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Germline variants and breast cancer survival in patients with distant metastases at primary breast cancer diagnosis

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Breast cancer metastasis accounts for most of the deaths from breast cancer. Identification of germline variants associated with survival in aggressive types of breast cancer may inform understanding of breast cancer progression and assist treatment. In this analysis, we studied the associations between germline variants and breast cancer survival for patients with distant metastases at primary breast cancer diagnosis. We used data from the Breast Cancer Association Consortium (BCAC) including 1062 women of European ancestry with metastatic breast cancer, 606 of whom died of breast cancer. We identified two germline variants on chromosome 1, rs138569520 and rs146023652, significantly associated with breast cancer-specific survival ($P = 3.19 \times 10^{-8}$ and 4.42×10^{-8}). In silico analysis suggested a potential regulatory effect of the variants on the nearby target genes *SDE2* and *H3F3A*. However, the variants showed no evidence of association in a smaller replication dataset. The validation dataset was obtained from the SNPs to Risk of Metastasis (StoRM) study and included 293 patients with metastatic primary breast cancer at diagnosis. Ultimately, larger replication studies are needed to confirm the identified associations.

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Breast cancer is the most common female cancer in the Western world and one of the most common causes of cancer death in women globally¹. Early detection and better treatments have helped to reduce breast cancer mortality in recent decades². Yet, when breast cancer metastasizes to distant sites, prognosis continues to be poor and for most cases treatment is only palliative³. Metastases in breast cancer can remain undetectable for many years after initial diagnosis, leading to incurable lesions⁴. Approximately 15% of patients with breast cancer will develop distant metastases within 3 years after diagnosis of the primary tumor⁵. Therefore, it is important to have the tools able to detect breast cancer metastases at earlier stages, in order to better manage and predict breast cancer progression. Prognostication models could benefit from the inclusion of germline genetic biomarkers that are capable of predicting tumor recurrence, second tumors or prognosis of second tumors. However, so far, it has been difficult to identify individual common germline variants associated with primary breast cancer survival due to the small effect size these variants are likely to have^{6,7}. Likewise, evidence as to whether or not germline variants can increase the probability of metastatic progression is currently limited to a few studies^{4,8}. For example, a candidate gene study identified common single nucleotide polymorphisms (SNPs) located within *SIP1* that were associated with metastasis and poor breast cancer prognosis⁹. Other studies have identified other metastasis susceptibility genes such as *RRP1b*¹⁰. Germline variants could specifically provide metastatic predisposition by affecting treatment response¹¹ or promoting tumor initiating events and providing new metastatic functions to tumor cells⁴.

The aim of this study was to identify associations between common germline variants and breast cancer-specific survival in patients with metastasis at primary breast cancer diagnosis. We hypothesized that germline variants might predispose to poorer survival after breast cancer metastasis, and that analyzing a set of patients with similar stage of the disease might help identify variants that do not show evidence of association in larger but more heterogeneous datasets.

Results

We used data from the Breast Cancer Association Consortium (BCAC): the dataset comprised data from 50 studies from which follow-up information for women diagnosed with distant metastases at primary breast cancer diagnosis was available. The results were based on the meta-analysis of two genome-wide SNP arrays (iCOGS¹² and OncoArray¹³ (see “Methods”). We analyzed variants that had a minor allele frequency (MAF) > 0.01 and an imputation quality $r^2 > 0.7$ for at least one of the two arrays. Details about the individual studies, the genotyping

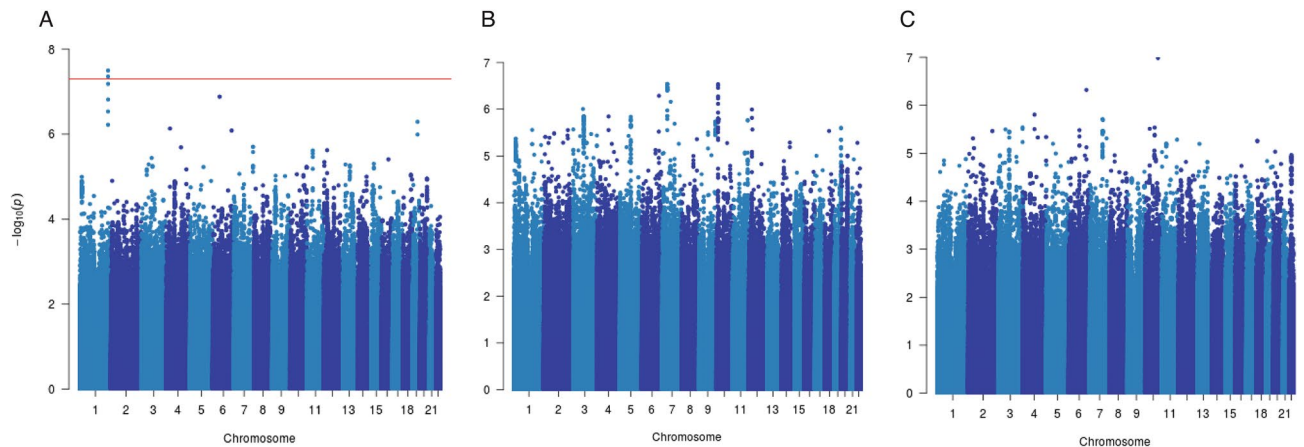


Figure 1. Manhattan plots of the meta-analysis of OncoArray and iCOGS datasets for the association of common germline variants and breast cancer-specific survival for patients with metastases at primary breast cancer diagnosis for (A) all breast tumors, (B) ER-positive tumors, and (C) ER-negative tumors. The y axis shows the $-\log_{10} P$ values of each variant analyzed, and the x axis shows their chromosome position. The red horizontal line represents $P = 5 \times 10^{-8}$.

array used and number of patients included are given in Supplementary Table 1. We analyzed the genotypes and clinico-pathological data of a total of 1062 breast cancer patients, 606 of whom died of breast cancer within 15 years of follow-up. Of these, 721 of the patients had estrogen receptor (ER)-positive disease (388 deaths) and 227 had ER-negative disease (148 deaths). All patients were women of European descent. The patients were diagnosed from 1979 to 2014 (median: 2004) and aged 26–92 (median: 60) years.

Manhattan plots showing the association between germline variants and breast cancer-specific survival of all, ER-positive and ER-negative metastasized breast cancers are shown in Fig. 1. We identified two genome-wide significant ($P < 5 \times 10^{-8}$) variants (SNPs: rs138569520 and rs146023652) on chromosome 1 associated with breast cancer-specific survival for all metastasized breast cancers (Table 1, Supplementary Table 2). The two variants were part of a set of six highly correlated SNPs (Table 1, $r^2 > 0.88$) based on European subjects in phase 3 of the 1000 Genomes Project¹⁴. No variant reached genome-wide significance for ER-positive or for ER-negative breast cancer tumors alone (Supplementary Tables 3 and 4).

The variant with the strongest association was the SNP rs138569520 (HR = 3.67, 95% CI 1.86–7.23 and $P = 3.19 \times 10^{-8}$). The HR estimates for rs138569520 in the ER-positive (HR = 3.38, 95% CI 1.48–7.70 and $P = 4.37 \times 10^{-4}$) and ER-negative (HR = 2.76, 95% CI 1.16–6.64 and $P = 8.70 \times 10^{-3}$) were similar ($P = 0.97$ for difference).

Several genes (*SDE2*, *LEFTY2*, *PYCR2* and *H3F3A*) were located within 100 kb of the most significant SNP rs138569520. We interrogated functional genomic data including annotations of enhancers, promoters and transcription factor binding sites and found evidence consistent with gene regulation in the regions containing the associated variants (Fig. 2). Hi-C analysis in HMEC cells¹⁵ showed that the lead variant rs138569520 is located in a genomic region interacting with the promoter region of *H3F3A*. SNPs rs146023652 and rs114512448 overlapped with transcription factor (TF) binding sites which might reflect the active transcription of *SDE2*. ChIP-seq signals from primary breast sub-populations¹⁶ also showed potential regulatory regions containing rs114512448. ChIA-PET analysis in MCF-7 cells from ENCODE¹⁷, detected an interaction between rs114512448 and the *PYCR2* gene. Finally, ChIA-PET also detected an interaction between rs72757046 and *SDE2* and *H3F3A*.

Using KMplotter (kmplotter.com/analysis)¹⁸, we tested the association of the mRNA tumor expression of *SDE2* and *H3F3A*, the genes in closest proximity to rs138569520, with overall survival in grade 3 breast tumors (to select the most aggressive subtype; selection for stage 4 was not available). Low mRNA expression levels of *SDE2* gene were significantly associated ($P = 0.01$) with poorer breast cancer survival (Fig. 3a), while, in contrast, high expression of *H3F3A* was associated with lower survival ($P = 6.7 \times 10^{-5}$) (Fig. 3b). These associations were not statistically significant, neither for grade 1 or for grade 2 disease ($P > 0.21$).

Lastly, we aimed to evaluate the significance of the two genome-wide significant SNPs using an independent set of 293 breast cancer patients with metastatic primary breast cancer at diagnosis from the SNPs to Risk of Metastasis (StoRM) study¹⁹. All patients were diagnosed in France from March 2012 to May 2014, aged 18 years or older (median: 59 years) and followed up to July 2017. A total of 293 patients were available for the validation study, 239 of whom had events, defined as progression and/or death occurring during follow-up. Both SNPs had good imputation quality ($r^2 \sim 0.7$) and similar MAFs to those in the BCAC dataset ($\sim 2\%$). However, neither of the two SNPs replicated in the survival analysis with the StoRM dataset (Table 2): rs138569520 (HR = 1.49, 95% CI 0.60–3.71, $P = 0.34$) and rs146023652 (HR = 1.25, 95% CI 0.46–3.37, $P = 0.66$). Although the HR estimates in the StoRM validation dataset were smaller than those from the BCAC analyses (HR = 3.67 and 3.64), the confidence limits overlapped.

Because the BCAC dataset also included prevalent cases ($n = 466$), we repeated the analysis with incident cases ($n = 596$) to match the study design in StoRM more closely. The HR estimates were similar to those for

SNP	Chr	Position	Ref	Alt	EAF	r ²	HR	LCL	UCL	P value
rs138569520	1	226193175	T	C	0.02	0.87	3.67	1.86	7.23	3.19 × 10 ⁻⁸
rs146023652	1	226158826	C	T	0.02	0.86	3.64	1.84	7.19	4.42 × 10 ⁻⁸
rs114512448	1	226173980	G	A	0.02	0.86	3.53	1.78	6.95	6.57 × 10 ⁻⁸
rs143653255	1	226157179	T	C	0.02	0.86	3.26	1.68	6.34	1.53 × 10 ⁻⁷
rs115086585	1	226154721	C	T	0.02	0.85	3.21	1.64	6.25	2.93 × 10 ⁻⁷
rs72757046	1	226235714	G	C	0.02	0.84	3.62	1.78	7.37	6.02 × 10 ⁻⁷

Table 1. Results for the six correlated variants associated with breast cancer-specific survival for patients with metastatic primary breast cancer at diagnosis. Genomic positions are based on the hg19 genome build. *ALT* alternate, *REF* reference, *EAF* effect allele frequency, *HR* hazard rate, *LCL* lower control limit, *UCL* upper control limit, *r²* imputation quality.

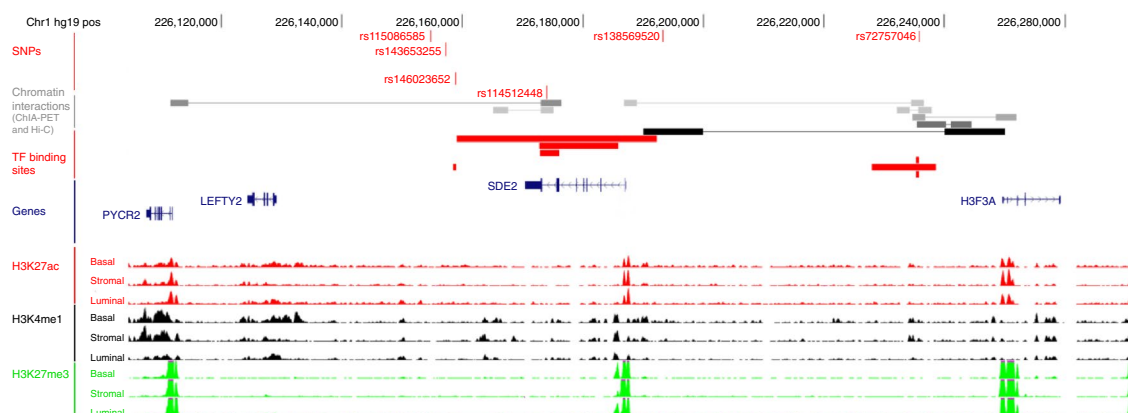


Figure 2. Functional annotation of the six highly correlated SNPs: rs138569520, rs146023652, rs114512448, rs143653255, rs115086585 and rs72757046. *TF* transcription factor.

the overall analysis (rs138569520: HR = 3.77, 95% CI 1.71–8.30, $P = 3.12 \times 10^{-5}$ and rs146023652: HR = 3.75, 95% CI 1.70–8.29, $P = 3.60 \times 10^{-5}$). Finally, since the maximum follow-up in the StoRM dataset was shorter (5 years, compared with a maximum of 15 years in the BCAC dataset), we repeated the main analysis in BCAC using a follow-up of 5 years ($n = 1031$, 476 deaths). The associations for the two SNPs were slightly less significant (rs138569520: HR = 3.43, 95% CI 1.74–6.80, $P = 1.83 \times 10^{-7}$ and rs146023652: HR = 3.41, 95% CI 1.72–6.76, $P = 2.55 \times 10^{-7}$) but the HR estimates were similar to those from the main analysis.

Discussion

In this analysis of breast cancer patients with metastatic primary breast cancer at diagnosis, involving 1062 patients with 606 breast cancer-specific deaths, we identified two variants on chromosome 1 (rs138569520 and rs146023652) associated with survival, at genome-wide levels of statistical significance. The most significant association was for the SNP rs138569520 ($P = 3.19 \times 10^{-8}$). The HR estimates were similar in patients with ER-positive and ER-negative disease.

Two genes, *SDE2* and *H3F3A*, were in closest proximity of rs138569520. Both genes have been previously associated with oncogenic processes relevant for metastatic progression: the *SDE2* gene (“silencing defective 2”) is known to be involved in DNA replication, telomere maintenance and cell cycle control^{20,21}. The functional roles of *SDE2* have been studied in a proteome dynamics analysis in prostate cancer cells; the results suggested that alterations of the gene might diminish the error-prone DNA repair pathway activation and promote missense mutations²². The gene *H3F3A* encodes for histone H3.3, and mutations in this protein have been linked to multiple cancer processes²³, including breast invasive ductal carcinoma²⁴. Additionally, the differential expression of these two genes was significantly associated with survival in grade 3 tumors based on KMplotter. Previous studies have also linked the expression of these genes to oncogenic processes. For example, downregulation of *SDE2* was associated with mutation disease phenotype as well as poorer mortality outcomes²². Likewise, overexpression of *H3F3A* was associated with lung cancer progression and promotion of lung cancer cell migration by activation of metastasis-related genes²⁵. Unfortunately, in KMplotter it was not possible to specifically select stage 4 tumors, which limits the interpretation of our findings. Future studies are needed in order to corroborate the association of *SDE2* and *H3F3A* expression with survival in this group of patients.

Additionally, there was predicted genomic activity in the locus based on the intersection of multiple genomic regulatory features in breast tissue. Although the SNPs appeared to cluster around *SDE2*, there was also *in-silico* evidence for two other potential target genes at this locus (*H3F3A* and *PYCR2*). *PYCR2* encodes for a mitochondrial protein involved in proline biosynthesis. While little is known about this proline form, studies for

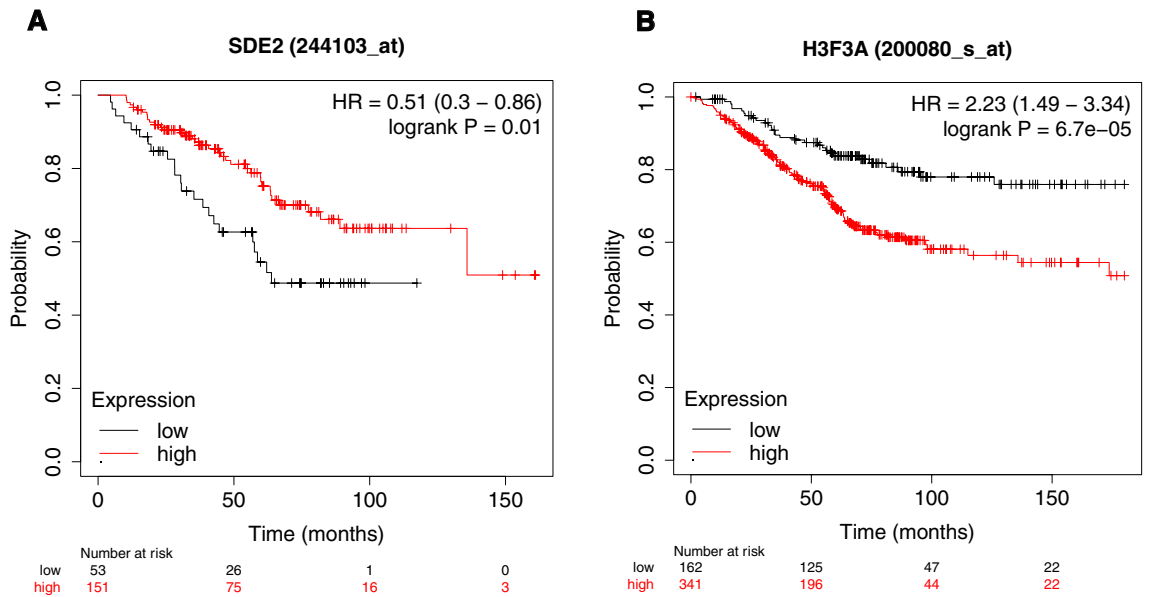


Figure 3. Kaplan–Meier overall survival plot for high versus low expression level of the genes (A) *SDE2* (n = 204) and (B) *H3F3A* (n = 503) restricted to patients with a grade 3 tumor and 15 years of follow-up. The differential expression analysis was performed in KMplotter.

SNP	Chr	Position	Ref	Alt	EAF	r ²	HR	LCL	UCL	P value
rs138569520	1	226193175	T	C	0.02	0.69	1.49	0.60	3.71	0.34
rs146023652	1	226158826	C	T	0.02	0.79	1.25	0.46	3.37	0.66

Table 2. Results for the validation of the two genome-wide significant variants in an independent dataset of breast cancer patients with metastatic primary breast cancer at diagnosis. *ALT* alternate, *REF* reference, *EAF* effect allele frequency, *HR* hazard rate, *LCL* lower control limit, *UCL* upper control limit, *r²* imputation quality.

the close family member *PYCR1* have found that higher levels of mRNA were associated with reduced survival from breast cancer patients²⁶. To support further our hypothesis that the two genome-wide significant SNPs (rs138569520 and rs146023652) were specific for survival in patients with metastatic disease, we confirmed that there were no associations (HR = 1.04, P = 0.58, MAF = 0.02 and HR = 1.03, P = 0.60, MAF = 0.02 respectively) with breast cancer-specific survival in the most recent BCAC dataset for all invasive early (stages I–III) breast cancers (OncoArray and iCOGS, n = 86,627)²⁷.

On the other hand, the two genome-wide significant variants, rs138569520 and rs146023652, were not replicated (P = 0.34 and P = 0.66, respectively) using an independent dataset of patients with metastatic primary breast cancer diagnosis (n = 293). The imputation quality and the minor allele frequency of the SNPs in the replication cohort were comparable to those in the BCAC analyses (MAF = 2% and r² > 7%), therefore the negative result could not be attributed to those factors. Age of the patients could also not explain the difference since both datasets had comparable median ages at diagnosis, 60 years for BCAC and 59 years for StoRM. On the other hand, it is important to state that there were several factors that varied between the datasets. First, the sample size differed considerably between BCAC (n = 1062) and the StoRM study (n = 293), the latter having a relatively small sample size which limits the power to detect associations. Total follow-up time also varied: for the BCAC dataset, patients were followed for a maximum of 15 years, while for the StoRM study the follow-up ended at 5 years. However, the results from the complementary analysis using the BCAC dataset and 5-year follow-up were comparable to the initial 15 years follow-up results. This finding suggests that the disparity in estimates between the two analyses is not due to shorter follow-up. There were several other differences between the main BCAC dataset and the StoRM cohort used for validation. For example, the BCAC dataset included multiple studies from several countries while the StoRM cohort included solely patients from France. Moreover, StoRM was a recent cohort with the earliest reported diagnosis starting in 2012. On the other hand, in BCAC, the year of patients' diagnosis ranged between 1979 and 2014 and included prevalent cases. While the analysis in BCAC using exclusively incident cases gave comparable estimates to the main analysis, the difference in the years of diagnosis could be related to differences in treatment strategies that were not considered in the current analysis. The lack of information about detailed treatment is a potential weakness of the current analysis and validation. Treatment strategy, together with characteristics of the tumor, will also influence the final prognosis of metastatic

breast cancer²⁸. It is important to note that the associations observed in the BCAC study may be false positives, and that further large replication studies will be required to confirm or refute the associations.

In conclusion, this analysis of BCAC patients with metastatic primary breast cancer at diagnosis from the BCAC dataset identified a new region in chromosome 1 associated with breast cancer-specific survival. The region includes six highly correlated SNPs that are predicted to be in an active region of the genome based on in-silico evidence from breast cancer tissues and that are located in close proximity to genes involved in oncogenic processes. However, we were unable to validate the association using a smaller, independent set of patients. Overall, the role of germline variants in metastasis and progression remains unclear. Further analyses with larger datasets including treatment information and functional analysis are needed to better understand the underlying biological processes and the links between this locus and the nearby genes. Prior validation of the reported associations is needed before these findings can be used in clinical-decision making. Therefore, a next step is to study these SNPs in a, preferably, prospective large series of metastasized breast cancer patients. Ultimately, germline variants could help identifying tailored treatments for patients with metastatic disease or better strategies for risk management stratification of aggressive forms of breast cancer.

Methods

Breast cancer samples and genotype data: Breast Cancer Association Consortium (BCAC). We used genotype and clinico-pathological data (database version 12) data from the Breast Cancer Association Consortium (BCAC). The dataset included 1062 breast cancer patients with metastatic primary breast cancer at diagnosis that were genotyped using one of the two different genotyping platforms: iCOGS¹² and OncoArray¹³, providing genome-wide coverage of common variants. The main analyses were based on imputed variants using the Haplotype Reference Consortium²⁹ as reference panel. All patients were women of European ancestry, aged 26–92 years (median: 60) years with metastasized breast cancer at diagnosis. Women were diagnosed between 1979 and 2014, with a median follow-up was three and a half years. Additional details about the genotype data and sample quality control have been described previously^{7,27,30}. We only analyzed variants that had a minor allele frequency (MAF) > 0.01 and an imputation quality $r^2 > 0.7$ for at least one of the two genotyping platforms (iCOGS or OncoArray). Details about the individual studies included in the analyses, including the array used, associated country and number of patients with metastatic primary breast cancer at diagnosis are given in Supplementary Table 1. The secondary use of data for the study was approved by the Data Access Committee of the BCAC, under the legal provisions of the Memorandum of Understanding and Data Transfer Agreements of Cambridge University which all the contributing institutions, which includes that all contributing institutions provided the data with the appropriate approval of their institutional review boards and informed consent of the participants of the individual studies.

Statistical and bioinformatic methods. We estimated the association of the germline variants with breast-cancer specific survival using Cox proportional hazards regression. We analyzed separately the OncoArray and iCOGS datasets and combined the estimates using fixed-effect meta-analyses. Follow-up was right censored on the date of death, last date known alive if death did not occur, or at 15 years after diagnosis, whichever came first²⁷. Time at risk was calculated from the date of diagnosis with left truncation for prevalent cases. The models were stratified by country and included the first two ancestry informative principal components¹². We performed the analysis for all breast cancers and for ER-positive and ER-negative tumors separately. To identify evidence of potential cis-regulatory activity, we intersected germline variants with numerous sources of genomic annotation information from primary breast cells (e.g., chromosome conformation, enhancer–promoter correlations, transcription factor and histone modification ChIP-seq). To assess the effect of gene expression on survival we used the Kaplan–Meier plotter on breast tissue data, grade 3 tumors and 15 years of follow-up (180 months)¹⁸.

Validation dataset: SNPs to risk of metastasis (StoRM). To attempt to validate our results we used data from the SNPs to Risk of Metastasis (StoRM) study. StoRM is a multicentric, prospective, cohort study of metastatic breast cancer patients in France that was originally designed to identify genetic and other factors associated with metastatic relapse and survival¹⁹. Patients aged 18 years or older, with a histologically proven breast cancer that was metastatic for less than 1 year were included. All patients that had another coexisting cancer or another cancer diagnosed within the last 5 years, were excluded from the study. Patients were followed from March 2012 to July 2017. Time to progression on the first metastatic treatment was recorded and patients were followed until death, every 6 months for 3 years, and then annually until July 2017. A total of 293 patients were available for the validation. The median follow-up was of 3.2 years. Because of the short total follow-up time (5 years) and the advanced disease stage of the patients in the cohort, both a recorded progression and/or death were considered as an event in the survival analyses. Of the whole set of 293 patients, 239 had a progression and/or died during the follow-up period.

Ethical 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.

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Author contributions

M.K.S. and M.E.G. conceived the study. M.E.G. performed the main data analyses and drafted the initial manuscript. M.K.S., S.C. and M.E.G. were involved in the interpretation of the data. R.K., Q.W., J.D. and M.K.B. provided database support. J.B. performed the functional analysis. M.K.S. and S.C. worked on revisions of the manuscript. All authors contributed data from their own studies, helped revise the manuscript, and approved the final version. All authors consented to this publication.

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Competing interests

The authors declare no competing interests.

Additional information

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