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Genetic modifiers of menopausal hormone replacement therapy and breast cancer risk: A genome-wide interaction study

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Abstract

Women using menopausal hormone therapy (MHT) are at increased risk to develop breast cancer (BC). To detect genetic modifiers of the association between current use of MHT and BC risk, we conducted a meta-analysis of four genome-wide case-only studies followed by replication in eleven case-control studies. We used a case-only design to assess interactions between single nucleotide polymorphisms (SNPs) and current MHT use on risk of overall and lobular BC. The discovery stage included 2,920 cases (541 lobular) from four genome-wide association studies. The top 1,391 SNPs showing *P*-values for interaction (P_{int}) $<3.0\times10^{-03}$ were selected for replication using pooled case-control data from eleven studies of the Breast Cancer Association Consortium, including 7,689 cases (676 lobular) and 9,266 controls. Fixed effects meta-analysis was used to derive combined P_{int}. No SNP reached genome-wide significance in either the discovery or combined stage. We observed effect modification of current MHT use on overall BC risk by two SNPs on chr13 near *POMP* (combined $P_{int}\leq8.9\times10^{-06}$), two SNPs in *SLC25A21* (combined $P_{int}\leq4.8\times10^{-05}$), and three SNPs in *PLCG2* (combined $P_{int}\leq4.5\times10^{-05}$). The association between lobular BC risk was potentially modified by one SNP in TMEFF2 (combined $P_{int}\leq2.7\times10^{-05}$), one SNP in *CD80* (combined $P_{int}\leq8.2\times10^{-06}$), three SNPs on chr17 near *TMEM132E* (combined $P_{int}\leq2.2\times10^{-06}$), and two SNPs on chr18 near *SLC25A52* (combined $P_{int}\leq4.6\times10^{-05}$). In conclusion, polymorphisms in genes related to solute transportation in mitochondria, transmembrane signaling and immune cell activation are potentially modifying BC risk associated with current use of MHT. These findings warrant replication in independent studies.

Keywords

breast cancer; genetic variation; menopausal hormone therapy; genome-wide

INTRODUCTION

Menopausal hormone therapy (MHT) is prescribed to women in order to alleviate climacteric symptoms and it is still commonly used despite evidence of associations with increased risk for cardiovascular diseases and breast cancer (Farquhar *et al.* 2009; Sprague *et al.* 2012; Tsai *et al.* 2011). Regarding breast cancer, only recent use of MHT increases

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

risk and the elevated risk dissipates within two years after cessation of use (Narod 2011). Furthermore, the associated risk varies by type of MHT preparation and is greater for use of combined estrogen-progestogen therapy than for use of estrogen-monotherapy (Chlebowski and Anderson 2012; Narod 2011). A meta-analysis conducted in 2005 reported an odds ratio (OR) of 1.39 (95% confidence interval (CI) 1.12–1.72) for the association between use of combined estrogen-progestogen therapy and breast cancer risk, whereas the respective OR for use of estrogen-monotherapy was 1.16 (95% CI 1.06–1.28) (Shah *et al.* 2005). Also, differences by histology have been observed, with a stronger increase in risk for lobular and tubular breast cancer compared with ductal breast cancer (Bakken *et al.* 2011; Flesch-Janys *et al.* 2008).

Understanding of the role of female sex hormones in breast carcinogenesis has already led to the development of therapeutic strategies such as the adjuvant endocrine therapy for estrogen receptor positive breast cancer (Smith and Dowsett 2003). By investigating genetic modifiers of MHT associated breast cancer, the underlying mechanisms could be further elucidated. The detection of genes involved in hormone-related breast carcinogenesis could lead to new strategies for breast cancer prevention and treatment. Knowledge of genetic modifiers could also contribute to safer use of MHT as the individual risk to develop breast cancer when using MHT may vary depending on the genetic background.

Previous studies investigating interactions between single nucleotide polymorphisms (SNPs) and use MHT regarding breast cancer risk predominantly pursued a candidate gene approach. Most of the reported interactions have not been followed up in further studies (Hein *et al.* 2012; Justenhoven *et al.* 2012; Lee *et al.* 2011). The possible interaction with variants of the known genetic susceptibility loci for breast cancer in *FGFR2* has not been clearly confirmed in subsequent studies (Campa *et al.* 2011; Kawase *et al.* 2009; Nickels *et al.* 2013; Prentice *et al.* 2009; Rebbeck *et al.* 2009; Travis *et al.* 2010). We previously failed to replicate the most significant interaction with MHT use observed for 2q36.3 in a genome-wide interaction study using case-only design (Hein *et al.* 2013).

We here expand our previous work (Hein *et al.* 2013) and conducted a meta-analysis of four case-only genome-wide gene-environment interaction studies for overall as well as for lobular breast cancer risk. We then evaluated the top 1,391 SNPs by case-control analyses utilizing data from eleven studies participating in the Breast Cancer Association Consortium (BCAC; http://ccge.medschl.cam.ac.uk/consortia/bcac/index.html).

MATERIALS AND METHODS

An overview of the included studies at each stage with respective numbers of cases and controls as well as the number of SNPs analyzed is displayed in Figure 1. All studies were approved by the relevant ethics committees and all participants gave informed consent.

Study population of case-only genome-wide studies

Under the assumption that the genetic and environmental factors are not associated in the population from which the cases were drawn, case-only studies provide a powerful and efficient way to detect gene-environment interactions (Piegorsch *et al.* 1994). We conducted

meta-analysis of four studies with quality control checked genome-wide data and information on current MHT use: the Mammary Carcinoma Risk Factor Investigation (MARIE) from Germany (Flesch-Janys *et al.* 2008), the Singapore and Sweden Breast Cancer Study (SASBAC) (Wedren *et al.* 2004), the Helsinki Breast Cancer Study (HEBCS) (Kilpivaara *et al.* 2004) and the Nurses' Health Study (NHS) from the US (Hunter *et al.* 2007). Details on all studies participating in the discovery as well as replication stage can be found in Supplementary Table 1. In total these studies contributed 2,920 cases (541 cases with lobular tumors) to the meta-analysis.

Briefly, the MARIE study is a population-based case-control study of postmenopausal women aged 50–74 years carried out in two regions in Germany with incident cases diagnosed 2001–2005 and controls matched by birth year and study region (Flesch-Janys *et al.* 2008). Initially, 800 MARIE cases with known age at menopause were randomly selected for genotyping, with lobular cases oversampled (Hein *et al.* 2013). After quality control checks, a total of 742 MARIE cases were included in the case-only genome-wide association analysis, of which 279 were lobular cases. SASBAC is a subset of a Swedish nationwide population-based case-control study (Wedren *et al.* 2004). The cases were incident breast cancer cases diagnosed 1993–1995 identified via the six regional cancer registries in Sweden, to which reporting is mandatory. Overall, 773 cases (36 lobular tumors) were included in the case-only genome-wide analyses (Li *et al.* 2011). A further 344 postmenopausal cases (88 lobular) were contributed by the hospital-based Finnish study HEBCS. In HEBCS, cases included both unselected breast cancer and familial breast cancer patients recruited at the Helsinki University Central Hospital, 1997–2004 (Kilpivaara *et al.* 2004). The NHS cohort was established in 1976 and comprised 121,700 female registered nurses. In 1989–1990, 32,826 participants donated a blood sample. Of this sub-cohort 1,061 participants of European descent with incident postmenopausal invasive breast cancer (138 lobular) were included in the case-only genome-wide association analysis (Hunter *et al.* 2007). All subjects were of European ancestry.

Study populations used in the replication stage

SNPs selected from the case-only genome-wide association studies for replication were evaluated using seven population-based studies (five case-control studies (CECILE, GENICA, MARIE, PBCS, SASBAC), one case-cohort (MCCS), and one nested case-control study (UKBGS)), and four non-population-based studies (MCBCS, kConFab/AOCS, OFBCR, pKARMA), including in total 7,689 cases (676 lobular) and 9,266 controls participating in the Breast Cancer Association Consortium (BCAC). Studies from BCAC were included if participants were of European descent and if genotype information, information on MHT use and information on reference age was available for at least 200 postmenopausal cases and 200 postmenopausal controls. A reference age of ≥54 years was used as surrogate for defining postmenopausal status if study derived information on menopausal status was missing. Participants of SASBAC and MARIE were excluded if they contributed already to the respective case-only genome-wide association studies. Additionally, cases in MCCS and pKARMA with prevalent breast cancer at time of enrollment were excluded. The reference date for controls was date of enrollment (MCCS) or date of interview (case-control studies). The reference date for cases was the date of

breast cancer diagnosis. The reference age was accordingly the age at reference date. In total, 7,698 cases (676 lobular) and 9,266 controls contributed to the replication analysis.

Menopausal hormone therapy exposure definition

Any type of MHT was taken into account when defining ever use of MHT. Only women using MHT more than three months were considered to be ever users. We defined current use of MHT as use within the last six months before reference date. Harmonization and plausibility checks of MHT information were conducted centrally for all studies participating in BCAC (Nickels *et al.* 2013).

Genotyping and Quality control

Genotyping was performed using the Illumina Humancnv370-duo chip (318,237 SNPs) in the MARIE study and the Illumina HumanHap550 chip I in SASBAC (500,007 SNPs), HEBCS (510,067 SNPs), and NHS (540,000 SNPs). Genotyping in NHS was part of the Cancer Genetic Markers of Susceptibility (CGEMS) project. All studies provided quality control checked genotype data.

SNPs selected for replication were genotyped on a custom Illumina iSelect genotyping array (iCOGS) that was designed by BCAC in collaboration with three other consortia (the Collaborative Oncological Gene-environment Study, COGS) (Michailidou *et al.* 2013). After genotyping the iCOGS data was centrally quality controlled, which lead to an exclusion of 56 SNPs selected for replication. We additionally excluded 9 SNPs with MAF < 0.05 in the replication dataset.

Imputation

All SNPs genotyped in the genome-wide studies, which were also contained in the HapMap phase II release 24 data, were used for imputation of additional genotypes using the software MACH 1.0.16 (Li *et al.* 2010). Employing the 'autoflip' option, the alleles are coded according to a unique reference scheme, so that the same allele was coded as reference in all four genome-wide case-only studies. For quality control of imputed data, imputed SNPs with minor allele frequency (MAF) <0.01 or r^2 <0.3 were excluded from the analysis.

SNP selection for replication

A list of 1,391 SNPs was generated based on the lowest P_{int} (cut-off P_{int} <3.0×10^{-03}) derived from the analysis of multiplicative interaction between MHT and breast cancer risk, after merging the results for overall and lobular breast cancer. A total of 3,277 SNPs were selected based on the interaction with overall breast cancer and 1,723 selected based on their association with lobular breast cancer. These SNPs were filtered according to the criteria MAF ≥0.05, *P*-value ≥0.05 for Cochran's Q or I^2 <30% and the availability of the respective SNP data in at least two case-only studies.

Statistical Analysis

We tested for multiplicative SNPxMHT interactions on the genome-wide level (2.5 million SNPs) in case-only analysis using logistic regression with MHT use (current use codes as 1,

never/past use coded as 0) as the outcome variable and the SNP as the explanatory variable. The SNP was assessed according to a log-additive genetic model, i.e. a 1 df test for trend by number of minor alleles (0, 1, or 2). Uncertainty of imputed SNPs was accounted for by using estimated genotype probabilities for imputed SNPs in the regression model. Covariates were not considered in the case-only analyses. These analyses were performed with the software ProbABEL version 0.1–2-plus (Aulchenko *et al.* 2010). Only genotyped SNPs that were also contained in the HapMap reference data and imputed SNPs were included in case-only analyses. Analyses were performed for all cases as well as separately for lobular cases. Since only individuals of European descent were included, the genomic inflation factor lambda was close to one (HEBCS $\lambda = 1.016$; MARIE $\lambda = 1.014$; SASBAC $\lambda = 1.009$) and, in case of NHS, there was also no indication for population stratification (Hunter *et al.* 2007). Therefore, the analyses were not corrected for population stratification. Combined results based on the four case-only analyses were obtained from a meta-analysis assuming a fixed effects model, using the software PLINK, version 1.07 (Purcell *et al.* 2007). Heterogeneity between studies was assessed using Cochran's Q statistic and I^2 (Higgins and Thompson 2002).

For the replication analysis, data from eleven studies were pooled and analyzed using case-control logistic regression. These analyses were performed using SAS software, version 9.2. SNPxMHT interactions were evaluated by means of a log-likelihood ratio test, comparing models with and without a multiplicative interaction term between SNP (coded according to log-additive mode of inheritance) and current use of MHT. The models were adjusted for study, reference age, former use of MHT, and six principal components to account for population stratification. The models included also interaction terms between study design (non-population-based vs. population based) and current use of MHT as well as former use of MHT. These interaction terms were included to account for possible differences in the estimates of the MHT effect according to study design.

Results from the case-only meta-analysis and the replication were combined in a meta-analysis assuming a fixed effects model, using the package "meta", version 2.1-2 (Schwarzer 2012) within the R software, version 2.15.0 (Team 2012). Linkage disequilibrium (LD) between selected SNPs was estimated in the control population of population-based studies using SNP_Tools, version 1.70 (Chen *et al.* 2009a) and Haploview, version 4.2 (Barrett *et al.* 2005).

The association between current MHT use and SNPs was assessed utilizing data of all studies of the replication stage as well as solely population-based studies. We fitted a logistic regression model adjusted for study with current MHT use as the outcome.

To illustrate the modification of overall as well as lobular breast cancer risk associated with current use of MHT by SNPs, the effect of current use of MHT was assessed in strata defined by the SNP genotype in pooled case-control data of the replication stage. The models were adjusted for study, reference age, former use of MHT and the two previously described interaction terms to account for possible differences in the estimates of the MHT effect according to study design.

To further evaluate the effect modification of current use of MHT by multiple modifying loci, a polygenic score was built for each individual. For the genetic score, we included the genetic loci found to modify the association with current use of MHT at a significance level of $P_{int} < 5.0 \times 10^{-05}$ and selected one SNP per region based on the effect estimate. The allele increasing the effect of MHT on breast cancer risk was used as risk allele and the polygenic score was derived by summing up the risk alleles (0, 1 or 2) for each SNP. Separate scores were constructed for overall and lobular breast cancer risk. To demonstrate the polygenic modifying effect, associations of current MHT use with breast cancer risk were calculated stratified by score categories (roughly quintiles for all breast cancers and tertiles for lobular tumors). The respective logistic regression models were adjusted for study, reference age, former use of MHT, an interaction term between former use of MHT and study design (non-population-based vs. population-based) and an interaction term between current use of MHT and study design.

RESULTS

Characteristics of the patients participating in the studies included in the discovery stage and the replication stage are shown in Supplementary Table 2 and Supplementary Table 3. The prevalence of current use of MHT varied between 10% and 60%. The estimated OR for the marginal effect of current use of MHT in the population-based studies of the replication stage was 1.51 (95% CI 1.21 – 1.88) for overall breast cancer and 1.83 (95% CI 1.34 – 2.47) for lobular breast cancer, as displayed in Supplementary Figure 1.

The meta-analysis of the case-only genome-wide studies did not identify any SNPxMHT interaction at genome-wide significance level (see Supplementary Figure 2 and Supplementary Figure 3, respectively, for quantile-quantile (QQ) and Manhattan plots of the results). The strongest association was observed for rs3824418 located in and intronic region of *TLE1* on 9q21.3 ($P_{int} = 6.7 \times 10^{-07}$.

Of the 1,391 SNPs carried forward for replication, 944 were selected based on their P_{int} for modification of overall breast cancer risk associated with MHT, and 447 based on their P_{int} for modification of lobular breast cancer risk. Overall, 7 SNPs showed effect modification of overall breast cancer with combined $P_{int} < 5.0 \times 10^{-05}$, as well as 7 SNPs with respect to lobular breast cancer risk (Table 1). There was no strong association between the SNPs selected for follow-up and current use of MHT, as can be seen from QQ-plots in Supplementary Figure 4. When analyzing the whole sample of the replication stage, the SNPs showing strongest association with current use of MHT were rs12538442, located in an intronic region of DOCK4 on chromosome 1 ($P = 3.1 \times 10^{-04}$), and rs17738984, located on chromosome 17q22 ($P = 8.8 \times 10^{-04}$). The SNPs showing the strongest association with MHT use when restricting the sample to population-based studies were rs10924245 in *KIF26B* ($P = 1.6 \times 10^{-04}$) and rs2102354 in *KCNN3* ($P = 7.7 \times 10^{-04}$), both located on chromosome 1.

Further information on SNPs, including their association with overall breast cancer risk in the replication dataset and MAFs in the different study populations can be found in Supplementary Table 4. The identified SNPs on each of the chromosomes 13, 14, 16, 17 and

18 are located in close proximity to each other and do not represent independent signals of genetic modification of the MHT effect. The respective LD plots can be found in Supplementary Figure 5.

For overall breast cancer risk, two SNPs (rs9578047 and rs9579199) near *POMP* on chromosome 13 showed an interaction with current use of MHT with combined P_{int} = 7.9×10^{-06} and 7.6×10^{-06}. SNPxMHT interactions with P_{int} of similar magnitude were also observed with rs7148646 and rs848694 in *SLC25A21* on chromosome 14 (combined P_{int} = 1.2×10^{-05} and 4.6×10^{-05}) and three SNPs (rs7192724, rs17202296, and rs4888190) in *PLCG2* on chromosome 16 (combined P_{int} = 3.3×10^{-06}, 2.8×10^{-05} and 4.5×10^{-05}) (Table 1). Associations between current use of MHT and overall breast cancer risk stratified by genotypes of these SNPs are displayed in Table 2. We did not observe significantly heterogeneous SNPxMHT interactions between studies that were pooled in the replication stage (P_{het} ranging from 0.16 to 0.91). The respective forest plots are shown in Supplementary Figure 6.

We combined rs7148646_G, rs9579199_G, and rs7192724_G and constructed a polygenic score to assess breast cancer risk associated with current use of MHT depending on the modifying genetic risk (number of risk modifying alleles) (Figure 2A). For women with a low polygenic score of two or less risk modifying alleles (16.7% of women), current use of MHT was not associated with an increased breast cancer risk (OR = 1.04, 95% CI 0.87 – 1.25). Among women carrying three (30.9% of women) or four (34.2% of women) risk modifying alleles, current use of MHT was associated with a significantly increased breast cancer risk (OR = 1.25, 95% CI 1.08 – 1.45 and OR = 1.57, 95% CI 1.36 – 1.80, respectively). The strongest association between current use of MHT and breast cancer risk was observed for women with a polygenic score of five or six (18.2% of women) (OR = 1.86, 95% CI 1.56 – 2.22), as expected.

With respect to lobular breast cancer, the variant rs11680872 located in an intronic region of *TMEFF2* on chromosome 2 showed a SNPxMHT interaction with combined P_{int} = 2.9×10^{-05} (Table 3). Also, rs7648642 in *CD80* on chromosome 3 modified MHT associated lobular breast cancer risk in both case-only and case-control analysis (combined P_{int} = 5.1×10^{-06}). The variants rs11654964, rs16970162 and rs11080292 located near *TMEM132E* on chromosome 17 yielded combined P_{int} of 2.7×10^{-06}, 8.6×10^{-06} and 9.3×10^{-06}, respectively. Further SNPxMHT interactions were observed for rs6506940 and rs594334 near *SLC25A52* on chromosome 18 (combined P_{int} = 1.2×10^{-05} and 3.4×10^{-05}, respectively) (Table 1). Table 2 shows the associations between current use of MHT and lobular breast cancer risk in strata defined by genotypes of these SNPs. There was no significant heterogeneity by study in the estimates for SNPxMHT interactions (P_{het} ranging from 0.22 to 0.94). The respective forest plots are shown in Supplementary Figure 7.

For lobular breast cancer risk, a polygenic score was constructed by combining rs11680872_A, rs7648642_C, rs11654964_A, and rs6506940_A (Figure 2B). Current use of MHT was not associated with increased lobular breast cancer risk in women carrying zero to four risk modifying alleles (15.2% of women, OR = 0.61, 95% CI 0.35 – 1.07), while the OR for lobular breast cancer risk was 1.64 (95% CI 1.18 – 2.26) in the subgroup (25.5% of

women) carrying five risk modifying alleles. The association with current MHT use increased with the polygenic score and the odds ratio for lobular breast cancer was 2.20 (95% CI 1.66 – 2.92) in women with a polygenic score of six (30.9% of women) and 2.38, 95% CI 1.78 – 3.18) in those carrying seven or eight risk modifying alleles (25.8% of women).

DISCUSSION

Using a two stage approach consisting of a meta-analysis of four case-only genome-wide association studies and a replication analysis in independent data of ten case-control studies, we attempted to identify SNPs that modify the effect of MHT use on breast cancer risk. Despite our large sample size in both discovery and replication stages, we did not identify any SNPs that reached genome-wide significance. We observed three loci on chromosome 13, 14 and 16 that modified MHT associated overall breast cancer risk in both the discovery and replication stage, with combined $P_{int} < 5.0 \times 10^{-05}$. Additionally, four genomic loci on chromosome 2, 3, 17 and 18 modified lobular breast cancer risk associated with MHT use in both study stages at the same significance level.

When combining variants that modify the effect of current use of MHT in a polygenic score, current MHT use was associated with an 86% increased breast cancer risk among women with five to six risk modifying alleles (18.2% of all women included in the study) and was not associated with breast cancer risk among women carrying two or less (16.7% of women). Women with a polygenic score of three or four that were currently using MHT were at an intermediate increased risk of breast cancer. Similar results were observed for lobular breast cancer risk. Given the observed interactions are confirmed, the polygenic score illustrates that even though the single detected interactions might be modest, the associated breast cancer risk for a woman using MHT may be appreciably increased if she carries a large number of adverse risk modifying alleles.

The loci of the identified polymorphisms provide indication of possible biological relevance for breast carcinogenesis. Two variants rs9579199 and rs9578047 close to *FLT1* and *POMP* on chromosome 13 showed modifying effects on MHT associated breast cancer risk. FLT1 is a vascular endothelial growth factor receptor and involved in tumor angiogenesis (Fischer *et al.* 2008). So far, no association has been reported between tumor development and POMP, a proteasome maturation protein. The variants on chromosome 14 (rs7148646, rs848694) lie in an intronic region of *SLC25A21*. *SLC25A21* encodes an oxodicarboxylate carrier, which transports C5–C7 oxodicarboxylates across the inner membranes of mitochondria (Fiermonte *et al.* 2001). Interestingly, the two SNPs rs6506940 and rs594334 found to modify risk of lobular breast cancer are located near another mitochondrial carrier gene, *SLC25A52*, on chromosome 18. Estrogen has been reported to be an important regulator of mitochondrial function (Chen *et al.* 2009b) and results of the present study suggest that mitochondrial related mechanisms may play a role in MHT associated breast carcinogenesis. The association between MHT and breast cancer was also modified by rs7192724, rs17202296 and rs4888190 located in intronic regions of *PLCG2*. PLCG2 is a member of the phosphoinositide-specific phospholipase C family and is involved in

transmitting activation signals across the cell membranes predominantly of B cells (Wang *et al.* 2000) as well as natural killer cells (Tassi *et al.* 2005).

With respect to lobular breast cancer, genetic variants in two transmembrane proteins were implicated. rs11680872 is located in an intron of *TMEFF2,* whose biological function is unclear but its promoter region has been commonly found to be hypermethylated in various cancers, including breast cancer (Lin *et al.* 2011; Park *et al.* 2011). Three variants (rs11654964, rs16970162 and rs11080292) are near (23–32kb 3′) *TMEM132E*, another transmembrane protein. We observed also an interaction with rs7648642, which lies in an intron of *CD80* on chromosome 3. CD80 is known to play an important role in T cell activation (Bhatia *et al.* 2006), and its expression has been found to be decreased in peripheral blood of breast cancer patients (Gong *et al.* 2012).

To account for differences by country with respect to types of preparations and dosages as well as different genotype platforms and laboratories, we used meta-analysis to combine the results in the discovery stage and adjusted for study in the replication stage. Heterogeneity of estimates regarding MHT use between studies due to differences in the study design were in part accounted for by adding an interaction term between MHT use and study design in the regression models (Supplementary Figure 1). The sensitivity analysis restricted to solely population-based studies of the replication stage for selected SNPxMHT interactions did not yield substantially altered estimates for interaction (less than 7.5% for overall breast cancer and less than 11.5% for lobular breast cancer, Supplementary Table 5). Utilizing the total study population of the replication stage, the study had 80% power to detect an interaction effect of 1.20, assuming an allele frequency of 20%, a marginal genetic effect of 1.15 and a marginal effect of current MHT use of 1.35. The power was reduced to 55% when restricting the sample to population-based studies. Furthermore, although the associations between current MHT use and breast cancer risk observed in the single studies were heterogeneous, this was not the case for the SNPxMHT interactions (Supplementary Figure 6 and 7). In general, estimates for gene-environment interaction are unlikely to be affected by selection bias (Morimoto *et al.* 2003) and more likely to be underestimated in the presence of non-differential or differential misclassification (Garcia-Closas *et al.* 1999).

Most of the reported genetic modifiers of MHT associated breast cancer risk have so far not been followed up in further studies (Justenhoven *et al.* 2012). One exception is with respect to variants in *FGFR2*, since it is also a know breast cancer susceptibility loci. Rebbeck et al. reported that the association between combined estrogen-progestogen therapy and breast cancer risk was modified by rs1219648 in postmenopausal women of European descent (P_{int} = 0.010) (Rebbeck *et al.* 2009). A study conducted in participants of the Women's Health Initiative trial could not replicate this finding (P_{int} = 0.661) but observed an interaction with rs3750817 in *FGFR2* (P_{int} = 0.033) (Prentice *et al.* 2009). A similar modifying effect of this SNP was also observed for estrogen-monotherapy (P_{int} = 0.046). The variants rs1219648 and rs3750817 are in moderate LD (r^2 = 0.44, D′ = 1.00). However, we did not observe an interaction regarding breast cancer risk with current use of any MHT and rs1219648 (P_{int} = 0.15) or rs3750817 (P_{int} = 0.23) in the genome-wide association study and the variants were not followed up in the replication stage. Similarly, no significant interactions were observed

with *FGRR2* variants in more recent studies (Andersen *et al.* 2012; Campa *et al.* 2011; Nickels *et al.* 2013).

Genome-wide studies of gene-environment interactions present challenges since the required sample size maybe inflated due to misclassification of environmental exposures and additional factors involved including effect size of gene-environment interaction and prevalence of environmental exposure(s) (Dempfle *et al.* 2008; Zondervan and Cardon 2004). To optimize power, we used the case-only approach in the discovery stage, which offers greater precision in estimating the interaction term, and the case-control approach in the replication stage to account for false positive results due to correlation of the environmental exposure with the genetic marker in the population (Piegorsch *et al.* 1994). There was no indication of strong associations between SNPs selected for follow-up and current use of MHT (Supplementary Figure 4), supporting the assumption of gene-environment independence. We minimized possible spurious associations due to differences in allele frequencies in the underlying populations by restriction to solely individuals of European descent. The observed genomic inflation in the case-only studies was close to one and the case-control analyses were controlled for population stratification by including genetic principal components.

In conclusion, the association between current use of MHT and risk of overall and lobular breast cancer is potentially modified by genetic variants in genes related to mitochondrial solute carriers, transmembrane signaling as well as immune cell activation. These findings need replication in independent studies of adequate power. The identified modest interaction effects are presently unlikely to be of clinical significance, but provide valuable insight into potential mechanisms of breast cancer development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Andersen SW, Trentham-Dietz A, Figueroa JD, Titus LJ, Cai Q, Long J, Hampton JM, Egan KM, Newcomb PA. Breast cancer susceptibility associated with rs1219648 (fibroblast growth factor receptor 2) and postmenopausal hormone therapy use in a population-based United States study. Menopause. 2012

Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. BMC Bioinformatics. 2010; 11:134. [PubMed: 20233392]

Bakken K, Fournier A, Lund E, Waaseth M, Dumeaux V, Clavel-Chapelon F, Fabre A, Hemon B, Rinaldi S, Chajes V, et al. Menopausal hormone therapy and breast cancer risk: impact of different treatments. The European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2011; 128:144–156. [PubMed: 20232395]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

Bhatia S, Edidin M, Almo SC, Nathenson SG. B7-1 and B7-2: similar costimulatory ligands with different biochemical, oligomeric and signaling properties. Immunol Lett. 2006; 104:70–75. [PubMed: 16413062]

Campa D, Kaaks R, Le Marchand L, Haiman CA, Travis RC, Berg CD, Buring JE, Chanock SJ, Diver WR, Dostal L, et al. Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium. J Natl Cancer Inst. 2011; 103:1252–1263. [PubMed: 21791674]

Chen B, Wilkening S, Drechsel M, Hemminki K. SNP_tools: A compact tool package for analysis and conversion of genotype data for MS-Excel. BMC Res Notes. 2009a; 2:214. [PubMed: 19852806]

Chen JQ, Cammarata PR, Baines CP, Yager JD. Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications. Biochim Biophys Acta. 2009b; 1793:1540–1570. [PubMed: 19559056]

Chlebowski RT, Anderson GL. Changing concepts: Menopausal hormone therapy and breast cancer. J Natl Cancer Inst. 2012; 104:517–527. [PubMed: 22427684]

Dempfle A, Scherag A, Hein R, Beckmann L, Chang-Claude J, Schafer H. Gene-environment interactions for complex traits: definitions, methodological requirements and challenges. Eur J Hum Genet. 2008; 16:1164–1172. [PubMed: 18523454]

Farquhar C, Marjoribanks J, Lethaby A, Suckling JA, Lamberts Q. Long term hormone therapy for perimenopausal and postmenopausal women. Cochrane Database Syst Rev. 2009:CD004143. [PubMed: 19370593]

Fiermonte G, Dolce V, Palmieri L, Ventura M, Runswick MJ, Palmieri F, Walker JE. Identification of the human mitochondrial oxodicarboxylate carrier. Bacterial expression, reconstitution, functional characterization, tissue distribution, and chromosomal location. J Biol Chem. 2001; 276:8225–8230. [PubMed: 11083877]

Fischer C, Mazzone M, Jonckx B, Carmeliet P. FLT1 and its ligands VEGFB and PlGF: drug targets for anti-angiogenic therapy? Nat Rev Cancer. 2008; 8:942–956. [PubMed: 19029957]

Flesch-Janys D, Slanger T, Mutschelknauss E, Kropp S, Obi N, Vettorazzi E, Braendle W, Bastert G, Hentschel S, Berger J, et al. Risk of different histological types of postmenopausal breast cancer by type and regimen of menopausal hormone therapy. Int J Cancer. 2008; 123:933–941. [PubMed: 18506692]

Garcia-Closas M, Rothman N, Lubin J. Misclassification in case-control studies of gene-environment interactions: assessment of bias and sample size. Cancer Epidemiol Biomarkers Prev. 1999; 8:1043–1050. [PubMed: 10613335]

Gong J, Pan W, Yang C, Guo F, Sun Y. Expression of co-stimulators CD28/B7-1 in peripheral blood of patients with breast cancer. Breast Cancer Res Treat. 2012; 136:621–622. [PubMed: 23053653]

Hein R, Abbas S, Seibold P, Salazar R, Flesch-Janys D, Chang-Claude J. Polymorphism Thr160Thr in SRD5A1, involved in the progesterone metabolism, modifies postmenopausal breast cancer risk associated with menopausal hormone therapy. Breast Cancer Res Treat. 2012; 131:653–661. [PubMed: 21947678]

Hein R, Flesch-Janys D, Dahmen N, Beckmann L, Lindstrom S, Schoof N, Czene K, Mittelstrass K, Illig T, Seibold P, et al. A genome-wide association study to identify genetic susceptibility loci that modify ductal and lobular postmenopausal breast cancer risk associated with menopausal hormone therapy use: a two-stage design with replication. Breast Cancer Res Treat. 2013

Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21:1539–1558. [PubMed: 12111919]

Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE, Wacholder S, Wang Z, Welch R, Hutchinson A, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet. 2007; 39:870–874. [PubMed: 17529973]

Justenhoven C, Obazee O, Brauch H. The pharmacogenomics of sex hormone metabolism: breast cancer risk in menopausal hormone therapy. Pharmacogenomics. 2012; 13:659–675. [PubMed: 22515609]

Kawase T, Matsuo K, Suzuki T, Hiraki A, Watanabe M, Iwata H, Tanaka H, Tajima K. FGFR2 intronic polymorphisms interact with reproductive risk factors of breast cancer: results of a case control study in Japan. Int J Cancer. 2009; 125:1946–1952. [PubMed: 19582883]

Kilpivaara O, Vahteristo P, Falck J, Syrjakoski K, Eerola H, Easton D, Bartkova J, Lukas J, Heikkila P, Aittomaki K, et al. CHEK2 variant I157T may be associated with increased breast cancer risk. Int J Cancer. 2004; 111:543–547. [PubMed: 15239132]

Lee E, Schumacher F, Lewinger JP, Neuhausen SL, Anton-Culver H, Horn-Ross PL, Henderson KD, Ziogas A, Van Den Berg D, Bernstein L, et al. The association of polymorphisms in hormone metabolism pathway genes, menopausal hormone therapy, and breast cancer risk: a nested case-control study in the California Teachers Study cohort. Breast Cancer Res. 2011; 13:R37. [PubMed: 21457551]

Li J, Humphreys K, Heikkinen T, Aittomaki K, Blomqvist C, Pharoah PD, Dunning AM, Ahmed S, Hooning MJ, Martens JW, et al. A combined analysis of genome-wide association studies in breast cancer. Breast Cancer Res Treat. 2011; 126:717–727. [PubMed: 20872241]

Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: Using Sequence and Genotype Data to Estimate Haplotypes and Unobserved Genotypes. Genet Epidemiol. 2010; 34:816–834. [PubMed: 21058334]

Lin K, Taylor JR Jr, Wu TD, Gutierrez J, Elliott JM, Vernes JM, Koeppen H, Phillips HS, de Sauvage FJ, Meng YG. TMEFF2 is a PDGF-AA binding protein with methylation-associated gene silencing in multiple cancer types including glioma. PLoS One. 2011; 6:e18608. [PubMed: 21559523]

Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, Schmidt MK, Chang-Claude J, Bojesen SE, Bolla MK, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013

Morimoto LM, White E, Newcomb PA. Selection bias in the assessment of gene-environment interaction in case-control studies. Am J Epidemiol. 2003; 158:259–263. [PubMed: 12882948]

Narod SA. Hormone replacement therapy and the risk of breast cancer. Nat Rev Clin Oncol. 2011; 8:669–676. [PubMed: 21808267]

Nickels S, Truong T, Hein R, Stevens K, Buck K, Behrens S, Eilber U, Schmidt M, Haberle L, Vrieling A, et al. Evidence of Gene-Environment Interactions between Common Breast Cancer Susceptibility Loci and Established Environmental Risk Factors. PLoS Genet. 2013; 9:e1003284. [PubMed: 23544014]

Park SY, Kwon HJ, Lee HE, Ryu HS, Kim SW, Kim JH, Kim IA, Jung N, Cho NY, Kang GH. Promoter CpG island hypermethylation during breast cancer progression. Virchows Arch. 2011; 458:73–84. [PubMed: 21120523]

Piegorsch WW, Weinberg CR, Taylor JA. Non-hierarchical logistic models and case-only designs for assessing susceptibility in population-based case-control studies. Stat Med. 1994; 13:153–162. [PubMed: 8122051]

Prentice RL, Huang Y, Hinds DA, Peters U, Pettinger M, Cox DR, Beilharz E, Chlebowski RT, Rossouw JE, Caan B, et al. Variation in the FGFR2 gene and the effects of postmenopausal hormone therapy on invasive breast cancer. Cancer Epidemiol Biomarkers Prev. 2009; 18:3079–3085. [PubMed: 19861516]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]

Rebbeck TR, DeMichele A, Tran TV, Panossian S, Bunin GR, Troxel AB, Strom BL. Hormone-dependent effects of FGFR2 and MAP3K1 in breast cancer susceptibility in a population-based sample of post-menopausal African-American and European-American women. Carcinogenesis. 2009; 30:269–274. [PubMed: 19028704]

Schwarzer G. meta: Meta-Analysis with R. 2012

Shah NR, Borenstein J, Dubois RW. Postmenopausal hormone therapy and breast cancer: a systematic review and meta-analysis. Menopause. 2005; 12:668–678. [PubMed: 16278609]

Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. N Engl J Med. 2003; 348:2431–2442. [PubMed: 12802030]

Sprague BL, Trentham-Dietz A, Cronin KA. A Sustained Decline in Postmenopausal Hormone Use Results From the National Health and Nutrition Examination Survey, 1999–2010. Obstet Gynecol. 2012; 120:595–603. [PubMed: 22914469]

Tassi I, Presti R, Kim S, Yokoyama WM, Gilfillan S, Colonna M. Phospholipase C-gamma 2 is a critical signaling mediator for murine NK cell activating receptors. J Immunol. 2005; 175:749–754. [PubMed: 16002670]

R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2012.

Travis RC, Reeves GK, Green J, Bull D, Tipper SJ, Baker K, Beral V, Peto R, Bell J, Zelenika D, et al. Gene-environment interactions in 7610 women with breast cancer: prospective evidence from the Million Women Study. Lancet. 2010; 375:2143–2151. [PubMed: 20605201]

Tsai SA, Stefanick ML, Stafford RS. Trends in menopausal hormone therapy use of US office-based physicians, 2000–2009. Menopause. 2011; 18:385–392. [PubMed: 21127439]

Wang D, Feng J, Wen R, Marine JC, Sangster MY, Parganas E, Hoffmeyer A, Jackson CW, Cleveland JL, Murray PJ, et al. Phospholipase Cgamma2 is essential in the functions of B cell and several Fc receptors. Immunity. 2000; 13:25–35. [PubMed: 10933392]

Wedren S, Lovmar L, Humphreys K, Magnusson C, Melhus H, Syvanen AC, Kindmark A, Landegren U, Fermer ML, Stiger F, et al. Oestrogen receptor alpha gene haplotype and postmenopausal breast cancer risk: a case control study. Breast Cancer Res. 2004; 6:R437–449. [PubMed: 15217512]

Zondervan KT, Cardon LR. The complex interplay among factors that influence allelic association. Nat Rev Genet. 2004; 5:89–100. [PubMed: 14735120]

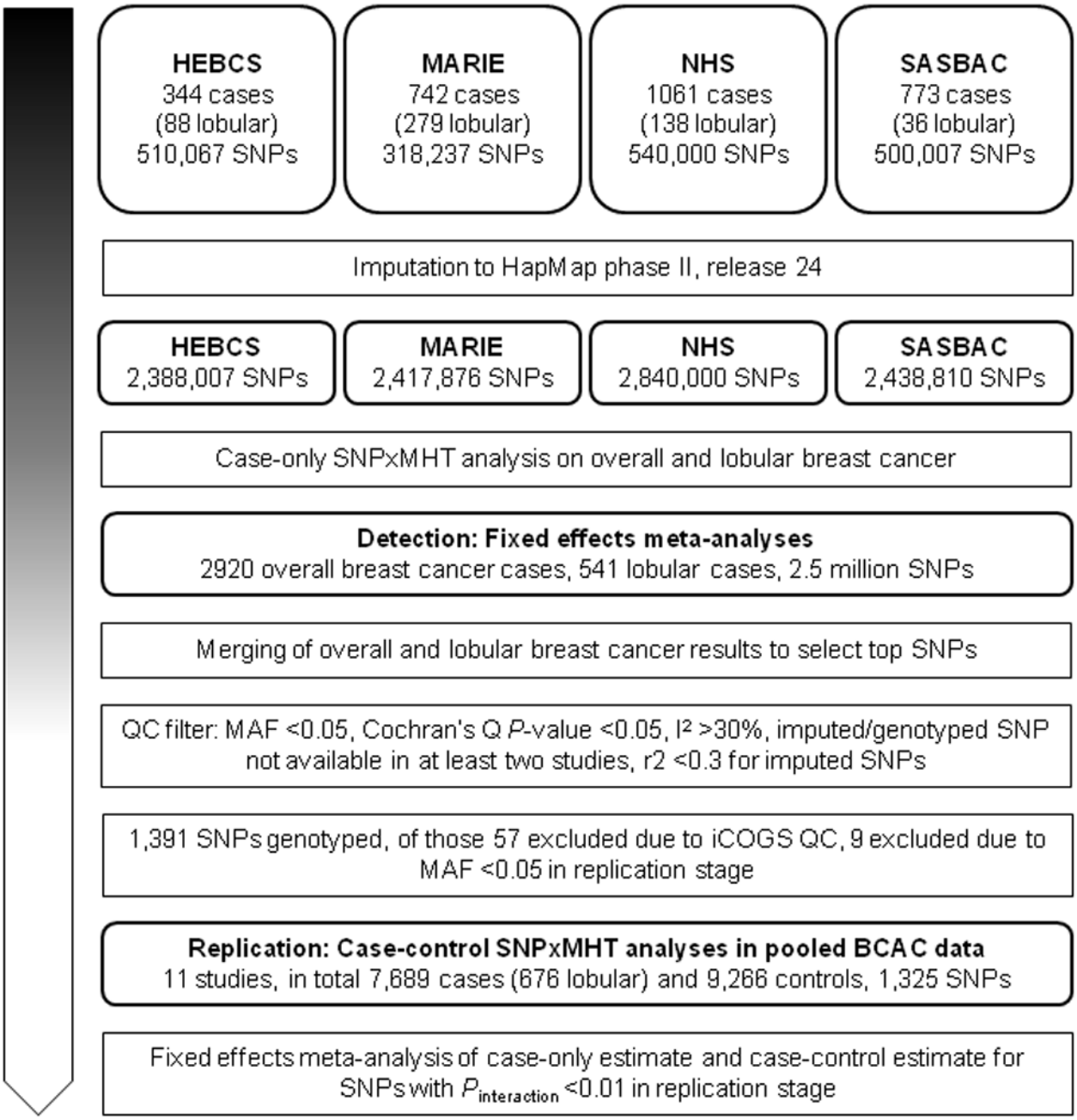

Figure 1.
Diagram describing numbers of participants, investigated SNPs and conducted analyses at each stage

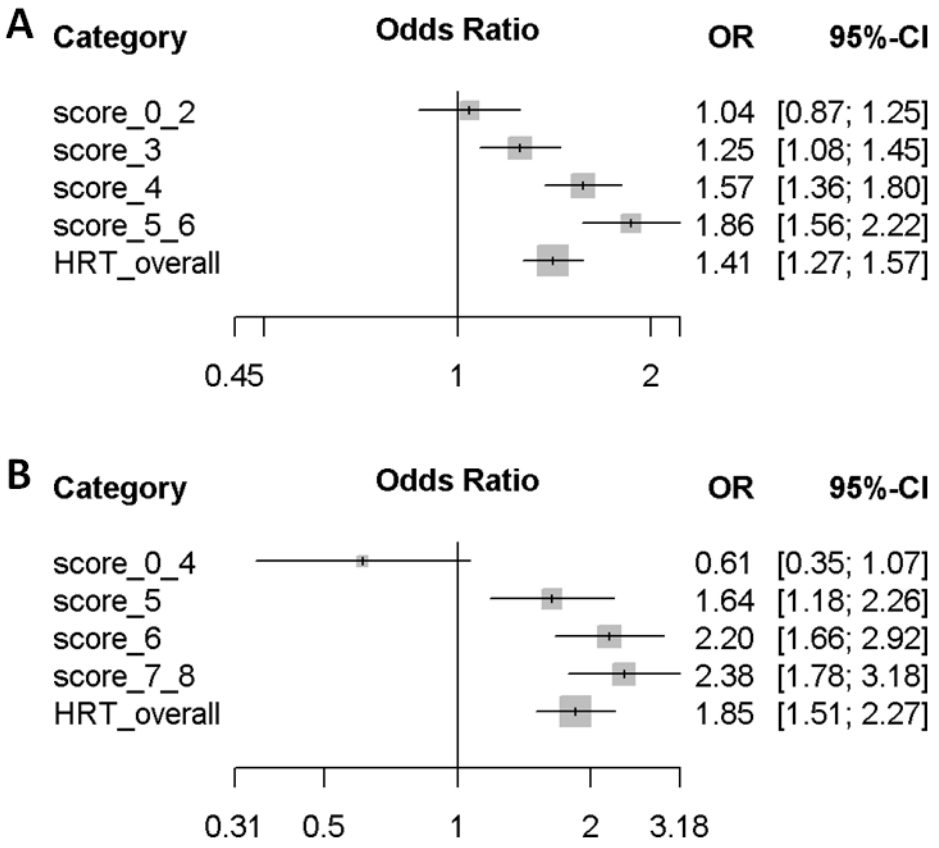

Figure 2.

Odd ratios for (A) overall breast cancer and (B) lobular breast cancer risk associated with current use of menopausal hormone therapy in categories defined by polygenic scores

Table 1

Odds ratios of multiplicative interaction between current menopausal hormone therapy use and SNPs for overall and lobular breast cancer risk

SNP	Chr	Position (build 37)	RefSeq Gene	Feature	GWAS[a]		REPLICATION		COMBINED[c]	
					OR (95% CI)	P_{int}	OR (95% CI)[b]	P_{int}	OR (95% CI)	P_{int}
Overall breast cancer										
rs9578047	13	29164731	68kb 5′ of POMP	-	0.81 (0.72 – 0.91)	6.3×10^{-04}	0.84 (0.75 – 0.95)	3.6×10^{-03}	0.83 (0.76 – 0.90)	7.9×10^{-06}
rs9579199	13	29164783	68kb 5′ of POMP	-	0.81 (0.72 – 0.91)	6.3×10^{-04}	0.84 (0.75 – 0.95)	3.4×10^{-03}	0.83 (0.76 – 0.90)	7.6×10^{-06}
rs7148646	14	37407036	SLC25A21	intronic	0.80 (0.70 – 0.90)	3.7×10^{-04}	0.85 (0.76 – 0.96)	7.4×10^{-03}	0.83 (0.76 – 0.90)	1.2×10^{-05}
rs848694	14	37371724	SLC25A21	intronic	0.80 (0.70 – 0.91)	9.4×10^{-04}	0.86 (0.76 – 0.97)	1.3×10^{-02}	0.83 (0.76 – 0.91)	4.6×10^{-05}
rs7192724	16	81958298	PLCG2	intronic	1.24 (1.09 – 1.42)	1.3×10^{-03}	1.24 (1.09 – 1.40)	7.8×10^{-04}	1.24 (1.13 – 1.36)	3.3×10^{-06}
rs17202296	16	81959191	PLCG2	intronic	1.28 (1.13 – 1.46)	1.4×10^{-04}	1.14 (1.01 – 1.29)	2.9×10^{-02}	1.21 (1.10 – 1.32)	2.8×10^{-05}
rs4888190	16	81963618	PLCG2	intronic	1.32 (1.16 – 1.5)	3.8×10^{-05}	1.11 (0.99 – 1.26)	8.0×10^{-02}	1.20 (1.10 – 1.31)	4.5×10^{-05}
Lobular breast cancer										
rs11680872	2	192830249	*TMEFF2*	intronic	2.01 (1.40 – 2.87)	1.5×10^{-04}	1.40 (1.05 – 1.87)	1.9×10^{-02}	1.61 (1.29 – 2.01)	2.9×10^{-05}
rs7648642	3	119261375	*CD80*	intronic	0.58 (0.43 – 0.79)	4.6×10^{-04}	0.68 (0.54 – 0.87)	2.3×10^{-03}	0.64 (0.53 – 0.78)	5.1×10^{-06}
rs11654964	17	32989538	23kb 3′ of *TMEM132E*		1.76 (1.30 – 2.38)	2.7×10^{-04}	1.58 (1.17 – 2.14)	2.4×10^{-03}	1.67 (1.35 – 2.06)	2.7×10^{-06}
rs16970162	17	32989786	23kb 3′ of *TMEM132E*		1.85 (1.34 – 2.54)	1.5×10^{-04}	1.49 (1.09 – 2.05)	1.1×10^{-02}	1.66 (1.33 – 2.07)	8.6×10^{-06}
rs11080292	17	32998577	32kb 3′ of *TMEM132E*		1.77 (1.29 – 2.44)	4.6×10^{-04}	1.57 (1.14 – 2.15)	4.9×10^{-03}	1.66 (1.33 – 2.08)	9.3×10^{-06}
rs6506940	18	29333635	6kb 3′ of *SLC25A52*		2.06 (1.35 – 3.13)	7.2×10^{-04}	1.80 (1.19 – 2.71)	4.0×10^{-03}	1.92 (1.43 – 2.58)	1.2×10^{-05}
rs594334	18	29364523	24kb 5′ of *SLC25A52*		1.92 (1.25 – 2.94)	3.0×10^{-03}	1.85 (1.22 – 2.81)	3.0×10^{-03}	1.88 (1.40 – 2.54)	3.4×10^{-05}

[a] fixed effects meta analysis of results from four case-only GWAS

[b] adjusted for study, reference age, former use of menopausal hormone therapy, interaction terms between study design (population-bases vs. non-population-based) and former use and current of menopausal hormone therapy, as well as genetic principal components

[c] fixed effects meta-analysis of results from GWAS and replication analysis

Table 2

Association between current use of menopausal hormone therapy and overall as well as lobular breast cancer risk stratified by genotype

SNP	Alleles[a]		Homozygous reference		Heterozygous		Homozygous coded	
	Reference	Coded	OR (95% CI)[b]	*P*	OR (95% CI)[b]	*P*	OR (95% CI)[b]	*P*
Overall breast cancer								
rs9578047	A	G	1.91 (1.46 – 2.50)	2.0×10^{-06}	1.50 (1.30 – 1.72)	1.3×10^{-08}	1.30 (1.14 – 1.48)	6.4×10^{-05}
rs9579199	G	A	1.92 (1.47 – 2.51)	1.8×10^{-06}	1.50 (1.30 – 1.72)	1.4×10^{-08}	1.30 (1.14 – 1.48)	6.5×10^{-05}
rs7148646	G	A	1.58 (1.39 – 1.80)	1.9×10^{-12}	1.24 (1.08 – 1.43)	2.6×10^{-03}	1.31 (1.10 – 1.73)	5.2×10^{-02}
rs848694	G	A	1.55 (1.37 – 1.75)	3.2×10^{-12}	1.23 (1.06 – 1.43)	5.8×10^{-03}	1.34 (0.97 – 1.85)	7.5×10^{-02}
rs7192724	C	G	1.10 (0.78 – 1.54)	5.9×10^{-01}	1.25 (1.09 – 1.44)	1.9×10^{-03}	1.58 (1.40 – 1.80)	7.8×10^{-13}
rs17202296	G	T	1.19 (0.88 – 1.62)	2.5×10^{-01}	1.32 (1.15 – 1.51)	1.1×10^{-04}	1.53 (1.35 – 1.74)	7.0×10^{-11}
rs4888190	C	G	1.22 (0.89 – 1.66)	2.1×10^{-01}	1.36 (1.18 – 1.57)	1.5×10^{-05}	1.52 (1.34 – 1.73)	1.3×10^{-10}
Lobular breast cancer								
rs11680872	G	A	1.33 (0.64 – 2.76)	4.5×10^{-01}	1.42 (1.04 – 1.94)	2.6×10^{-02}	2.19 (1.70 – 2.82)	1.3×10^{-09}
rs7648642	C	A	3.05 (2.10 – 4.42)	4.1×10^{-09}	1.62 (1.23 – 2.14)	6.0×10^{-04}	1.43 (1.01 – 2.02)	4.3×10^{-02}
rs11654964	C	A	0.24 (0.06 – 1.04)	5.7×10^{-02}	1.61 (1.18 – 2.19)	2.8×10^{-03}	2.15 (1.68 – 2.76)	1.7×10^{-09}
rs16970162	C	G	0.33 (0.07 – 1.47)	1.5×10^{-01}	1.57 (1.14 – 2.17)	6.3×10^{-03}	2.09 (1.64 – 2.66)	3.0×10^{-09}
rs11080292	T	C	0.38 (0.08 – 1.70)	2.0×10^{-01}	1.47 (1.06 – 2.03)	2.1×10^{-02}	2.15 (1.69 – 2.74)	6.5×10^{-10}
rs6506940	G	A	0.94 (0.10 – 8.90)	9.6×10^{-01}	1.08 (0.72 – 1.61)	7.2×10^{-01}	2.05 (1.63 – 2.58)	7.4×10^{-10}
rs594334	C	T	1.40 (0.24 – 7.98)	7.1×10^{-01}	1.03 (0.68 – 1.58)	8.7×10^{-01}	2.09 (1.67 – 2.61)	1.6×10^{-10}

[a] the coded allele was not necessarily the minor allele.

[b] adjusted for study, reference age, former use of menopausal hormone therapy, interaction between former use of menopausal hormone therapy and study design (non-population-based vs. population-based) and interaction between current use of menopausal hormone therapy and study design