Full title: Physical activity, sedentary time and breast cancer risk: A Mendelian randomization study

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ABSTRACT

Objectives: Physical inactivity and sedentary behaviour are associated with higher breast cancer risk in observational studies, but ascribing causality is difficult. Mendelian randomization (MR) assesses causality by simulating randomized trial groups using genotype. We assessed whether lifelong physical activity or sedentary time, assessed using genotype, may be causally associated with breast cancer risk overall, pre/post-menopause, and by case-groups defined by tumour characteristics.

Methods: We performed two-sample inverse-variance-weighted MR using individual-level Breast Cancer Association Consortium case-control data from 130,957 European-ancestry women (69,838 invasive cases), and published UK Biobank data (n=91,105-377,234). Genetic instruments were single nucleotide polymorphisms (SNPs) associated in UK Biobank with wrist-worn accelerometer-measured overall physical activity (n_{snps}=5) or sedentary time (n_{snps}=6), or accelerometer-measured (n_{snps}=1) or self-reported (n_{snps}=5) vigorous physical activity.

Results: Greater genetically-predicted overall activity was associated with lower breast cancer risk, overall (OR=0.59; 95%CI 0.42-0.83 per-standard deviation [SD; ~8 milligravities acceleration]) and for most case-groups. Genetically-predicted vigorous activity was associated with lower risk of pre/perimenopausal breast cancer (OR=0.62; 95%CI 0.45-0.87, ≥3 vs. 0 self-reported days/week), with consistent estimates for most case-groups. Greater genetically-predicted sedentary time was associated with higher hormone-receptor-negative tumour risk (OR=1.77; 95%CI 1.07-2.92 per-SD [~7% time spent sedentary]), with elevated estimates for most case-groups. Results were robust to sensitivity analyses examining pleiotropy (including weighted-median-MR, MR-Egger).

Conclusion: Our study provides strong evidence that greater overall physical activity, greater vigorous activity, and lower sedentary time are likely to reduce breast cancer risk. More widespread adoption of active lifestyles may reduce the burden from the most common cancer in women.

Keywords: Breast cancer; Physical activity; Sedentary time; Mendelian randomization; Causal inference

KEY MESSAGES

What is already known on this topic:

- Observational studies have reported that active lifestyles are associated with lower breast cancer risk, but whether activity is the protective (causative) factor cannot be conclusively determined from observational evidence.

What this study adds:

- This study, using individual-level data from the Breast Cancer Association Consortium, provides strong evidence that greater levels of physical activity and less sedentary time are likely to reduce breast cancer risk, with results generally consistent across breast cancer subtypes.
- A systematic Mendelian randomization approach enhanced the ability to draw causal conclusions by minimising the effect of biases such as confounding, which are likely to have affected previous studies.

How this study might affect research, practice or policy:

- Upon triangulating multiple evidence types, there is now robust evidence that insufficiently active lifestyles are a modifiable cause of breast cancer risk, and a

stronger focus on promoting active lifestyles is likely to reduce the high burden from breast cancer.

- It would be of public health benefit for physical activity researchers to establish whether Mendelian randomization supports the observational findings regarding active lifestyles and cancer risk for other cancer types.

Introduction

Greater physical activity and less sedentary time are associated with lower breast cancer risk in observational studies. International and national cancer agencies have concluded that physical activity may reduce breast cancer risk, particularly postmenopausal disease, with associations strongest for vigorous activity.(1-3) Sedentary (sitting/reclining) time, a distinct exposure affecting 'active' and 'inactive' people, has been less well-studied, with conflicting findings.(4, 5) Physical inactivity or excess sitting may plausibly influence breast cancer initiation and/or growth. However, whether observed associations are causal or produced by biases (e.g. confounding, selection bias, reverse causation) is unclear. Mendelian randomization (MR) can simulate randomized controlled trials using observational data by substituting genotypes, which are randomly assigned at meiosis (before conception), as instruments (proxies) for exposures of interest.(6) Subject to meeting specific assumptions of instrumental variable analysis,(7) some of which can be investigated using sensitivity analyses (see Methods), MR can minimise confounding and reverse causation, potentially providing stronger evidence for causal inference.

A recent MR study assessed physical activity and breast cancer risk overall and by oestrogen-receptor (ER) status,(8) but did not examine other breast tumour types, vigorous activity, or sedentary time. We aimed to appraise the causal nature of associations between overall activity, vigorous activity, and sedentary time, and breast cancer risk, overall and by menopausal status, stage, grade, morphology, and molecular subtypes defined by hormone-receptor (ER, progesterone [PR]) and human epidermal growth factor receptor-2 (HER2) status.

Methods

Data sources

We performed two-sample MR using individual-level data from 130,957 European-ancestry women (69,838 with invasive breast cancers; 6,667 with in situ breast cancers; 54,452 controls) from 76 Breast Cancer Association Consortium (BCAC) studies (Tables 1, S1)(outcome dataset), and genetic estimates for movement-related exposures from published genome-wide association studies (GWAS) using UK Biobank data (exposure datasets; n=91,105-377,234).(9-11) Instruments were single-nucleotide polymorphisms (SNPs) associated in the UK Biobank GWAS with overall physical activity (all movement), vigorous physical activity, or sedentary time (Table S2).

Exposures

Overall physical activity

As our primary physical activity instrument we used five SNPs associated with overall activity ($p<5x10^{-8}$) in a prior GWAS of accelerometer-assessed movement in the UK Biobank (n=91,105) (9), which explain 0.10% of the variance in activity. Doherty and colleagues assessed overall activity as average vector magnitude (milligravities) per 30-second period,(9, 12) with mean (standard deviation, SD) 29.0 (8.0) milligravities among women in UK Biobank.(13) One SD (8 milligravities) corresponds to ~50 minutes of moderate (e.g. brisk walking) activity per week.(8)

For comparability with the previous MR study on this topic,(8) we used an expanded set of ten SNPs as a secondary instrument for overall activity. These SNPs were associated at relaxed significance ($p<5x10^{-7}$) with the accelerometer-assessed overall activity phenotype in a separate UK Biobank GWAS of physical activity by Klimentidis and colleagues.(10, 11)

Vigorous physical activity

Klimentidis and colleagues identified one SNP associated ($p<5x10^{-9}$) with high-intensity movement, assessed as the fraction of 30-second intervals containing accelerations over 425 milligravities.(10) This threshold approximates expenditure output for vigorous activity (>6 metabolic equivalents of task [METs]).(14) This SNP explains approximately 0.02% of variance in high-intensity movement. They identified five SNPs associated ($p<5x10^{-9}$) with self-reported engagement in vigorous activity for at least ten minutes ≥3 vs. 0 days/week (n=377,234), (10) which explain approximately 0.06% of variance in this exposure. We examined both instruments as complementary measures for vigorous activity, each likely subject to different error (weak instrument or reporting bias).

Sedentary time

Doherty and colleagues applied machine-learning models, trained using body-camera and diary data, to UK Biobank accelerometry data to identify sedentary periods (sitting/reclining; MET-value typically ≤1.5).(9, 13) They identified six SNPs associated ($p<5x10^{-8}$) with the probability of engaging in sedentary behaviours, defined as the ratio of sedentary-to-total 30-second periods.(9) On average UK Biobank women spent 34.6% (SD=7.2%) of their time sedentary.(13) We used these six variants, explaining 0.12% of variance in sedentariness, as our sedentary time instrument.

Outcomes

We estimated breast cancer risk overall, by menopausal status, and by case-groups defined by molecular/morphological subtype, stage, or grade at diagnosis, using BCAC clinical data to assign case-groups according to hypotheses arising from the literature. We defined separate case/control groups for invasive pre/peri-menopausal (n=23,999 cases;

17,686 controls) and postmenopausal (n=45,839 cases; 36,766 controls) breast cancers, using age at diagnosis/interview (</≥50 years) to assign missing menopausal status (27%). We examined subtypes separately by hormone-receptor (HR) status (ER+/- n=46,528/11,246; PR+/- n=34,891/16,432) and HER2 status (+/- n=6,945/33,214), and jointly including HER2-enriched (ER-/PR-/HER2+; n=1,974) and triple-negative (ER-/PR-/HER2-; n=4,964) cancers. We examined invasive ductal/lobular cancers (n=42,223/8,795), ductal carcinoma in situ (n=3,510), and risk by stage (stage I, n=17,583; stage II, n=15,992; stages III/IV, n=4,553) and grade (well/moderately differentiated, n=34,647; poorly/undifferentiated, n=16,432).

SNP-exposure (UK Biobank) and SNP-outcome (BCAC) associations

We extracted or derived estimates of association (beta coefficients, standard errors [SEs]) between SNPs and exposures from the UK Biobank GWAS publications,(9, 10) standardised to refer to the trait-increasing allele. Where required,(10) we converted estimates to per-SD changes in activity/sedentary time using UK Biobank activity data.

Genotypes in BCAC were determined using the OncoArray, an Illumina custom array, and imputed using IMPUTE2.(15) We harmonised UK Biobank and BCAC data so SNP-exposure and SNP-outcome estimates related to the same allele, using allele frequency information to resolve strand-ambiguous SNPs where possible (i.e., unless allele frequencies were 45%-55%). For each SNP, we derived trait-specific effect-allele dosages (range 0-2) by summing alleles predicting more activity (activity instruments) or sedentary time (sitting instrument). We assessed the association between each SNP and each outcome from individual-level BCAC data by fitting logistic regression models, adjusted for age at diagnosis (cases) or interview (controls), country, and ten principal components of genetic population structure (accounting for genetic substructure within Europeans), obtaining beta

coefficients and SEs for use in the MR analysis. Table 1 summarises the BCAC studies and participants.

Statistical analysis

We used SNP-exposure and SNP-outcome beta coefficients and SEs to estimate odds ratios (OR) and 95% confidence intervals (CI) of the effect of each trait on each outcome. For single SNPs, we divided the SNP-outcome association by the SNP-exposure association to obtain the causal estimate (Wald ratio). For multi-SNP instruments, we used inverse-variance weighted (IVW)-MR, which averages Wald ratios across SNPs, weighted by SNP-exposure beta coefficients.(16-18) IVW-MR assumes all instruments are valid or that pleiotropy is balanced,(17) and we assumed linearity in the associations between the SNPs and exposure, and between SNPs and outcome. We performed case-only analyses to test for differences between subtypes.

Core assumptions of MR, which can be investigated using sensitivity analyses, are that the instrument: predicts exposure; is not associated with confounders of the exposure/outcome association; and influences the outcome only via the exposure (no horizontal pleiotropy) (6, 7, 19), summarised in Figure S1. We undertook sensitivity analyses to assess the robustness of our findings and the potential for violations of assumptions, most critically horizontal pleiotropy. We calculated Cochran's Q-statistic for between-SNP heterogeneity of effects. We applied complementary methods relaxing different MR assumptions, weighted-median MR (allows invalid instruments)(20) and MR-Egger (allows horizontal pleiotropy, although prone to imprecision)(19, 21). We inspected per-SNP causal estimates (scatter, forest plots) and leave-one-out analyses to identify SNPs distorting results. We performed MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) to identify outlying SNPs with evidence of horizontal pleiotropy (global-pleiotropy and SNP-outlier tests

p<0.05).(22) We examined the effect of excluding two SNPs with imputation quality <0.9. We checked whether SNPs are associated with other relevant traits (possible confounders, adiposity, cancer risk) or gene expression using the NHGRI-EBI GWAS Catalog(23) and PhenoScanner.(24, 25)

Data preparation and analyses were performed using R software (R Foundation for Statistical Computing, Vienna), including the 'MendelianRandomization'(18) and 'MR-PRESSO' packages.(22) Statistical power was calculated using the mRnd Mendelian randomization power calculation online tool.(26) Further details are in Supplementary Methods (Online Resource).

Results

Overall physical activity

Greater genetically-predicted physical activity was associated with lower risk of invasive breast cancer (OR=0.48;95%CI 0.30-0.78 per-SD [~8 milligravities] in overall activity), with no clearly differential effects by menopausal status, molecular subtype, morphology, stage, or grade (Table 2). We observed ORs less than 1 for all outcomes, including ER+ (OR=0.45;95%CI 0.25-0.83), PR+ (OR=0.43;95%CI 0.22-0.85), HER2+ (OR=0.48;95%CI 0.26-0.89), and HR+/HER2+ (OR=0.42;95%CI 0.20-0.88) disease. Weighted-median MR and MR-Egger results were broadly consistent (Table S3).

Heterogeneity of causal effects between SNPs was evident for some outcomes (Cochran's-Q p_{het}<0.05)(Table 2); this was resolved after removing outliers rs564819152 (associated previously with ovarian cancer; outlying for six outcomes) or rs6775319 (one outcome), detected by MR-PRESSO, per-SNP, and leave-one-out analyses (Figures S2-S3; Table S4). Evidence of protective associations remained strong after excluding rs564819152

(Table 2). Outlier-corrected results (OR [95%CI]) were 0.59 (0.42-0.83) for all invasive breast cancer, 0.60 (0.43-0.85) for ER+, and 0.58 (0.37-0.91) for PR+ disease (HER2+ and HR+/HER2+ analyses had no outlying SNPs).

The protective effects were consistent across leave-one-out analyses (Table S4). SNPs were not associated in prior GWAS with confounders of the exposure/outcome relationship, but two had been identified in an ovarian cancer GWAS (Table S5). Excluding these made little difference to results (Table S4). Two SNPs have been reported to be associated ($p<5x10^{-8}$) with adiposity in UK Biobank,(24, 25, 27) consistent with reduced adiposity being a downstream effect of increased activity (Table S5).

Results were similar although slightly attenuated using the expanded ten-SNP instrument(10)(Table S6-S7). Estimates generally remained protective upon removing outlying SNPs detected by pleiotropy investigations (IVW heterogeneity tests [Table S6], MR-PRESSO, per-SNP effects [Figures S4-S5], leave-one-out analysis [Table S8]). Most estimates were similar (Table S8) upon excluding one SNP with imputation quality <0.9 (Table S8). Four of the ten SNPs were associated in prior GWAS with confounders (including height, alcohol intake, education) or cancer risk. Furthermore, rs55657917 is associated with gene expression in breast tissue, including in two genes associated with breast cancer risk (Table S5).(23-25) However, results excluding potentially confounded SNPs were relatively unchanged (Table S8). For four SNPs, the activity-increasing allele is associated with reduced adiposity in UK Biobank.(27)

Vigorous physical activity

There was little evidence that genetically-predicted acceleration over 425 milligravities (one SNP) was associated with risk of breast cancer, with wide confidence intervals crossing one, although most estimates were in the protective direction (Table 3).

The activity-increasing allele has been associated in GWAS(24, 25, 27) with greater height and decreased adiposity (Table S5).

There was weak evidence that genetically-predicted self-reported vigorous activity was associated with decreased breast cancer risk overall (OR=0.83;95%CI 0.69-1.01, ≥3 days/week vs. none), and ORs for most case-groups were less than 1 (Table 3). A protective association was seen for pre/perimenopausal breast cancer (OR=0.62;95%CI 0.45-0.87), with little evidence for an association with postmenopausal breast cancer risk (OR=0.95;95%CI 0.75-1.19) (p=0.82 for the difference in pre/peri- vs. post-menopausal estimates). A protective relationship was seen for PR+ disease (OR=0.77;95%CI 0.61-0.98). There was little evidence of pleiotropic effects (Table 3, S9-S10) except one outlier in modelling in situ cancers (Figures S6-S7), a SNP previously associated with height, age at menarche, and adiposity (Table S5).(24, 25, 27) After excluding this SNP, the in situ OR was elevated (from OR=0.94;95%CI 0.43-2.08 to OR=1.30;0.72-2.34)(Table 3); other estimates remained similar (Table S10). Excluding one SNP associated in UK Biobank GWAS with past smoking and childhood height (Table S5)(24, 25, 27) attenuated estimates slightly (Table S10). The association with pre/perimenopausal cancers remained substantially inverse (protective), with confidence intervals that did not cross the null, in all sensitivity analyses (Table S10).

Sedentary time

The estimates for genetically-predicted sedentary time were elevated (in the direction of increased risk) for almost every case-group, although CIs were wide (Table 4). Greater sedentary time was associated with higher risk of hormone-receptor-negative (HR-) tumours (OR=1.77;95%CI 1.07-2.92 per-SD [~7% time spent sedentary]), including triple-negative (ER-/PR-/HER2-) cancers (OR=2.04;95%CI 1.06-3.93) (p=0.11 for the difference in ORs by HR-status). ORs were substantially elevated for in situ cancers (OR=1.75;95%CI 1.00-3.07),

specifically ductal carcinoma in situ (OR=2.11;95%CI 0.99-4.49). The point estimate was elevated for stage I tumours (OR=1.62;95%CI 0.99-2.65), with little evidence of association with stage III/IV (OR=0.91;95%CI 0.45-1.84) (p=0.25 for the difference in estimates for risk of stage I vs stage III/IV tumours).

Heterogeneity between SNPs was not detected (all p_{het}>0.2)(Table 4), all MR methods produced broadly consistent results (Table S11), and MR-PRESSO did not identify outlying SNPs. Estimates were consistently elevated across leave-one-out analyses, including after omitting: one SNP correlated with a physical activity variant; one SNP predicting greater education and adiposity in prior GWAS(24, 25, 27, 28); or one strand-ambiguous SNP with minor allele frequency ~50%, for which effect-allele harmonisation was not definitive (Table S12). After excluding a SNP with imputation quality <0.9, which may have been an outlier for PR+ analyses (Figures S8-S9; MR-Egger $p_{pleiotropy}$=0.046 for PR+), point estimates for PR+ and most other outcomes including HR-, triple-negative, and in situ cancers, moved further from null (Table S12). Estimates for HR- and in situ cancers remained substantially elevated in all sensitivity analyses (Table S12).

Discussion

Main findings

We conducted a Mendelian randomization study using individual-level data on 130,957 women. We found that women with genetic variants predisposing them to be more active had lower breast cancer risk overall and for most case-groups defined by tumour subtypes, stage, or grade. Effect estimates for vigorous physical activity were in the protective direction for most types of breast cancer; reporting more frequent vigorous activity was associated with reduced risk of pre/perimenopausal breast cancer. Women with genetic variants predisposing them to more sedentary time had higher risk of HR- breast cancer, but

there was no strong evidence of differences in association by subtypes and weak evidence of an increased risk overall.

Strengths and limitations

A strength of our study is the use of individual-level BCAC data, which permitted examination of more outcomes than previously possible. Large sample sizes are another strength. BCAC is the largest collaboration of breast cancer studies, and we employed the most powerful available genetic instruments identified by the largest GWAS for movement-related behaviours, likely improving precision of our estimates. While statistical power was limited by the limited proportion of variation in exposure explained by the genetic instruments available (we had 52% power to detect expected effects for overall activity and overall breast cancer risk, and less power for other exposure/outcome combinations; Table S13), there were no larger datasets available to increase power. The UK Biobank studies are the only GWAS of accelerometer-assessed movement, which substantially decreases measurement error compared to self-report. Measurement error in assessing genotype is typically very low (often estimated as less than 1% (29, 30)).

The UK Biobank GWAS which identified our instruments used wrist-worn accelerometers, which may not capture ambulation as well as hip-worn accelerometers;(31) while this may have slightly affected precision, no superior data are available. Gene-exposure associations were estimated from a population (UK Biobank) including men, but no strong evidence of sexual dimorphism was reported in UK Biobank,(9) so we assume that SNP-exposure estimates adequately reflect associations in women. While our instruments predict only a small fraction of variance in exposure, any weak-instrument bias would have biased estimates towards the null and cannot explain our findings.(19) Some contributing studies within BCAC did not provide sufficient data on cancer diagnosis to classify cases into case

groups (for example tumour subtype or stage), and therefore numbers (32)included in these analyses were much lower. Women without these tumour-specific outcome data may have differed from those included in analyses. Our analyses took a conventional approach of assuming linearity in SNP-exposure and SNP-outcome relationships. Satisfying this assumption is not required for valid causal inference, so even in the presence of nonlinearity our results would still provide information on probable causality, approximating an population-average causal effect of intervening on the exposure.(32-34)

Due to the nature of the data and study design, we estimated odds ratios as the measure of effect, which in some circumstances can be prone to non-collapsibility and sparse-data bias.(35, 36) These issues are most severe when many covariates are included in models (which was not the case for the current analysis), and when outcomes are neither rare nor very common (many of the outcomes we investigated are rare, limiting the extent of noncollapsibility). Overall activity and sedentary time results for pre/peri- and post-menopausal breast cancer (the only sub-outcome where all participants could be classified), demonstrate a slight pattern of noncollapsibility, where the odds ratio for all invasive breast cancers does not lie between the odds ratios for each group separately. This is not a bias but a mathematical property of odds ratios.(35)

Implications

This analysis extends findings from a recent MR study of overall physical activity and breast cancer risk overall and by ER-status, using BCAC summary data.(8) Our study, using individual-level data, confirmed those findings, and showed that the risk reduction holds across multiple subtypes. Our study also examined vigorous activity and sedentary time, not previously studied in relation to breast cancer risk using MR. We assessed associations with

multiple outcomes (overall and by case-group) and our results may be subject to false positives. There was no strong evidence of differences in association by case-group.

While MR may provide estimates which more closely reflect underlying causal relationships, core assumptions must be satisfied before causal conclusions can be drawn. We satisfied the first (instrument predicts exposure) by selecting genome-wide significant SNPs identified by the largest GWAS of our traits of interest. We maximised the possibility of meeting the second (no confounding) by checking whether the SNPs were reported in prior GWAS of possible confounders (known breast cancer risk factors), and confirming that results remained consistent after excluding any SNPs that were (e.g., smoking [vigorous activity analyses], education [sedentary behaviour analyses]). We interrogated the third assumption (instrument influences outcome only through exposure) using several pleiotropy-detection approaches, acting on detected violations, and confirming consistency of results from methods relaxing this assumption. Our conclusions remained unchanged following exclusion of potentially-pleiotropic SNPs.

Several SNPs in the analyses were associated with adiposity in previous GWAS. While we cannot rule out horizontal pleiotropy (SNPs influencing adiposity independently of physical activity/sedentary time), vertical pleiotropy (same causal pathway) is more plausible; reduced adiposity is a downstream effect of increased physical activity. Vertical pleiotropy does not violate MR assumptions and excluding vertically-pleiotropic variants may distort causal estimates.(19) Nevertheless, previous MR analysis has shown evidence of a bi-directional relationship between overall activity and adiposity.(9)

Although it is possible that our findings arose by chance, our results for physical activity are consistent with observational studies, which have suggested a 20-25% breast cancer risk reduction for the most vs. least active women, with evidence of dose-response.(3,

37) Our findings support this and furthermore suggest that these relationships are likely to be causal. The observational evidence for risk reduction, particularly for premenopausal breast cancer, is strongest for vigorous physical activity, suggesting that vigorous activity may be particularly important in preventing carcinogenesis.(3, 38) Short bouts of intense activity may be more protective than equivalent energy expenditure accumulated from light activity. We found that self-reported vigorous activity was associated with lower pre/perimenopausal breast cancer risk and found weak evidence for a protective effect of vigorous activity overall. Future studies should continue to explore this with more powerful instruments.

For sedentary time, the observational evidence is sparse and inconsistent. Our results, which minimise likelihood of confounding (e.g. by unhealthy diet), are suggestive of a causal association with elevated risk of breast cancer, particularly for HR- and in situ cancer. While there is debate about the independence of physical activity and sedentary behaviour, they have different determinants and correlates and are often treated as separate traits. In our study the genetic instruments for sedentary behaviour and physical activity were mostly distinct; removing one SNP which predicted both traits did not change our findings, suggesting that both behaviours independently influence breast cancer risk.

Robust causal inference should triangulate findings across methods.(39) Our findings must be considered in light of biological plausibility. A reasonable body of mechanistic evidence supports numerous causal pathways between physical activity and breast cancer risk. Pathways involving adiposity, metabolic dysfunction, sex hormones, and inflammation have been most thoroughly described.(40-42) Mechanisms linking sedentary time and cancer are likely to at least partially overlap with those underpinning the physical activity relationship.(43, 44) Our findings cannot shed light on drivers of carcinogenesis. We saw suggestive differences by HR-status, but this may be a chance finding. Known adiposity-

related SNPs did not seem to unduly influence our results, perhaps indicating that multiple pathways are important.

Conclusion

Increasing physical activity and reducing sedentary time are already recommended for cancer prevention. Our study adds further evidence that such behavioural changes are likely to lower future breast cancer incidence. A stronger cancer-control focus on physical activity and sedentary time as modifiable cancer risk factors is warranted, given the heavy burden of disease attributed to the most common cancer in women.

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Project conception – BML, RLM; Project design – SCD, BML, RLM, SJL, RMM, DRE, TB; Acquisition, analysis, or interpretation of data for the work – all authors; initial drafting of manuscript – SCD, BML, RLM, SJL, RMM, DRE, TB; critical input – all authors; final approval of manuscript – all authors.

Ethics approval

This analysis and each contributing study received approval from the appropriate institutional review board or committee.

Data sharing statement

The data used in this study are de-identified patient data from 76 studies participating in the Breast Cancer Association Consortium (BCAC). Enquiries about accessing BCAC data can be directed to the BCAC coordinators at the University of Cambridge: https://bcac.ccge.medschl.cam.ac.uk/

Patient involvement

Patient co-production was not adopted for this large multi-study analysis. We thank all participants for providing their data to the contributing BCAC studies.

Tables

Table 1. Characteristics of 76 Breast Cancer Association Consortium studies, and 130,957 study participants, included in the individual-level analysis

Study acronym [a]	Country	Diagnosis years	Invasive cases (N)	In situ cases (N)	Controls (N)
ABCFS	Australia	1963-2013	1,117	-	187
ABCTB	Australia	2004-2013	920	6	375
BCEES	Australia	2009-2011	783	-	834
MCCS	Australia	1981-2012	870	180	978
HMBCS	Belarus	1994-2007	212	-	249
LMBC	Belgium	1994-2011	784	21	1,268
CBCS	Canada	2005-2009	568	108	817
MTLGEBCS	Canada	2007-2011	341	-	170
OFBCR	Canada	1967-2015	1,721	2	643
CGPS	Denmark	1981-2012	1,408	3	716
EPIC	Europe (Multiple countries)	n.r.	3,435	412	3,597
HEBCS	Finland	1997-2012	281	-	177
KBCP	Finland	1990-2012	522	34	245
CECILE	France	2005-2007	280	26	159
BBCC	Germany	1988-2013	403	8	253
BSUCH	Germany	1990-2013	252	1	168
ESTHER	Germany	2001-2004	291	3	187
GC-HBOC	Germany	1947-2014	3,378	256	1,593
GENICA	Germany	2000-2004	459	1	284
GEPARSIXTO	Germany	n.r.	386	-	-
GESBC	Germany	1992-1995	312	39	181
HABCS	Germany	1984-2010	909	19	863
MARIE	Germany	2001-2005	506	6	289
PREFACE	Germany	2001-2011	2,923	-	-
SKKDKFZS	Germany	1993-2005	1,086	9	-
SUCCESSB	Germany	2008-2011	440	-	-
SUCCESSC	Germany	2001-2011	2,836	-	-
CCGP	Greece	1983-2013	667	5	322
BCINIS	Israel	1999-2012	1,337	100	724
MBCSG	Italy	1977-2012	549	72	366
ABCS	Netherlands	2003-2011	347	-	189
ORIGO	Netherlands	1991-2005	921	113	-
RBCS	Netherlands	1975-2009	444	23	-
NBCS	Norway	1973-2011	1,163	38	-
PBCS	Poland	1998-2003	1,740	111	2,045
SZBCS	Poland	2010-2012	352	9	174
MABCS	Republic of North Macedonia	1993-2013	89	1	90
HUBCS	Russia	1977-2009	211	-	116
BREOGAN	Spain	1991-2019	1,535	129	910
HCSC	Spain	1975-2013	423	3	-
KARBAC	Sweden	1966-2013	499	3	-
KARMA	Sweden	1969-2017	2,839	339	6,983
MISS	Sweden	1983-2013	633	68	1,529
pKARMA	Sweden	1980-2015	748	86	48
SMC	Sweden	1987-2013	1,509	-	661
BBCS	UK	1985-2009	122	-	440

Study acronym [a]	Country	Diagnosis years	Invasive cases (N)	In situ cases (N)	Controls (N)
DIETCOMPLYF	UK	2004-2007	708	3	-
FHRISK	UK	1987-2015	146	31	644
POSH	UK	2000-2007	1,088	-	-
PROCAS	UK	1988-2018	380	93	1,648
SBCS	UK	2012-2015	126	2	-
SEARCH	UK	2003-2012	4,057	-	2,653
UKBGS	UK	1985-2014	1,048	584	705
UKOPS	UK	n.a.	-	-	974
2SISTER	USA	n.r.	919	151	-
AHS	USA	1994-2013	513	1	1,137
BCFR-NY	USA	1949-2011	401	53	27
BCFR-PA	USA	1969-2011	67	6	-
BCFR-UTAH	USA	1952-2009	100	1	-
CPSII	USA	1992-2009	2,393	598	3,028
CTS	USA	1998-2010	1,156	-	610
MCBCS	USA	1998-2014	749	167	212
MEC	USA	1972-2012	668	5	724
MMHS	USA	2003-2013	275	99	1,635
MSKCC	USA	1982-2012	136	2	-
NBHS	USA	2001-2009	483	112	652
NC-BCFR	USA	1967-2012	759	15	150
NCBCS	USA	1993-2012	2,074	315	1,006
NHS	USA	1976-2012	1,103	333	1,804
NHS2	USA	1989-2011	1,112	409	1,905
PLCO	USA	1994-2013	1,822	483	2,595
SISTER	USA	2003-2008	1,504	498	1,556
TNBCC	USA	2003-2013	113	-	-
UBCS	USA	1960-2015	606	60	-
UCIBCS	USA	1994-2003	427	74	258
USRT	USA	1945-2005	1,354	338	1,699
Total		**1945-2019**	**69,838**	**6,667**	**54,452**

n.a., not applicable; n.r., not recorded

a See Supplementary Table S1 (Online Resource) for study names and references.

Table 2. Association between the primary instrumental genetic variables for overall physical activity (per standard deviation) and risk of breast cancer

		Full instrument (five SNPs)		Excluding one pleiotropic SNP for outcomes with detected pleiotropy[a]	
Type of breast cancer	**N cases (vs. 54,452 controls)**	**Odds ratios (95% CI) [b]**	***P* for heterogeneity [c]**	**Odds ratios (95% CI) [b]**	***P* for heterogeneity [c]**
Invasive cancers					
All invasive	69,838	0.48 (0.30-0.78)	0.016	0.59 (0.42-0.83)	0.312
Pre/perimenopausal	[d] 23,999	0.51 (0.31-0.83)	0.419	--	
Postmenopausal	[e] 45,839	0.48 (0.28-0.80)	0.054	--	
By receptor status					
ER+	46,528	0.45 (0.25-0.83)	0.004	0.60 (0.43-0.85)	0.459
ER-	11,246	0.79 (0.37-1.66)	0.069	--	
PR+	34,891	0.43 (0.22-0.85)	0.003	0.58 (0.37-0.91)	0.223
PR-	16,432	0.65 (0.38-1.13)	0.186	--	
HER2+	6,945	0.48 (0.26-0.89)	0.479	--	
HER2-	33,214	0.58 (0.35-0.98)	0.060	--	
Combined hormone receptor- and/or HER2-defined subtypes					
ER+ or PR+; HER2+	4,816	0.42 (0.20-0.88)	0.478	--	
ER+ or PR+; HER2-	27,874	0.57 (0.28-1.18)	0.004	0.79 (0.49-1.26)	0.254
ER-; PR-; HER2+	1,974	0.53 (0.18-1.57)	0.700	--	
ER-; PR-; HER2-	4,964	0.60 (0.17-2.12)	0.015	0.95 (0.37-2.44)	0.224
ER- and PR- (all)	9,215	0.65 (0.27-1.56)	0.036	0.46 (0.22-0.96)	0.226
By morphology					
Ductal	42,223	0.52 (0.32-0.84)	0.053	--	
Lobular	8,795	0.32 (0.18-0.58)	0.500	--	
By stage at diagnosis					
Stage I	17,583	0.51 (0.32-0.82)	0.333	--	
Stage II	15,992	0.36 (0.22-0.58)	0.576	--	
Stage III/IV	4,553	0.37 (0.17-0.81)	0.499	--	
By tumour grade					
Grade 1/2	34,647	0.43 (0.23-0.81)	0.011	0.58 (0.39-0.85)	0.514
Grade 3	16,432	0.46 (0.30-0.72)	0.552	--	
In situ cancers					
All in situ	6,667	0.63 (0.34-1.18)	0.390	--	
Ductal carcinoma in situ	3,510	[f] 0.92 (0.25-3.43)	0.039	--	

Abbreviations: CI, confidence interval; ER+/-, oestrogen receptor positive/negative; GWAS, genome wide association study; HER2+/-, human epidermal growth factor receptor 2 positive/negative; PR+/-, progesterone receptor positive/negative; SNP, single nucleotide polymorphism.

a Outlying SNP rs564819152 was excluded from analyses of all invasive, ER+, PR+, HR+/HER2-, and well/moderately differentiated cancers (outlier identified by MR-PRESSO, global-pleiotropy test $p<0.05$), and HR- cancers (outlier suggested by scatter plots and leave-one-out analyses; MR-PRESSO global-pleiotropy test $p=0.053$). Outlying SNP rs6775319 was excluded from analyses of triple negative cancers (ER-/PR-/HER2-), and was identified by MR-PRESSO.

b Causal odds ratios were estimated by inverse-variance weighted Mendelian randomization, using SNPs identified in a GWAS of accelerometer-measured movement traits by Doherty et al (9)

c p-value associated with the heterogeneity test statistic (Cochran's Q statistic) measuring heterogeneity of causal effects between SNPs

d vs pre/perimenopausal controls (n=17,686), assigned using age (<50 years) if menopause status was unknown

e vs postmenopausal controls (n=36,766), assigned using age (≥50 years) if menopause status was unknown

f For analyses of ductal carcinoma in situ, likely pleiotropy was indicated by the Cochran's Q statistic (p_{het}=0.04) and the MR-Egger intercept test for horizontal pleiotropy ($p_{intercept}$=0.01). However, a clear outlying SNP could not be identified, although leave-one-out analyses suggested substantial variation in results by instrument composition.

-- No outlying SNPs were identified.

Table 3. Association between instrumental genetic variables for vigorous physical activity, assessed in two ways, and risk of breast cancer

		Accelerometer-measured activity over 425 milligravities, per fraction of time, using one SNP [a]	Self-reported vigorous physical activity (≥ 3 vs. 0 days/week)			
			Full instrument (five SNPs)		Excluding one pleiotropic SNP for outcome with detected pleiotropy [b]	
Type of breast cancer	N cases (vs. 54,452 controls)	Odds ratios (95% CI) [c]	Odds ratios (95% CI) [c]	*P* for heterogeneity [d]	Odds ratios (95% CI) [c]	*P* for heterogeneity [d]
Invasive cancers						
All invasive	69,838	0.63 (0.32-1.22)	0.83 (0.69-1.01)	0.650	--	
Pre/perimenopausal	[e] 23,999	0.80 (0.25-2.58)	0.62 (0.45-0.87)	0.788	--	
Postmenopausal	[f] 45,839	0.53 (0.24-1.21)	0.95 (0.75-1.19)	0.630	--	
By receptor status						
ER+	46,528	0.74 (0.35-1.55)	0.86 (0.70-1.07)	0.917	--	
ER-	11,246	0.58 (0.17-1.94)	0.86 (0.61-1.21)	0.418	--	
PR+	34,891	0.68 (0.30-1.54)	0.77 (0.61-0.98)	0.544	--	
PR-	16,432	0.56 (0.19-1.59)	0.95 (0.70-1.28)	0.948	--	
HER2+	6,945	0.31 (0.07-1.39)	0.83 (0.53-1.31)	0.327	--	
HER2-	33,214	1.01 (0.44-2.31)	0.86 (0.68-1.10)	0.550	--	
Combined hormone receptor- and/or HER2-defined subtypes						
ER+ or PR+; HER2+	4,816	0.41 (0.07-2.35)	1.00 (0.58-1.70)	0.321	--	
ER+ or PR+; HER2-	27,874	0.84 (0.35-2.02)	0.82 (0.64-1.06)	0.560	--	
ER-; PR-; HER2+	1,974	0.21 (0.02-2.87)	0.57 (0.27-1.20)	0.727	--	
ER-; PR-; HER2-	4,964	2.16 (0.39-12.1)	1.30 (0.79-2.12)	0.593	--	
ER- and PR- (all)	9,215	0.78 (0.21-2.91)	0.95 (0.66-1.39)	0.559	--	
By morphology						
Ductal	42,223	0.62 (0.29-1.32)	0.81 (0.65-1.00)	0.932	--	
Lobular	8,795	0.60 (0.15-2.45)	0.78 (0.53-1.17)	0.809	--	
By stage at diagnosis						
Stage I	17,583	0.47 (0.16-1.36)	0.88 (0.65-1.19)	0.598	--	
Stage II	15,992	0.66 (0.21-2.07)	0.82 (0.59-1.14)	0.788	--	
Stage III/IV	4,553	0.41 (0.06-2.63)	0.75 (0.44-1.27)	0.910	--	

		Accelerometer-measured activity over 425 milligravities, per fraction of time, using one SNP [a]	Self-reported vigorous physical activity (≥ 3 vs. 0 days/week)			
			Full instrument (five SNPs)		Excluding one pleiotropic SNP for outcome with detected pleiotropy [b]	
Type of breast cancer	**N cases (vs. 54,452 controls)**	**Odds ratios (95% CI) [c]**	**Odds ratios (95% CI) [c]**	***P* for heterogeneity [d]**	**Odds ratios (95% CI) [c]**	***P* for heterogeneity [d]**
By tumour grade						
Grade 1/2	34,647	0.54 (0.24-1.22)	0.84 (0.66-1.06)	0.640	--	
Grade 3	16,432	0.51 (0.18-1.46)	0.99 (0.73-1.33)	0.557	--	
In situ cancers						
All in situ	6,667	0.47 (0.11-2.09)	0.94 (0.43-2.08)	0.007	1.30 (0.72-2.34)	0.189
Ductal carcinoma in situ	3,510	0.65 (0.09-4.72)	0.85 (0.42-1.69)	0.204	--	

Abbreviations: CI, confidence interval; ER+/-, oestrogen receptor positive/negative; GWAS, genome wide association study; HER2+/-, human epidermal growth factor receptor 2 positive/negative; PR+/-, progesterone receptor positive/negative; SNP, single nucleotide polymorphism.

a This SNP is a missense mutation in the gene *PML*, which plays a role in tumour suppression and is associated with height. *PML* is not expressed in breast tissue, but highly expressed in adipose tissue, suggesting that inverse (protective) associations observed do not derive from direct oncosuppression.

b Excluding one outlying SNP identified by MR-PRESSO: rs2764261 (for analyses modelling the association with in situ tumours)

c Causal odds ratios were estimated by inverse-variance weighted Mendelian randomization, using SNPs identified in a GWAS of physical activity by Klimentidis et al (10)

d *p*-value associated with the heterogeneity test statistic (Cochran's Q statistic) measuring heterogeneity of causal effects between SNPs

e vs pre/perimenopausal controls (n=17,686), assigned using age (<50 years) if menopause status was unknown

f vs postmenopausal controls (n=36,766), assigned using age (≥50 years) if menopause status was unknown

-- No outlying SNPs were identified by MR-PRESSO.

Table 4. Association between instrumental genetic variables for sedentary time (per standard deviation in percent time spent sedentary) and risk of breast cancer

Type of breast cancer	N cases (vs. 54,452 controls)	Odds ratios (95% CI) [a]	*P* for heterogeneity [b]
Invasive cancers			
All invasive	69,838	1.20 (0.93-1.55)	0.962
Pre/perimenopausal	[c] 23,999	1.22 (0.78-1.90)	0.589
Postmenopausal	[d] 45,839	1.21 (0.89-1.65)	0.983
By receptor status			
ER+	46,528	1.19 (0.90-1.57)	0.992
ER-	11,246	1.43 (0.90-2.26)	0.926
PR+	34,891	1.19 (0.87-1.63)	0.386
PR-	16,432	1.40 (0.94-2.09)	0.435
HER2+	6,945	1.17 (0.67-2.06)	0.718
HER2-	33,214	1.27 (0.93-1.74)	0.955
Combined hormone receptor- and/or HER2-defined subtypes			
ER+ or PR+; HER2+	4,816	0.86 (0.44-1.67)	0.585
ER+ or PR+; HER2-	27,874	1.12 (0.80-1.56)	0.801
ER-; PR-; HER2+	1,974	1.94 (0.71-5.25)	0.646
ER-; PR-; HER2-	4,964	2.04 (1.06-3.93)	0.500
ER- and PR- (all)	9,215	1.77 (1.07-2.92)	0.819
By morphology			
Ductal	42,223	1.21 (0.91-1.62)	0.992
Lobular	8,795	1.12 (0.66-1.91)	0.695
By stage at diagnosis			
Stage I	17,583	1.62 (0.99-2.65)	0.187
Stage II	15,992	1.23 (0.79-1.90)	0.820
Stage III/IV	4,553	0.91 (0.45-1.84)	0.640
By tumour grade			
Grade 1/2	34,647	1.15 (0.84-1.57)	0.901
Grade 3	16,432	1.32 (0.88-1.97)	0.967
In situ cancers			
All in situ	6,667	1.75 (1.00-3.07)	0.933
Ductal carcinoma in situ	3,510	2.11 (0.99-4.49)	0.487

Abbreviations: CI, confidence interval; ER+/-, oestrogen receptor positive/negative; GWAS, genome wide association study; HER2+/-, human epidermal growth factor receptor 2 positive/negative; PR+/-, progesterone receptor positive/negative; SNP, single nucleotide polymorphism.

a Causal odds ratios were estimated by inverse-variance weighted Mendelian randomization, using six SNPs identified in a GWAS of accelerometer-measured movement traits by Doherty et al (9)

b *p*-value associated with the heterogeneity test statistic (Cochran's Q statistic) measuring heterogeneity of causal effects between SNPs

c vs pre/perimenopausal controls (n=17,686), assigned using age (<50 years) if menopause status was unknown

d vs postmenopausal controls (n=36,766), assigned using age (≥50 years) if menopause status was unknown

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