breast cancer prognosis comprising data for 84,457 female breast cancer patients of European ancestry. To account for potential subtype-speciﬁc associations, we also performed separate analyses for ER-positive and ER-negative breast cancer. An overview of all data is given in the Methods section & **Supplementary Table 1a**. As a first step in our analysis, we tested the association of ~7.3 million imputed genetic variants with breast cancer-speciﬁc survival using a Cox proportional hazard model **(Fig. 1a)**. Based on a genome-wide statistical significance P value threshold of 5 × 10-8, we identified two variants at 8q13, in high linkage disequilibrium with each other, associated with survival in ER-positive breast cancer. The top variant was rs6990375 (chr8:70571531, P = 6.35 x 10-9) followed by rs13272847 (chr8:70573316, P = 1.07 x 10-8) . We did not find significant variants for ER-negative or all breast cancer cases.

Next, we aggregated the summary statistics of the individual variants into gene-level P values (~21,800 genes in total) using the Pascal algorithm15 **(Fig. 1b)**. We computed the gene score based on the maximum chi-squared signal within a window size of 50-kb around the gene region (see Methods) **(Fig. 2)**. Two genes were associated with survival in ER-positive breast cancer at P < 0.05 after Bonferroni correction: *SLCO5A1* (P = 4 × 10-7, corrected P = 0.01) and *SULF1* (P = 7 × 10-7, corrected P = 0.02) **(Fig. 2c)**. These two genes are located in close proximity to each other around the significant variants at 8q13 identified in the single variant analysis. Their significance is therefore likely driven by a single causal genetic variant. The top variant rs6990375 is situated in the 3’ UTR of *SULF1* where it may affect the binding of regulatory micro-RNAs. While the association of this variant with breast cancer survival has not been identified previously, it has been reported to be associated with age of onset of ovarian cancer23. *SULF1* has been found to be involved in cell proliferation, migration, and invasion as well as drug-induced apoptosis in cancer cell lines24, most likely due to its regulatory role in FGF25 and Wnt signaling26. Less is known about the function of *SLCO5A1*, although a role in cell proliferation has been suggested27 . In line with the single variant analysis, we found no significant genes for all breast cancer or ER-negative breast cancer **(Fig. 2a,b)** when aggregating individual variants into genes.

**Network analysis of genetic variants identifies multiple germline-related prognostic modules (GRPMs)**

To explore whether weaker signals of association were hidden in our data, we investigated the hypothesis that the germline genetic variants associated with breast cancer prognosis target particular biological processes, but within those processes do not uniquely target one particular gene. Different subgroups of patients might harbor variants in different genes, which ultimately affect the same biological process. Such polygenic signals, unless they have very big effects, may remain undetected if only individual variants or even individual genes are tested. We therefore applied network propagation28, a technique that maps gene association scores onto a protein-protein interaction (PPI) network and uses the network topology to detect sub-networks, or modules, of closely interacting, high-scoring proteins **(Fig. 1c)**. In the context of this paper, we will refer to these modules also as germline-related prognostic modules (GRPMs).

For the network propagation, we used the HotNet2 method17, which has been used previously with GWAS data29. We based the gene scores on the aggregate gene P values computed by the Pascal method (see Methods). The protein interaction network used by HotNet2 was obtained from iRefIndex30.

When considering all breast cancers, the HotNet2 analysis identified no significant GRPMs (lowest P value = 0.06). In contrast, several GRPMs were associated with prognosis in the analyses by ER subtype. For ER-positive patients, the best HotNet2 result (P value < 0.01) comprised 31 GRPMs of seven or more genes. For ER-negative patients, the best HotNet2 results (P value < 0.01) included 116 GRPMs of four or more genes. A list of all significant prognostic modules is presented in **Supplementary Table 2**.

To help the interpretation of the identified GRPMs, we developed an extension to HotNet2 that maps the module genes to the specific genetic variants that are most strongly associated with prognosis. This was done by performing a Lasso-penalized Cox regression on the genetic variants assigned to the module genes. Using those selected variants and their effect sizes, a polygenic hazard score (PHS) was computed and used to identify a set of high-confidence GRPMs **(Fig. 1d)**, as well as to perform a functional characterization of the downstream effects of the prognostic variants **(Fig. 1e)**.

**Identification and functional characterization of high-confidence germline-related prognostic modules link them to known pathways in breast cancer biology**

We restricted our scope to a subset of high-confidence GRPMs. This subset was identified by testing the association of each module’s PHS with breast cancer prognosis in an independent set of 12,381 patients (with 1,120 events) **(Supplementary Table 1b)** that were not used previously in the HotNet2 analysis or in the construction of the PHS score. GRPMs with a significant association between PHS and prognosis (P value < 0.05, based on a one-sided test; see Methods) in this independent set were considered high-confidence and will be discussed in the remainder of this section. Following this procedure, we found four high-confidence GRPMs for ER-negative breast cancer **(Fig. 3a-c) and** one high-confidence GRPM for ER-positive breast cancer **(Fig. 3d).** Hazard ratios of the association of the PHSs with breast cancer-specific survival ranged from 1.09 to 1.28 **(Fig. 3e)**.

To provide a functional characterization of the five high-confidence GRPMs found in the ER-negative and ER-positive subtypes, we tested each module for enriched biological processes on two levels. The first, which we call the module-level, considers the direct functions of the GRPM proteins themselves. These were identified by an enrichment analysis of the annotated biological functions of the module proteins and their direct interactors in a PPI network annotation (see Methods). For the high-confidence GRPMs in ER-negative breast cancer we identified enriched processes related to G-alpha signaling, cell growth and angiogenesis, insulin secretion and circadian clock **(Supplementary Fig. 1a-d)**. For the ER-positive high-confidence GRPM, the enriched processes included signaling by Rho GTPases and apoptosis **(Supplementary Fig. 1e)**.

The module-level enrichment provides a general summary of the biological functions of the GRPM genes. However, it is based on functional annotations that have been derived from studies in many different cell types and biological environments. To study the specific downstream effects of the identified prognostic variants in breast cancer tumors, we performed enrichment analyses on the downstream transcriptional changes due to the prognostic variants affecting the module proteins.

We estimated these downstream transcriptional effects using genetic variants and RNA expression data of female breast cancer patients from The Cancer Genome Atlas (TCGA)31. For each of the five GRPMs, the downstream analysis was performed on the subset of TCGA patients matching the ER subtype in which the GRPM was identified, 118 patients with ER-negative and 440 with ER-positive tumors. Using the germline genotype data of these TCGA patients, we computed the PHS for each GRPM **(Supplementary Table 3)**. Based on these PHSs, we then computed GRPM downstream transcriptional effect scores, which reflect the correlation between a module’s PHS and the mRNA expression level of every gene **(Fig. 1e)** (see Methods). Using the obtained downstream transcriptional effect scores, we performed Gene Set Enrichment Analysis (GSEA)32 with gene sets based on Reactome33 and the MSigDB34 Hallmark gene sets. The enrichment results for the MSigDB Hallmark gene sets are shown in **Figure 3**, only pathways with a P value < 0.001 and FDR < 0.01 were included in the visualization. The full list of enriched processes per high-confidence GRPM can be found in **Supplementary Tables 5-9** and **Supplementary Figure 2**.

The enriched pathways in the downstream analysis included biological processes such as cell cycle, DNA repair, metabolism of RNA, lipids or proteins, apoptosis, and translation of proteins. Importantly, we observed overlap of the biological processes enriched in the downstream analysis and those found for the module proteins. This observation has two important implications. First, it provides additional support for the biological role assigned to the module proteins. In addition to this, in cases where module proteins may serve several roles, it helps identify which of those roles is affected by the prognostic variants at a transcriptional level. The enriched biological processes assigned to the modules and the related downstream processes are described below.

***ER-negative tumors: G-alpha signaling events***

Two high-confidence GRPMs found for patients with ER-negative tumors **(Fig. 3a)** suggested, from the module-level analysis, G-alpha signaling and G-protein activation as biological processes associated with survival. The first GRPM (P = 0.0096) includes *ADCY10, GNA11, PTGIR* and *RGS3* **(Fig. 3a, right)** and the other GRPM (P = 0.0082) is a larger module of 19 genes: *ADRBK2, CCL16, CNR2, CXCR5, DNAJB4, F2R, GNA15, GNAT1, GRM4, GUCA1A, GUCA1B, GUCA2B, GUCY2D, HRH4, LTB4R, OPRK1, OPRM1, RGS9* and *RGS9BP* **(Fig. 3a, left)**.

On closer inspection of the genetic variants selected for the two modules’ PHSs, we observed that one genetic variant was shared by both modules. The other variants in the PHSs, three variants in total for GNA15 PHS and two variants for the GNA11 PHS, were also located in the same genomic region on chromosome 19p13.3 **(Fig. 4a)**. These variants are upstream of *GNA11* in one module and *GNA15* in the other. For the other genes in these two GRPMs, no genetic variants were selected as part of the modules’ PHSs. This may be due to lack of statistical power: although the gene scores were high enough to be included in the module, none of their individual genetic variants had a strong enough association. The co-location of *GNA11* and *GNA15* provides an explanation for why the identified variants were selected for both modules. It also suggests that the genetic associations of these two genes and hence of the two modules are not independent. Indeed, the patients’ PHSs for both GRPMs are highly correlated **(Fig. 4b)**, which supports a shared genetic association. This raises the question of whether the putative germline genetic effect on survival is mediated through both genes or only one of the two. In the downstream analyses of both modules, changes of *GNA15* expression were identified as one of the strongest downstream transcriptional effects, whereas this is not the case for *GNA11*. Conversely, in an independent gene expression dataset using KMplotter (kmplot.com/analysis), we found that expression of *GNA11* is significantly associated with recurrence free survival in ER-negative breast cancer **(Supplementary Fig. 3)**, while a similar effect was not seen for *GNA15*. These preliminary observations leave open the hypothesis of a role for both genes. A definitive answer will require more functional analyses.

In the module-level analysis, the GRPM formed by four genes also showed enrichment for insulin secretion. It has been shown that there is a close relationship between G-proteins and their coupled receptors (GPCR), insulin and the insulin-like growth factor I receptor (IGFIR). Altered versions of this crosstalk could play a role in cancer cells35,36. For example, it has been proposed that in cancer cells, insulin can increase the activity of GPCRs in cancer tissues via the mTOR (mammalian target of rapamycin) pathway36, which was also one of the enriched processes in the downstream analysis. The highest scoring gene in the module, *GNA11*, codes for the alpha subunit of the G11 protein, which has been linked to insulin secretion and signaling37,38.

For the 19-gene GRPM, we also identified thrombin signaling and platelet aggregation as two of the main module-level enriched pathways. Thrombin is a type of the above mentioned GPCRs with the capacity to upregulate genes able to induce, or contribute to oncogenesis and angiogenesis, and is known to be able to stimulate the adhesion of tumor cells to platelets39. In the downstream analysis, we identified processes such as GPCR ligand binding and hemostasis which contributes to the thrombosis process and therefore is also linked to GPCRs40 **(Supplementary Fig. 2a** and **Supplementary Table 5)**. It has been reported that hemostatic elements such as platelets, coagulation and the fibrinolytic system might play an important role in breast cancer progression and metastasis41.

***ER-negative tumors: circadian clock***

Another module identified by our network analysis consists of four genes with a strong link to the circadian clock mechanism: *PER1*, *PER3*, *TIMELESS*, and *TIPIN* (P =0.030) **(Fig. 3b)**. Having an important role in the regulation of the cell cycle42, the circadian clock is believed to be important in the development of cancer. Disrupted sleep patterns and associated changes to the body’s circadian rhythm have long been implicated in the risk of developing several cancers including breast cancer42–44. Although long-term night-shift work has not consistently been found to be associated with breast cancer45, one study reported an increased risk of ER-negative breast cancer46. More recently, genetic variants in circadian clock genes have been reported to be associated with breast cancer risk47,48. In addition to risk, the circadian clock has also been suggested to be involved in breast cancer progression and prognosis49,50.

More specifically, the circadian clock genes in this module have also individually been implicated in the biology of cancer in general and breast cancer in particular. The period genes *PER1* and *PER3* have been found to suppress cancer cell growth51,52 and have also been observed to be deregulated in breast cancer53. *TIMELESS* and its interactor *TIPIN* are believed to be central players in the connection between the circadian clock and the cell cycle and apoptosis54,55. The importance of these genes in the regulation of cell cycle was supported by the downstream analysis, which pointed out that cell cycle-related processes are strongly enriched among the downstream transcriptional changes.

***ER-negative tumors: regulators of cell growth and angiogenesis***

The last high-confidence GRPM identified for ER-negative breast cancer contains proteins that have been linked to regulation of cell growth or angiogenesis: *CHCHD4*, *PDE9A*, *SLC36A1*, and *PHYHIPL* (P = 0.027) **(Fig. 3c)**. Knock down of *CHCHD4* has been found to reduce tumor growth and angiogenesis in vivo56. In addition, *CHCHD4* has been observed to mediate the mitochondrial translocation of p5357 through which it may trigger apoptosis via the p53 mitochondrial pathway58. *PDE9A* is a regulator of cGMP signaling, a pathway that is increasingly being recognized as an important player in breast cancer biology59. Inhibition of *PDE9A* has been found to trigger apoptosis in both ER-positive and ER-negative breast cancer cell lines60. *SLC36A1*, also known as *PAT1*, has been linked to tumor cell growth through its involvement in the activation of mTORC1. *PHYHIPL* (or *PAHX-AP1*) has mostly been described in the context of neuronal cells, but no role in cancer has been described.

***ER-positive tumors: Rho GTPases to cell growth and regulation of apoptosis***

For ER-positive tumors, we identified one high-confidence module (P value = 0.020) **(Fig. 3d)**. The module was predicted to be involved in Rho GTPases effectors, which typically function as binary switches controlling a variety of biological processes. Because of their ability to control cell motility they have been hypothesized to play a role in progression and metastatic dissemination of cancer cells61. This GRPM contains seven genes: *ARHGAP10*, *CCNT2*, *CDR2*, *HEXIM1*, *NEUROD2*, *PKN1* and *ZFAND6*. *ARHGAP10* (rho GTPase Activating Protein 10 ) was previously reported as the most significant locus (P value = 2.3 × 10−7) in a GWAS of breast cancer survival18. The top scoring gene in the module, *PKN1* (protein-kinase-C-related kinase), controls processes such as regulation of the intermediate filaments of the actin cytoskeleton, tumor cell invasion and cell migration62. It is activated by the Rho family of small G-proteins and might mediate the Rho-dependent signaling pathway63, which was one of the main enriched pathways in the module-level analysis. *PKN1* has also been described as an important player in other cancers: in androgen-associated prostate cancer by controlling migration and metastasis62, or in melanomas by inhibiting Wnt/b-catenin signaling and apoptosis63.

From the module-level analysis, another enriched main process was the pathway linked to *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) regulation, which is a well characterized tumor suppressor64. *PTEN* is directly involved in the metabolism of phospholipids and lipoproteins65, adaptive immune system and B-cell receptor associated events,66 which were all hits in the downstream analysis. One of the six genes in the module, *HEXIM1* (hexamethylene bisacetamide-inducible protein 1), is a positive regulator of p53 and has been identified as a potential novel therapeutic target modulating cell death in breast cancer cells67. In the downstream analysis of this module we also identified processes present in the module-level analysis that highlighted key tumorigenic biological processes **(Supplementary Table 9)**, for instance pathways related to p53 activity, WNT signaling, regulation of mRNA stability by proteins that bind AU-rich elements or apoptotic execution phase.

**DISCUSSION**

There is evidence that breast cancer prognosis has a heritable component2,68,69. Exploring the possible link between germline genetic variants and breast cancer survival may help to develop better criteria for breast cancer stratification, which might have implications for breast cancer prognostication and treatment70. However, identifying germline genetic variants associated with breast cancer prognosis has been challenging so far, mainly because the current sample sizes have been insufficient to detect small effect signals.

In this work, we started with a survival analysis based on individual germline variants similar to the previous GWAS we have undertaken5. While in the previous analyses no variants reached genome-wide significance, here, we identified two genome-wide significant variants for ER-positive tumors (rs6990375: P < 6.35 x 10-9 and rs13272847: P = 1.07 x 10-8) located in 8q13. More complete follow-up and more conservative variant filtering per dataset (only including variants with imputation r2 > 0.8) may have enabled identification of these variants that remained below genome-wide significance in our previous study (P = 3.02 x 10-5 and P = 1.73 x 10-5, respectively). In the gene-level analysis, we found two significant genes (*SLCO5A1* and *SULF1*, P < 0.05 after Bonferroni correction) associated with breast cancer survival. It is likely that both associations were driven by the identified leading variant rs6990375.

To address the lack of power in the individual germline variant and gene-level analyses, we developed a network analysis method that revealed five high-confidence GRPMs associated with breast cancer prognosis. We identified four modules specific for ER-negative breast cancer and one for ER-positive breast cancer. The GRPMs comprise crucial processes such as cell cycle and progression, regulation of apoptosis, signaling by mTOR, immune system, G-alpha signaling, and the circadian clock. These processes are already known to play a role in cancer biology in general and breast cancer prognosis specifically. However, our results highlight the possible regulatory impact of germline variants on these processes, which traditionally has received little attention in cancer survival studies. The broad range of genes and functions seems to indicate, as already hypothesized, that breast cancer survival is a complex phenotype influenced by many factors and biological mechanisms.

The analysis by ER-status subtypes identified significant associations that were not present when analyzing all patients together. This is in line with the breast cancer risk analyses undertaken in this same dataset, where the ER-subtype analyses also identified new associations4. Additionally, the main classiﬁcation of breast cancer tumors used for prognosis and treatment selection is based on immunohistochemical markers such as ER-, PR- and HER2-status71, reflecting the fact that each group has a different etiology and prognosis. This assumption is further supported by a comparison of the gene association scores between the ER-status subtypes. The gene scores for ER-positive and ER-negative breast cancer are uncorrelated **(Supplementary Fig. 4c)** (Pearson correlation = -0.002), while the gene scores for all breast cancer cases seem to resemble the ER-positive subtype more **(Supplementary Fig. 4a)** (Pearson correlation = 0.366) than the ER-negative subtype **(Supplementary Fig. 4b)** (Pearson correlation = 0.197). In addition, we found that the distribution of PHSs across patients was similar for ER-positive and ER-negative breast cancer patients **(Supplementary Fig. 5)**, butimportantly, each PHS was associated with prognosis only for the subtype in which it was found **(Supplementary Table 4)**. These differential associations across subtypes suggest that prognosis is inherited differently for these two different disease classes.

The network-based approach and the stratification of patients by ER-status enabled a refined interpretation of the GWAS results9,72, but the findings are still limited due to the number of deaths observed, limited follow-up, missing treatment information, and possibly remaining heterogeneity of tumor subtype within the ER classes. Increased sensitivity and specificity of the results could be achieved by including additional patients, and by adjusting for more fine-grained tumor characteristics and the treatment received. Moreover, the network propagation results are dependent on the completeness of the PPI network used. As a notable consequence of this, we did not identify modules containing the two gene-level significant hits *SLCO5A1* and *SULF1*, due to the fact that the PPI network did not contain the proteins they code for.

Using curated protein interaction networks such as iRefIndex in propagation analyses may cause a subtle type of ascertainment bias: more interactions tend to be known for better studied proteins, which proteins involved in in tumor initiation and progression often are. As a result, genes scores may correlate positively with the number of interactions in the protein interaction network. This is the case, for example, when gene scores are based on somatic mutation frequencies in cancer. HotNet2 only controls for this partially, whereas a recent extension to the HotNet2 method provides a more rigorous solution73. We tested whether our analysis is vulnerable to this ascertainment bias by computing the correlation between the gene scores computed by Pascal and the number of interactions recorded by iRefIndex. For all, ER-positive, and ER-negative breast cancer, these correlations were close to zero (Pearson r2 = -0.012, r2 = -0.006, and r2 = 0.003 respectively) showing no evidence of ascertainment bias.

In summary, our network propagation analysis shows a germline genetic link to breast cancer survival and proposes a mechanism by which multiple loci with small individual effects might inﬂuence breast cancer-speciﬁc prognosis. Experimental follow-up of the high-confidence GRPMs identified is required to better understand the role of these modules. While we focused on the subset of high-confidence modules, the other modules may also yield new insights if assessed in the context of larger independent datasets. Together the results presented here may feed future hypotheses about the contribution of germline variation to breast cancer survival.

**Methods**

**Breast cancer patient data.** We used data from 12 genome-wide association studies (GWAS) that together account for 84,457 invasive breast cancer patients with 5,413 breast cancer-speciﬁc deaths within 10 years (events). These included 55,701 patients with ER-positive breast cancer (2,854 events) and 14,529 patients with ER-negative breast cancer (1,724 events), while the ER-status was unknown for the remaining 14,227 patients. All patients were females of European ancestry. A summary of the studies with the numbers of patients and events by study is given in **(Supplementary Table 1a)**. The GWAS sample sets were genotyped using a variety of genotyping arrays, targeting between 200,000 and 900,000 variants across the genome, and subsequently imputed using a common reference (details given below). The majority of patients came from the Breast Cancer Association Consortium (BCAC), which itself comprised 69 studies from across the world that underwent a uniform data harmonization and quality control (data freeze 10). Genotyping in BCAC was performed in two rounds using two different genotyping platforms: iCOGS and OncoArray. In subsequent analyses, we treated these two platforms as different studies. The OncoArray dataset is the largest in BCAC, with higher quality imputed genotypes compared to the iCOGS data. As an independent dataset, we separated out the entire SEARCH study, comprising 12,381 patients and 1,120 events, from the BCAC data. Patients in the SEARCH study were recruited in the United Kingdom. Their genotypes were obtained using either iCOGS or OncoArray **(Supplementary Table 1b)**. Participants of all the studies provided written informed consent and studies were approved by local medical ethical committees.

**Genotype data and sample quality control.** Quality checks were performed for all studies. The methodology used for the genotype and sample quality control can be found elsewhere4,9,74,75. Genotypes for all 12 datasets were imputed using a reference panel from the 1000 Genomes Project76 March 2012 release. Imputation was performed by a two-stage procedure75 using SHAPEIT77 for pre-phasing and IMPUTE278 for genotype imputation. The genome-wide analyses were performed on ~7.3 million variants that had a minor allele frequency (MAF) > 0.05 and were imputed with imputation quality r2 > 0.8 in at least one of the studies.

**GWAS survival analysis and summary statistics.** The survival analysis was performed for all invasive breast cancer cases combined and for each of the ER-status subtypes (ER-positive and ER-negative) individually. A Cox proportional hazards model was ﬁtted to assess the association of the genotype with breast cancer-speciﬁc survival. Time-to-event was calculated from the date of diagnosis. Yet, because patients were recruited at different times before or after diagnosis, time under observation was calculated from the recruitment date (left truncation) in order to avoid possible bias produced by prevalent cases. Follow-up was right censored on the date of death if the patient died from a cause other than breast cancer, the last date the patient was known to be alive if death did not occur, or at 10 years after diagnosis, whatever came ﬁrst. To control for cryptic population substructure, we adjusted for principal components (for the number of principal components per study see **Supplementary Table 1a**). Details of the principal component analysis are described elsewhere4. Since BCAC-OncoArray and BCAC-iCOGS comprised data from large international cohort studies, the Cox models for these datasets were stratiﬁed by country. Separate survival analyses were performed for each of the 12 main studies, after which overall results per variant were obtained by combining the results of all studies with imputation quality r2 > 0.8 for that variant using a ﬁxed-effects meta-analysis. All statistical tests were two-sided.

**From variant P values to gene scores.** We used the GWAS summary statistics from the survival analysis as input for computing gene scores. To obtain gene scores, we used the Pascal algorithm15 which combines variant P values while taking into account dependence due to linkage disequilibrium (LD) structure. The Pascal method implements two gene-level statistics, corresponding to the strongest single association per gene (maximum of chi-squared statistics), or the average of all associations across the gene (sum of chi-squared statistics). After computing both statistics we tested which one had more power. To this end, we represented the set of P values into a quantile-quantile (QQ)-plot **(Supplementary Fig. 6)**. For all breast cancer cases and for both ER-status groups, the QQ-plots suggested that the maximum statistic has more power than the sum statistic. Therefore, of the two gene statistics we chose the maximum of chi-squared statistics for the gene-level statistic.

For the LD-reference population used in the gene computation, we created an extended version that included more variants than the default library provided with Pascal. This reference population was based on 503 European genomes from the 1000 Genomes Project (1KG)76. For the remaining parameters, we used the default settings. First, only variants with an imputation quality r2 > 0.8 and MAF > 5% in the patient data were considered. Second, the mapping of the variants to genes was based on the Pascal’s default 50-kb window size from the start and end of the gene. Finally, when computing gene scores, HLA genes were excluded. After the gene score computation, we obtained 21,815 gene scores for all invasive breast cancer, 21,789 for ER-positive and 21,797 for ER-negative. The slightly different numbers of gene scores between groups are due to the distinct selection of variants, which may have different allele frequencies across groups. The gene scores used in the HotNet2 analysis were obtained by taking the -log10 of the gene P values computed with Pascal.

**Network propagation with HotNet2.** We performed a network propagation analysis using the HotNet2 algorithm13 and the protein-protein interaction network iRefIndex30 applied to the -log10 gene scores obtained from the previous step. For edge removal on the created modules, HotNet2 automatically selects four different values which determine four different edge removal thresholds. The signiﬁcance test is a two-stage statistical test based on the number and size of the identified modules compared to those found using a permutation test. We used 500 permutations and a minimum network size of 2 for statistical testing. Further details are provided in the original HotNet publication79,80.

**Construction of polygenic hazard scores.** To summarize the total prognostic effect of the hereditary variants within the signiﬁcant germline-regulated prognostic modules (GRPMs), we constructed polygenic hazard scores (PHS), using a two-step approach. First, we selected the set of variants that best represented the genetic association of breast cancer survival with each GRPM. This variant selection was performed on the BCAC-OncoArray data, since this was the largest study and had the highest imputation quality. We performed the selection using the *glmnet* R package81, ﬁtting a Lasso (alpha = 1) model with 10-fold cross-validation to tune the sparsity penalty and the same selection of input variants as used for the computation of the Pascal gene scores, that is, picking those variants with MAF > 5% and within a 50-kb window around the start and end of the gene. With the set of germline variants selected using the Lasso procedure **(Supplementary Table 3)**, we fitted a Cox model to estimate unpenalized coefficients, and extracted their effect size estimates to compute a PHS per GRPM, which characterized the whole set of variants for the speciﬁc module in a unique score. For a set of selected variants , the PHS is deﬁned as:

PHS=

where is the genotype for the *i*th variant and its associated coefﬁcient.

**Identification of high-confidence GRPMs.** We obtained a selection of high-confidence GRPMs from among all modules identified using HotNet2 by testing the association of each module’s PHS in two datasets. The first dataset was the BCAC-OncoArray data minus the SEARCH data component of BCAC, i.e. the same data on which the PHS was derived, which was also a subset of the data used in the HotNet2 analysis. The second dataset consisted of the SEARCH study, which was held out of the BCAC data to serve as a truly independent set. Only GRPMs that had a PHS signiﬁcantly associated (P < 0.05) with breast cancer-specific survival in both the BCAC-OncoArray and the independent SEARCH data were considered high-confidence GRPMs and kept for further analysis. To test the association of a PHS with prognosis, we fitted a Cox model to the PHS, adjusted for the ﬁrst two genetic principal components and stratified by country. We then calculated a one-sided P value for the association of the PHS covariate with survival, taking advantage of the fact that the direction of association of the PHS is predefined, i.e. lower PHS means better survival. For the BCAC OncoArray data, the P value was corrected for multiple testing using Bonferroni correction based on the number of modules tested. The independent SEARCH data comprised two subsets using either OncoArray or iCOGS data. We analyzed these two subsets separately, and then combined the results of both groups using a ﬁxed-effect meta-analysis.

**Functional enrichment analysis of GRPM members.** Using Cytoscape version 3.4.0 software82 we extended the GRPMs by adding the ﬁrst direct neighboring genes in the Mentha83 human protein-protein interaction network. With the extension of the GRPMs we obtained bigger modules placed in a functional context. We then used the Cytoscape app ClueGO84. ClueGO uses kappa statistics to group the elements of the network and creates organized pathway categories based on the integrated pathway annotation. We based the analysis on human Reactome33 pathways, a Kappa Score Threshold of 0.4, and Bonferroni correction for the computed enrichment P values. For the visualization, we selected the fusion feature that groups pathways according to overlapping genes to facilitate interpretation of the results. We selected pathways with a P value < 0.05.

**Downstream functional enrichment.** In order to add biological and functional interpretation to the GRPMs we looked for associations between the modules’ PHSs and the expression patterns of potential downstream genes **(Fig. 1e)**. From The Cancer Genome Atlas (TCGA)31 library we extracted matched RNA-seq and genotype data of female breast cancer patients of European ancestry. This resulted in 118 patients with ER-negative breast cancer and 440 patients with ER-positive breast cancer. For each GRPM, we computed the previously obtained PHS for the subset of TCGA patients with a tumor matching the subtype for which the GRPM was found. Next, we aimed to quantify the downstream transcriptional effect of the GRPM on the expression of every individual gene. To do so, we computed the Pearson correlation between the GRPM’s PHS and the RNA expression of each gene. Finally, we performed gene set enrichment analysis (GSEA)32 to test for enrichment of biological pathways among the highly correlating genes. We used an annotation set of Reactome pathways33 and MSigDB34 Hallmark gene sets to perform the pre-ranked GSEA. We visualized the Reactome results with the EnrichmentMap85 Cytoscape app. Only biological processes with P value < 0.001 and FDR < 0.05 were considered as signiﬁcantly enriched.

**Data availability**

All 10-year breast cancer-specific survival summary estimates are available via the BCAC website (http://bcac.ccge.medschl.cam.ac.uk/bcacdata/). Data may be requested for analyses through a formal request (<http://bcac.ccge.medschl.cam.ac.uk>) or via the BCAC coordinator ([mkh39@medschl.cam.ac.uk](mailto:mkh39@medschl.cam.ac.uk)). Requests are subject to review of the BCAC Data Access Committee.

**References**

1. Lindström, L. S. *et al.* Prognostic information of a previously diagnosed sister is an independent prognosticator for a newly diagnosed sister with breast cancer. *Ann. Oncol.* **25**, 1966–1972 (2014).

2. Verkooijen, H. M. *et al.* Breast cancer prognosis is inherited independently of patient, tumor and treatment characteristics. *Int. J. Cancer* **130**, 2103–2110 (2012).

3. Milne, R. L. *et al.* Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. *Nat. Genet.* **49**, 1767–1778 (2017).

4. Michailidou, K. *et al.* Association analysis identifies 65 new breast cancer risk loci. *Nature* **551**, 92–94 (2017).

5. Maria Escala-Garcia\*, Qi Guo\*, Thilo Dörk, Sander Canisius, Renske Keeman, Joe Dennis, Jonathan Beesley, Julie Lecarpentier5, Manjeet K. Bolla, Qin Wang, BCAC authors, Douglas F. Easton, Peter A. Fasching, Heli Nevanlinna, Diana M. Eccles, P. D. P. P. and M. K. S. Genome-wide association study of germline variants and breast cancer-specific mortality. *Br. J. Cancer* (2018). doi:https://doi.org/10.1038/s41416-019-0393-x

6. Pirie, A. *et al.* Common germline polymorphisms associated with breast cancer-specific survival. *Breast Cancer Res.* **17**, 58 (2015).

7. Hartman, M. *et al.* Identification of novel genetic markers of breast cancer survival. *Breast Cancer Res.* **9**, 1–9 (2015).

8. Wang, K., Li, M. & Hakonarson, H. Analysing biological pathways in genome-wide association studies. *Nat. Rev. Genet.* **11**, 843–854 (2010).

9. Guo, Q. *et al.* Identification of novel genetic markers of breast cancer survival. *J. Natl. Cancer Inst.* **107**, djv081–djv081 (2015).

10. Menashe, I. *et al.* Pathway analysis of breast cancer genome-wide association study highlights three pathways and one canonical signaling cascade. *Cancer Res.* **70**, 4453–9 (2010).

11. Baranzini, S. E. *et al.* Pathway and network-based analysis of genome-wide association studies in multiple sclerosis. *Hum. Mol. Genet.* **18**, 2078–90 (2009).

12. Luo, L. *et al.* Genome-wide gene and pathway analysis. *Eur. J. Hum. Genet.* **18**, 1045–1053 (2010).

13. Leiserson, M. D. M. *et al.* Pan-cancer network analysis identifies combinations of rare somatic mutations across pathways and protein complexes. *Nat. Genet.* **47**, 106–14 (2015).

14. Petersen, A., Alvarez, C., DeClaire, S. & Tintle, N. L. Assessing methods for assigning SNPs to genes in gene-based tests of association using common variants. *PLoS One* **8**, e62161 (2013).

15. Lamparter, D., Marbach, D., Rueedi, R., Kutalik, Z. & Bergmann, S. Fast and Rigorous Computation of Gene and Pathway Scores from SNP-Based Summary Statistics. *PLOS Comput. Biol.* **12**, e1004714 (2016).

16. Creixell, P. *et al.* Pathway and network analysis of cancer genomes. *Nat. Methods* **2**, 1–6 (2015).

17. Marbach, D. *et al.* Tissue-specific regulatory circuits reveal variable modular perturbations across complex diseases. *Nat. Methods* **13**, 366–370 (2016).

18. Azzato, E. M. *et al.* A Genome-Wide Association Study of Prognosis in Breast Cancer. *Cancer Epidemiol. Biomarkers Prev.* **19**, 1140–1143 (2010).

19. Azzato, E. M. *et al.* Association between a germline OCA2 polymorphism at chromosome 15q13.1 and estrogen receptor-negative breast cancer survival. *J. Natl. Cancer Inst.* **102**, 650–62 (2010).

20. Kiyotani, K. *et al.* A genome-wide association study identifies locus at 10q22 associated with clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients in Japanese. *Hum. Mol. Genet.* **21**, 1665–72 (2012).

21. Shu, X. O. *et al.* Novel genetic markers of breast cancer survival identified by a genome-wide association study. *Cancer Res.* **72**, 1182–9 (2012).

22. Rafiq, S. *et al.* Identification of inherited genetic variations influencing prognosis in early-onset breast cancer. *Cancer Res.* **73**, 1883–91 (2013).

23. Han, C. H. *et al.* Polymorphisms in the SULF1 gene are associated with early age of onset and survival of ovarian cancer. *J. Exp. Clin. Cancer Res.* **30**, 5 (2011).

24. Lai, J.-P., Sandhu, D. S., Shire, A. M. & Roberts, L. R. The tumor suppressor function of human sulfatase 1 (SULF1) in carcinogenesis. *J. Gastrointest. Cancer* **39**, 149–58 (2008).

25. Emerson, C. P. *et al.* QSulf1, a heparan sulfate 6-O-endosulfatase, inhibits fibroblast growth factor signaling in mesoderm induction and angiogenesis. *Proc. Natl. Acad. Sci.* **101**, 4833–4838 (2004).

26. Ai, X. *et al.* QSulf1 remodels the 6-O sulfation states of cell surface heparan sulfate proteoglycans to promote Wnt signaling. *J. Cell Biol.* **162**, 341–351 (2003).

27. Sebastian, K. *et al.* Characterization of SLCO5A1/OATP5A1, a solute carrier transport protein with non-classical function. *PLoS One* **8**, e83257 (2013).

28. Cowen, L., Ideker, T., Raphael, B. J. & Sharan, R. Network propagation: A universal amplifier of genetic associations. *Nat. Rev. Genet.* **18**, 551–562 (2017).

29. Nakka, P., Raphael, B. J. & Ramachandran, S. Gene and network analysis of common variants reveals novel associations in multiple complex diseases. *Genetics* **204**, 783–798 (2016).

30. Razick, S., Magklaras, G. & Donaldson, I. M. iRefIndex: A consolidated protein interaction database with provenance. *BMC Bioinformatics* **9**, 405 (2008).

31. Cancer Genome Atlas Research Network *et al.* The Cancer Genome Atlas Pan-Cancer analysis project. *Nat. Genet.* **45**, 1113–20 (2013).

32. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 15545–50 (2005).

33. Joshi-Tope, G. *et al.* Reactome: a knowledgebase of biological pathways. *Nucleic Acids Res.* **33**, D428-32 (2005).

34. Liberzon, A. *et al.* The Molecular Signatures Database Hallmark Gene Set Collection. *Cell Syst.* **1**, 417–425 (2015).

35. Lappano, R. & Maggiolini, M. G protein-coupled receptors: novel targets for drug discovery in cancer. *Nat. Rev. Drug Discov.* **10**, 47–60 (2011).

36. Kisfalvi, K., Rey, O., Young, S. H., Sinnett-Smith, J. & Rozengurt, E. Insulin Potentiates Ca 2+ Signaling and Phosphatidylinositol 4,5-Bisphosphate Hydrolysis Induced by G q Protein-Coupled Receptor Agonists through an mTOR-Dependent Pathway. *Endocrinology* **148**, 3246–3257 (2007).

37. Sassmann, A. *et al.* The Gq/G11-mediated signaling pathway is critical for autocrine potentiation of insulin secretion in mice. *J. Clin. Invest.* **120**, 2184–93 (2010).

38. Imamura, T. *et al.* G alpha-q/11 protein plays a key role in insulin-induced glucose transport in 3T3-L1 adipocytes. *Mol. Cell. Biol.* **19**, 6765–74 (1999).

39. Nierodzik, M. L. & Karpatkin, S. Thrombin induces tumor growth, metastasis, and angiogenesis: Evidence for a thrombin-regulated dormant tumor phenotype. *Cancer Cell* **10**, 355–62 (2006).

40. Woulfe, D. S. Platelet G protein-coupled receptors in hemostasis and thrombosis. *J. Thromb. Haemost.* **3**, 2193–200 (2005).

41. Lal, I., Dittus, K. & Holmes, C. E. Platelets, coagulation and fibrinolysis in breast cancer progression. *Breast Cancer Res.* **15**, 207 (2013).

42. Gaucher, J., Montellier, E. & Sassone-Corsi, P. Molecular Cogs: Interplay between Circadian Clock and Cell Cycle. *Trends Cell Biol.* **28**, 368–379 (2018).

43. Hansen, J. & Stevens, R. G. Case-control study of shift-work and breast cancer risk in Danish nurses: impact of shift systems. *Eur. J. Cancer* **48**, 1722–9 (2012).

44. Knutsson, A. *et al.* Breast cancer among shift workers: results of the WOLF longitudinal cohort study. *Scand. J. Work. Environ. Health* **39**, 170–7 (2013).

45. Travis, R. C. *et al.* Night shift work and breast cancer incidence: Three prospective studies and meta-analysis of published studies. *J. Natl. Cancer Inst.* **108**, djw169 (2016).

46. Rabstein, S. *et al.* Night work and breast cancer estrogen receptor status--results from the German GENICA study. *Scand. J. Work. Environ. Health* **39**, 448–55 (2013).

47. Zienolddiny, S. *et al.* Analysis of polymorphisms in the circadian-related genes and breast cancer risk in Norwegian nurses working night shifts. *Breast Cancer Res.* **15**, R53 (2013).

48. Reszka, E., Przybek, M., Muurlink, O. & Pepłonska, B. Circadian gene variants and breast cancer. *Cancer Lett.* **390**, 137–145 (2017).

49. Cadenas, C. *et al.* Loss of circadian clock gene expression is associated with tumor progression in breast cancer. *Cell Cycle* **13**, 3282–91 (2014).

50. Ha, N.-H., Long, J., Cai, Q., Shu, X. O. & Hunter, K. W. The Circadian Rhythm Gene Arntl2 Is a Metastasis Susceptibility Gene for Estrogen Receptor-Negative Breast Cancer. *PLoS Genet.* **12**, e1006267 (2016).

51. Yang, X. *et al.* The circadian clock gene per1 suppresses cancer cell proliferation and tumor growth at specific times of day. *Chronobiol. Int.* **26**, 1323–1339 (2009).

52. Komatsu, N. *et al.* The Circadian Gene Per1 Plays an Important Role in Cell Growth and DNA Damage Control in Human Cancer Cells. *Mol. Cell* **22**, 375–382 (2006).

53. Kuo, S.-J. *et al.* Deregulated expression of the PER1 , PER2 and PER3 genes in breast cancers. *Carcinogenesis* **26**, 1241–1246 (2005).

54. Unsal-Kacmaz, K., Mullen, T. E., Kaufmann, W. K. & Sancar, A. Coupling of Human Circadian and Cell Cycles by the Timeless Protein. *Mol. Cell. Biol.* **25**, 3109–3116 (2005).

55. Rigaill, G. *et al.* TIPIN depletion leads to apoptosis in breast cancer cells. *Mol. Oncol.* **9**, 1580–1598 (2015).

56. Ashcroft, M. *et al.* Human CHCHD4 mitochondrial proteins regulate cellular oxygen consumption rate and metabolism and provide a critical role in hypoxia signaling and tumor progression. *J. Clin. Invest.* **122**, 600–611 (2012).

57. Zhuang, J. *et al.* Mitochondrial disulfide relay mediates translocation of p53 and partitions its subcellular activity. *Proc. Natl. Acad. Sci.* **110**, 17356–17361 (2013).

58. Sun, Y. *et al.* Haploinsufficiency in the mitochondrial protein CHCHD4 reduces brain injury in a mouse model of neonatal hypoxia-ischemia. *Cell Death Dis.* **8**, e2781 (2017).

59. Windham, P. F. & Tinsley, H. N. CGMP signaling as a target for the prevention and treatment of breast cancer. *Semin. Cancer Biol.* **31**, 106–110 (2015).

60. Saravani, R., Karami-Tehrani, F., Hashemi, M., Aghaei, M. & Edalat, R. Inhibition of phosphodiestrase 9 induces cGMP accumulation and apoptosis in human breast cancer cell lines, MCF-7 and MDA-MB-468. *Cell Prolif.* **45**, 199–206 (2012).

61. Aznar, S. & Lacal, J. C. Rho signals to cell growth and apoptosis. *Cancer Lett.* **165**, 1–10 (2001).

62. Jilg, C. A. *et al.* PRK1/PKN1 controls migration and metastasis of androgen-independent prostate cancer cells. *Oncotarget* **5**, 12646–64 (2014).

63. James, R. G. *et al.* Protein Kinase PKN1 Represses Wnt/β-Catenin Signaling in Human Melanoma Cells. *J. Biol. Chem.* **288**, 34658–34670 (2013).

64. Dillon, L. & Miller, T. Therapeutic Targeting of Cancers with Loss of PTEN Function. *Curr. Drug Targets* **15**, 65–79 (2014).

65. Garcia-Cao, I. *et al.* Systemic elevation of PTEN induces a tumor-suppressive metabolic state. *Cell* **149**, 49–62 (2012).

66. Chen, L. & Guo, D. The functions of tumor suppressor PTEN in innate and adaptive immunity. *Cell. Mol. Immunol.* **14**, 581–589 (2017).

67. Neo, S. H. *et al.* Use of a novel cytotoxic HEXIM1 peptide in the directed breast cancer therapy. *Oncotarget* **7**, 5483–94 (2016).

68. Hartman, M. *et al.* Is breast cancer prognosis inherited? *Breast Cancer Res.* **9**, R39 (2007).

69. Möller, S. *et al.* The heritability of breast cancer among women in the nordic twin study of cancer. *Cancer Epidemiol. Biomarkers Prev.* **25**, 145–150 (2016).

70. Anderson, W. F., Rosenberg, P. S., Prat, A., Perou, C. M. & Sherman, M. E. How many etiological subtypes of breast cancer: Two, three, four, or more? *J. Natl. Cancer Inst.* **106**, 1–11 (2014).

71. Dai, X., Chen, A. & Bai, Z. Integrative investigation on breast cancer in ER, PR and HER2-defined subgroups using mRNA and miRNA expression profiling. *Sci. Rep.* **4**, 6566 (2014).

72. Kao, P. Y. P., Leung, K. H., Chan, L. W. C., Yip, S. P. & Yap, M. K. H. Pathway analysis of complex diseases for GWAS, extending to consider rare variants, multi-omics and interactions. *Biochim. Biophys. Acta* **1861**, 335–353 (2017).

73. Reyna, M. A., Leiserson, M. D. M. & Raphael, B. J. Hierarchical HotNet: identifying hierarchies of altered subnetworks. *Bioinformatics* **34**, i972–i980 (2018).

74. Michailidou, K. *et al.* Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat. Genet.* **45**, 353–61, 361e1-2 (2013).

75. van den Broek, A. J. *et al.* Impact of Age at Primary Breast Cancer on Contralateral Breast Cancer Risk in BRCA1/2 Mutation Carriers. *J. Clin. Oncol.* **34**, 409–18 (2016).

76. 1000 Genomes Project Consortium *et al.* A map of human genome variation from population-scale sequencing. *Nature* **467**, 1061–73 (2010).

77. Delaneau, O., Marchini, J. & Zagury, J.-F. A linear complexity phasing method for thousands of genomes. *Nat. Methods* **9**, 179–81 (2011).

78. Howie, B., Marchini, J. & Stephens, M. Genotype imputation with thousands of genomes. *G3 (Bethesda).* **1**, 457–70 (2011).

79. Vandin, F., Clay, P., Upfal, E. & Raphael, B. J. Discovery of mutated subnetworks associated with clinical data in cancer. *Pac. Symp. Biocomput.* 55–66 (2012). doi:9789814366496\_0006 [pii]

80. Vandin, F., Upfal, E. & Raphael, B. J. Algorithms for detecting significantly mutated pathways in cancer. *Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics)* **6044 LNBI**, 506–521 (2010).

81. Kodama, I. *et al.* Estrogen regulates the production of VEGF for osteoclast formation and activity in op/op mice. *J. Bone Miner. Res.* **19**, 200–6 (2004).

82. Shannon, P. *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–504 (2003).

83. Calderone, A., Castagnoli, L. & Cesareni, G. mentha: a resource for browsing integrated protein-interaction networks. *Nat. Methods* **10**, 690–691 (2013).

84. Bindea, G. *et al.* ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* **25**, 1091–3 (2009).

85. Merico, D., Isserlin, R., Stueker, O., Emili, A. & Bader, G. D. Enrichment Map: A Network-Based Method for Gene-Set Enrichment Visualization and Interpretation. *PLoS One* **5**, e13984 (2010).

**Supplementary Information**

**Acknowledgements**

**BCAC:** We thank all the individuals who took part in these studies and all the researchers, clinicians, technicians and administrative staff who have enabled this work to be carried out. We acknowledge all contributors to the COGS and OncoArray study design, chip design, genotyping, and genotype analyses. **ABCFS** thank Maggie Angelakos, Judi Maskiell, Gillian Dite. **ABCS** thanks Frans Hogervorst, Sten Cornelissen and Annegien Broeks. **ABCTB** Investigators: Christine Clarke,Rosemary Balleine, Robert Baxter,Stephen Braye, Jane Carpenter, Jane Dahlstrom, John Forbes, Soon Lee, Debbie Marsh, Adrienne Morey, Nirmala Pathmanathan, Rodney Scott, Allan Spigelman, Nicholas Wilcken, Desmond Yip. Samples are made available to researchers on a non-exclusive basis. **BBCS** thanks Eileen Williams, Elaine Ryder-Mills, Kara Sargus. The **BCINIS** study would not have been possible without the contributions of Dr. K. Landsman, Dr. N. Gronich, Dr. A. Flugelman, Dr. W. Saliba, Dr. E. Liani, Dr. I. Cohen, Dr. S. Kalet, Dr. V. Friedman, Dr. O. Barnet of the NICCC in Haifa, and all the contributing family medicine, surgery, pathology and oncology teams in all medical institutes in Northern Israel. **BIGGS** thanks Niall McInerney, Gabrielle Colleran, Andrew Rowan, Angela Jones. The **BREOGAN** study would not have been possible without the contributions of the following: Manuela Gago-Dominguez, Jose Esteban Castelao, Angel Carracedo, Victor Muñoz Garzón, Alejandro Novo Domínguez, Maria Elena Martinez, Sara Miranda Ponte, Carmen Redondo Marey, Maite Peña Fernández, Manuel Enguix Castelo, Maria Torres, Manuel Calaza, José Antúnez, Máximo Fraga and the staff of the Department of Pathology and Biobank of the University Hospital Complex of Santiago-CHUS, Instituto de Investigación Sanitaria de Santiago, IDIS, Xerencia de Xestion Integrada de Santiago-SERGAS; Joaquín González-Carreró and the staff of the Department of Pathology and Biobank of University Hospital Complex of Vigo, Instituto de Investigacion Biomedica Galicia Sur, SERGAS, Vigo, Spain. **BSUCH** thanks Peter Bugert, Medical Faculty Mannheim. **CCGP** thanks Styliani Apostolaki, Anna Margiolaki, Georgios Nintos, Maria Perraki, Georgia Saloustrou, Georgia Sevastaki, Konstantinos Pompodakis. **CGPS** thanks staff and participants of the Copenhagen General Population Study. For the excellent technical assistance: Dorthe Uldall Andersen, Maria Birna Arnadottir, Anne Bank, Dorthe Kjeldgård Hansen. The Danish Cancer Biobank is acknowledged for providing infrastructure for the collection of blood samples for the cases. **CNIO-BCS** thanks Guillermo Pita, Charo Alonso, Nuria Álvarez, Pilar Zamora, Primitiva Menendez, the Human Genotyping-CEGEN Unit (CNIO). Investigators from the **CPS-II** cohort thank the participants and Study Management Group for their invaluable contributions to this research. They also acknowledge the contribution to this study from central cancer registries supported through the Centers for Disease Control and Prevention National Program of Cancer Registries, as well as cancer registries supported by the National Cancer Institute Surveillance Epidemiology and End Results program. The **CTS** Steering Committee includes Leslie Bernstein, Susan Neuhausen, James Lacey, Sophia Wang, Huiyan Ma, and Jessica Clague DeHart at the Beckman Research Institute of City of Hope, Dennis Deapen, Rich Pinder, and Eunjung Lee at the University of Southern California, Pam Horn-Ross, Peggy Reynolds, Christina Clarke Dur and David Nelson at the Cancer Prevention Institute of California, Hoda Anton-Culver, Argyrios Ziogas, and Hannah Park at the University of California Irvine, and Fred Schumacher at Case Western University. **DIETCOMPLYF** thanks the patients, nurses and clinical staff involved in the study. The DietCompLyf study was funded by the charity Against Breast Cancer (Registered Charity Number 1121258) and the NCRN. We thank the participants and the investigators of EPIC (European Prospective Investigation into Cancer and Nutrition). **ESTHER** thanks Hartwig Ziegler, Sonja Wolf, Volker Hermann, Christa Stegmaier, Katja Butterbach. **FHRISK** thanks NIHR for funding. **GC-HBOC** thanks Stefanie Engert, Heide Hellebrand, Sandra Kröber and LIFE - Leipzig Research Centre for Civilization Diseases (Markus Loeffler, Joachim Thiery, Matthias Nüchter, Ronny Baber). The **GENICA** Network: Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tübingen, Germany [HB, Wing-Yee Lo], German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ) [HB], Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn, Germany [Yon-Dschun Ko, Christian Baisch], Institute of Pathology, University of Bonn, Germany [Hans-Peter Fischer], Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany [UH], Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum (IPA), Bochum, Germany [Thomas Brüning, Beate Pesch, Sylvia Rabstein, Anne Lotz]; and Institute of Occupational Medicine and Maritime Medicine, University Medical Center Hamburg-Eppendorf, Germany [Volker Harth]. **HABCS** thanks Michael Bremer. **HEBCS** thanks, Rainer Fagerholm, Kirsimari Aaltonen, Karl von Smitten, Irja Erkkilä. **HUBCS** thanks Shamil Gantsev. **KARMA** and **SASBAC** thank the Swedish Medical Research Counsel. **KBCP** thanks Eija Myöhänen, Helena Kemiläinen. **kConFab/AOCS** wish to thank Heather Thorne, Eveline Niedermayr, all the kConFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the Clinical Follow Up Study (which has received funding from the NHMRC, the National Breast Cancer Foundation, Cancer Australia, and the National Institute of Health (USA)) for their contributions to this resource, and the many families who contribute to kConFab. **LMBC** thanks Gilian Peuteman, Thomas Van Brussel, EvyVanderheyden and Kathleen Corthouts. **MARIE** thanks Petra Seibold, Judith Heinz, Nadia Obi, Sabine Behrens, Ursula Eilber, Muhabbet Celik and Til Olchers. **MBCSG**: Paolo Radice, Jacopo Azzollini, Bernardo Bonanni, Bernard Peissel, Roberto Villa, Giulia Cagnoli, Irene Feroce, and the personnel of the Cogentech Cancer Genetic Test Laboratory. We thank the coordinators, the research staff and especially the MMHS participants for their continued collaboration on research studies in breast cancer. The following are NBCS Collaborators: Kristine K. Sahlberg (PhD), Lars Ottestad (MD), Rolf Kåresen (Prof. Em.) Dr. Ellen Schlichting (MD), Marit Muri Holmen (MD), Toril Sauer (MD), Vilde Haakensen (MD), Olav Engebråten (MD), Bjørn Naume (MD), Alexander Fosså (MD), Cecile E. Kiserud (MD), Kristin V. Reinertsen (MD), Åslaug Helland (MD), Margit Riis (MD), Jürgen Geisler (MD) and OSBREAC. **NHS/NHS2** would like to thank the participants and staff of the NHS and NHS2 for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. **OBCS** thanks Arja Jukkola-Vuorinen, Mervi Grip, Saila Kauppila, Meeri Otsukka, Leena Keskitalo and Kari Mononen for their contributions to this study. **OFBCR** thanks Teresa Selander, Nayana Weerasooriya. **ORIGO** thanks E. Krol-Warmerdam, and J. Blom for patient accrual, administering questionnaires, and managing clinical information. **PBCS** thanks Louise Brinton, Mark Sherman, Neonila Szeszenia-Dabrowska, Beata Peplonska, Witold Zatonski, Pei Chao, Michael Stagner. The ethical approval for the **POSH** study is MREC /00/6/69, UKCRN ID: 1137. We thank staff in the Experimental Cancer Medicine Centre (ECMC) supported Faculty of Medicine Tissue Bank and the Faculty of Medicine DNA Banking resource. **PREFACE** thanks Sonja Oeser and Silke Landrith. **PROCAS** thanks NIHR for funding. **RBCS** thanks Petra Bos, Jannet Blom, Ellen Crepin, Elisabeth Huijskens, Anja Kromwijk-Nieuwlaat, Annette Heemskerk, the Erasmus MC Family Cancer Clinic. **SBCS** thanks Sue Higham, Helen Cramp, Dan Connley, Ian Brock, Sabapathy Balasubramanian and Malcolm W.R. Reed. We thank the **SEARCH** and **EPIC** teams. **SKKDKFZS** thanks all study participants, clinicians, family doctors, researchers and technicians for their contributions and commitment to this study. We thank the SUCCESS Study teams in Munich, Duessldorf, Erlangen and Ulm. We thank the **SUCCESS** Study teams in Munich, Duessldorf, Erlangen and Ulm. **SZBCS** thanks Ewa Putresza. **UCIBCS** thanks Irene Masunaka. **UKBGS** thanks Breast Cancer Now and the Institute of Cancer Research for support and funding of the Breakthrough Generations Study, and the study participants, study staff, and the doctors, nurses and other health care providers and health information sources who have contributed to the study. We acknowledge NHS funding to the Royal Marsden/ICR NIHR Biomedical Research Centre. The authors thank the **WHI** investigators and staff for their dedication and the study participants for making the program possible.

**Funding: BCAC** is funded by Cancer Research UK [C1287/A16563, C1287/A10118], the European Union's Horizon 2020 Research and Innovation Programme (grant numbers 634935 and 633784 for BRIDGES and B-CAST respectively), and by the European Community´s Seventh Framework Programme under grant agreement number 223175 (grant number HEALTH-F2-2009-223175) (COGS). The EU Horizon 2020 Research and Innovation Programme funding source had no role in study design, data collection, data analysis, data interpretation or writing of the report.

Genotyping of the **OncoArray** was funded by the NIH Grant U19 CA148065, and Cancer UK Grant C1287/A16563 and the PERSPECTIVE project supported by the Government of Canada through Genome Canada and the Canadian Institutes of Health Research (grant GPH-129344) and, the Ministère de l’Économie, Science et Innovation du Québec through Genome Québec and the PSRSIIRI-701 grant, and the Quebec Breast Cancer Foundation. Funding for the **iCOGS** infrastructure came from: the European Community's Seventh Framework Programme under grant agreement n° 223175 (HEALTH-F2-2009-223175) (COGS), Cancer Research UK (C1287/A10118, C1287/A10710, C12292/A11174, C1281/A12014, C5047/A8384, C5047/A15007, C5047/A10692, C8197/A16565), the National Institutes of Health (CA128978) and Post-Cancer GWAS initiative (1U19 CA148537, 1U19 CA148065 and 1U19 CA148112 - the GAME-ON initiative), the Department of Defence (W81XWH-10-1-0341), the Canadian Institutes of Health Research (CIHR) for the CIHR Team in Familial Risks of Breast Cancer, and Komen Foundation for the Cure, the Breast Cancer Research Foundation, and the Ovarian Cancer Research Fund. The DRIVE Consortium was funded by U19 CA148065.

**ABCFS** was supported by grant UM1 CA164920 from the National Cancer Institute (USA). The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centres in the in the Breast Cancer Family Registry (**BCFR)**, nor does mention of trade names, commercial products, or organizations imply endorsement by the USA Government or the BCFR. The **ABCFS** was also supported by the National Health and Medical Research Council of Australia, the New South Wales Cancer Council, the Victorian Health Promotion Foundation (Australia) and the Victorian Breast Cancer Research Consortium. J.L.H. is a National Health and Medical Research Council (NHMRC) Senior Principal Research Fellow. M.C.S. is a NHMRC Senior Research Fellow. The **ABCS** study was supported by the Dutch Cancer Society [grants NKI 2007-3839; 2009-4363; 2015-7632]. The **ABCTB** was supported by the National Health and Medical Research Council of Australia, The Cancer Institute NSW and the National Breast Cancer Foundation. The work of the **BBCC** was partly funded by ELAN-Fond of the University Hospital of Erlangen. The **BBCS** is funded by Cancer Research UK and Breast Cancer Now and acknowledges NHS funding to the NIHR Biomedical Research Centre, and the National Cancer Research Network (NCRN). For the **BCFR-NY, BCFR-PA, BCFR-UT** this work was supported by grant UM1 CA164920 from the National Cancer Institute. For **BIGGS**, ES is supported by NIHR Comprehensive Biomedical Research Centre, Guy's & St. Thomas' NHS Foundation Trust in partnership with King's College London, United Kingdom. IT is supported by the Oxford Biomedical Research Centre. The **BREOGAN** is funded by Acción Estratégica de Salud del Instituto de Salud Carlos III FIS PI12/02125/Cofinanciado FEDER; Acción Estratégica de Salud del Instituto de Salud Carlos III FIS PI17/00918/Cofinanciado FEDER; Acción Estratégica de Salud del Instituto de Salud Carlos III FIS Intrasalud (PI13/01136); Programa Grupos Emergentes, Cancer Genetics Unit, Instituto de Investigacion Biomedica Galicia Sur. Xerencia de Xestion Integrada de Vigo-SERGAS, Instituto de Salud Carlos III, Spain; Grant 10CSA012E, Consellería de Industria Programa Sectorial de Investigación Aplicada, PEME I + D e I + D Suma del Plan Gallego de Investigación, Desarrollo e Innovación Tecnológica de la Consellería de Industria de la Xunta de Galicia, Spain; Grant EC11-192. Fomento de la Investigación Clínica Independiente, Ministerio de Sanidad, Servicios Sociales e Igualdad, Spain; and Grant FEDER-Innterconecta. Ministerio de Economia y Competitividad, Xunta de Galicia, Spain. The **BSUCH** study was supported by the Dietmar-Hopp Foundation, the Helmholtz Society and the German Cancer Research Center (DKFZ). **CCGP** is supported by funding from the University of Crete. The **CECILE** study was supported by Fondation de France, Institut National du Cancer (INCa), Ligue Nationale contre le Cancer, Agence Nationale de Sécurité Sanitaire, de l'Alimentation, de l'Environnement et du Travail (ANSES), Agence Nationale de la Recherche (ANR). The **CGPS** was supported by the Chief Physician Johan Boserup and Lise Boserup Fund, the Danish Medical Research Council, and Herlev and Gentofte Hospital. The **CNIO-BCS** was supported by the Instituto de Salud Carlos III, the Red Temática de Investigación Cooperativa en Cáncer and grants from the Asociación Española Contra el Cáncer and the Fondo de Investigación Sanitario (PI11/00923 and PI12/00070). The American Cancer Society funds the creation, maintenance, and updating of the **CPS-II** cohort. The **CTS** was initially supported by the California Breast Cancer Act of 1993 and the California Breast Cancer Research Fund (contract 97-10500) and is currently funded through the National Institutes of Health (R01 CA77398, UM1 CA164917, and U01 CA199277). Collection of cancer incidence data was supported by the California Department of Public Health as part of the statewide cancer reporting program mandated by California Health and Safety Code Section 103885. The University of Westminster curates the **DietCompLyf** database funded by Against Breast Cancer Registered Charity No. 1121258 and the NCRN. The coordination of **EPIC** is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by: Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l’Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF) (Germany); the Hellenic Health Foundation, the Stavros Niarchos Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); Health Research Fund (FIS), PI13/00061 to Granada, PI13/01162 to EPIC-Murcia, Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (RD06/0020) (Spain); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk, MR/M012190/1 to EPIC-Oxford) (United Kingdom). The **ESTHER** study was supported by a grant from the Baden Württemberg Ministry of Science, Research and Arts. Additional cases were recruited in the context of the VERDI study, which was supported by a grant from the German Cancer Aid (Deutsche Krebshilfe). **FHRISK** is funded from NIHR grant PGfAR 0707-10031. Prof D Gareth Evans is supported by the NIHR Manchester Biomedical Research Centre (IS-BRC-1215-20007). The **GC-HBOC** is supported by the German Cancer Aid (grant no 110837, coordinator: Rita K. Schmutzler, Cologne). This work was also funded by the European Regional Development Fund and Free State of Saxony, Germany (LIFE - Leipzig Research Centre for Civilization Diseases, project numbers 713-241202, 713-241202, 14505/2470, 14575/2470). The **GENICA** was funded by the Federal Ministry of Education and Research (BMBF) Germany grants 01KW9975/5, 01KW9976/8, 01KW9977/0 and 01KW0114, the Robert Bosch Foundation, Stuttgart, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum (IPA), Bochum, as well as the Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn, Germany. The **GESBC** was supported by the Deutsche Krebshilfe e. V. [70492] and the German Cancer Research Center (DKFZ). The **HABCS** study was supported by the Claudia von Schilling Foundation for Breast Cancer Research, by the Lower Saxonian Cancer Society, and by the Rudolf Bartling Foundation. The **HEBCS** was financially supported by the Helsinki University Central Hospital Research Fund, Academy of Finland (266528), the Finnish Cancer Society, and the Sigrid Juselius Foundation. The **HUBCS** was supported by a grant from the German Federal Ministry of Research and Education (RUS08/017), and by the Russian Foundation for Basic Research and the Federal Agency for Scientific Organizations for support the Bioresource collections and RFBR grants 14-04-97088, 17-29-06014 and 17-44-020498. Financial support for **KARBAC** was provided through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet, the Swedish Cancer Society, The Gustav V Jubilee foundation and Bert von Kantzows foundation. The **KARMA** study was supported by Märit and Hans Rausings Initiative Against Breast Cancer. The **KBCP** was financially supported by the special Government Funding (EVO) of Kuopio University Hospital grants, Cancer Fund of North Savo, the Finnish Cancer Organizations, and by the strategic funding of the University of Eastern Finland. **kConFab** is supported by a grant from the National Breast Cancer Foundation, and previously by the National Health and Medical Research Council (NHMRC), the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the Cancer Foundation of Western Australia. **LMBC** is supported by the 'Stichting tegen Kanker'. The **MARIE** study was supported by the Deutsche Krebshilfe e.V. [70-2892-BR I, 106332, 108253, 108419, 110826, 110828], the Hamburg Cancer Society, the German Cancer Research Center (DKFZ) and the Federal Ministry of Education and Research (BMBF) Germany [01KH0402]. **MBCSG** is supported by grants from the Italian Association for Cancer Research (AIRC) and by funds from the Italian citizens who allocated the 5/1000 share of their tax payment in support of the Fondazione IRCCS Istituto Nazionale Tumori, according to Italian laws (INT-Institutional strategic projects “5x1000”). The **MCBCS** was supported by the NIH grants CA192393, CA116167, CA176785 an NIH Specialized Program of Research Excellence (SPORE) in Breast Cancer [CA116201], and the Breast Cancer Research Foundation and a generous gift from the David F. and Margaret T. Grohne Family Foundation. **MCCS** cohort recruitment was funded by VicHealth and Cancer Council Victoria. The **MCCS** was further supported by Australian NHMRC grants 209057 and 396414, and by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry (VCR) and the Australian Institute of Health and Welfare (AIHW), including the National Death Index and the Australian Cancer Database. The **MEC** was supported by NIH grants CA63464, CA54281, CA098758, CA132839 and CA164973. The **MISS** study is supported by funding from ERC-2011-294576 Advanced grant, Swedish Cancer Society, Swedish Research Council, Local hospital funds, Berta Kamprad Foundation, Gunnar Nilsson. The **MMHS** study was supported by NIH grants CA97396, CA128931, CA116201, CA140286 and CA177150. The **NBCS** has received funding from the K.G. Jebsen Centre for Breast Cancer Research; the Research Council of Norway grant 193387/V50 (to A-L Børresen-Dale and V.N. Kristensen) and grant 193387/H10 (to A-L Børresen-Dale and V.N. Kristensen), South Eastern Norway Health Authority (grant 39346 to A-L Børresen-Dale) and the Norwegian Cancer Society (to A-L Børresen-Dale and V.N. Kristensen). The **NC-BCFR** and **OFBCR** were supported by grant UM1 CA164920 from the National Cancer Institute (USA). The **NCBCS** was funded by Komen Foundation, the National Cancer Institute (P50 CA058223, U54 CA156733, U01 CA179715), and the North Carolina University Cancer Research Fund. The **NHS** was supported by NIH grants P01 CA87969, UM1 CA186107, and U19 CA148065. The **NHS2** was supported by NIH grants UM1 CA176726 and U19 CA148065. The **OBCS** was supported by research grants from the Finnish Cancer Foundation, the Academy of Finland (grant number 250083, 122715 and Center of Excellence grant number 251314), the Finnish Cancer Foundation, the Sigrid Juselius Foundation, the University of Oulu, the University of Oulu Support Foundation and the special Governmental EVO funds for Oulu University Hospital-based research activities. The **ORIGO** study was supported by the Dutch Cancer Society (RUL 1997-1505) and the Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL CP16). The **PBCS** was funded by Intramural Research Funds of the National Cancer Institute, Department of Health and Human Services, USA. Genotyping for **PLCO** was supported by the Intramural Research Program of the National Institutes of Health, NCI, Division of Cancer Epidemiology and Genetics. The **PLCO** is supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics and supported by contracts from the Division of Cancer Prevention, National Cancer Institute, National Institutes of Health. The **POSH** study is funded by Cancer Research UK (grants C1275/A11699, C1275/C22524, C1275/A19187, C1275/A15956 and Breast Cancer Campaign 2010PR62, 2013PR044. PROCAS is funded from NIHR grant PGfAR 0707-10031. **PROCAS** is funded from NIHR grant PGfAR 0707-10031. The **RBCS** was funded by the Dutch Cancer Society (DDHK 2004-3124, DDHK 2009-4318). The **SASBAC** study was supported by funding from the Agency for Science, Technology and Research of Singapore (A\*STAR), the US National Institute of Health (NIH) and the Susan G. Komen Breast Cancer Foundation. The **SBCS** was supported by Sheffield Experimental Cancer Medicine Centre and Breast Cancer Now Tissue Bank. **SEARCH** is funded by Cancer Research UK [C490/A10124, C490/A16561] and supported by the UK National Institute for Health Research Biomedical Research Centre at the University of Cambridge. The University of Cambridge has received salary support for PDPP from the NHS in the East of England through the Clinical Academic Reserve. **SKKDKFZS** is supported by the DKFZ. The **SMC** is funded by the Swedish Cancer Foundation and the Swedish Research Council (SIMPLER, VR 2017-00644). The **SZBCS** was supported by Grant PBZ\_KBN\_122/P05/2004. The **UCIBCS** component of this research was supported by the NIH [CA58860, CA92044] and the Lon V Smith Foundation [LVS39420]. The **UKBGS** is funded by Breast Cancer Now and the Institute of Cancer Research (ICR), London. ICR acknowledges NHS funding to the NIHR Biomedical Research Centre. The **USRT** Study was funded by Intramural Research Funds of the National Cancer Institute, Department of Health and Human Services, USA. The **WHI** program is funded by the National Heart, Lung, and Blood Institute, the US National Institutes of Health and the US Department of Health and Human Services (HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C and HHSN271201100004C). This work was also funded by NCI U19 CA148065-01.

**Author contributions:** M.K.S. and S.C. conceived the study. M.E.G. performed the data analyses. M.K.S., S.C. and M.E.G. were involved in the interpretation of the data. S.C. provided statistical and computational support for the data analyses. R.K., Q.W., M.K.B. and J.D. provided database support. M.E.G., M.K.S. and S.C wrote the manuscript. All authors contributed data from their own studies, helped revise the manuscript and approved the final version.

**Ethics approval:** The study was performed in accordance with the Declaration of Helsinki. All individual studies, from which data was used, were approved by the appropriate medical ethical committees and/or institutional review boards. All study participants provided informed consent.

**Consent for publication:** All authors consented to this publication.

**Competing interests:** The authors declare no competing interests.

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