Cancer

Reassessing the causal role of obesity in breast cancer susceptibility: a comprehensive multivariable Mendelian randomization investigating the distribution and timing of exposure

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Abstract

Background: Previous Mendelian randomization (MR) studies on obesity and risk of breast cancer adopted a small number of instrumental variables and focused mainly on the crude total effect. We aim to investigate the independent causal effect of obesity on breast cancer susceptibility, considering the distribution of fat, covering both early and late life.

Methods: Using an enlarged set of female-specific genetic variants associated with adult general [body mass index (BMI)] and abdominal obesity [waist-to-hip ratio (WHR) with and without adjustment for BMI, WHR and $WHR_{adj}BMI$] as well as using sex-combined genetic variants of childhood obesity (childhood BMI), we performed a two-sample univariable MR to re-evaluate the total effect of each obesity-related exposure on overall breast cancer ($N_{case} = 133\,384$, $N_{control} = 113\,789$). We further looked into its oestrogen receptor (ER)-defined subtypes ($N_{ER+} = 69\,501$, $N_{ER-} = 21\,468$, $N_{control} = 105\,974$). Multivariable MR

was applied to estimate the independent causal effect of each obesity-related exposure on breast cancer taking into account confounders as well as to investigate the independent effect of adult and childhood obesity considering their inter-correlation.

Results: In univariable MR, the protective effects of both adult BMI [odds ratio (OR) = 0.89, 95% CI = 0.83–0.96, $P = 2.06 \times 10^{-3}$] and childhood BMI (OR = 0.78, 95% CI = 0.70–0.87, $P = 4.58 \times 10^{-6}$) were observed for breast cancer overall. Comparable effects were found in ER+ and ER− subtypes. Similarly, genetically predicted adult WHR was also associated with a decreased risk of breast cancer overall (OR = 0.87, 95% CI = 0.80–0.96, $P = 3.77 \times 10^{-3}$), restricting to ER+ subtype (OR = 0.88, 95% CI = 0.80–0.98, $P = 1.84 \times 10^{-2}$). Conditional on childhood BMI, the effect of adult general obesity on breast cancer overall attenuated to null (BMI: OR = 1.00, 95% CI = 0.90–1.10, $P = 0.96$), whereas the effect of adult abdominal obesity attenuated to some extent (WHR: OR = 0.90, 95% CI = 0.82–0.98, $P = 1.49 \times 10^{-2}$; WHR$_{adj}$BMI: OR = 0.92, 95% CI = 0.86–0.99, $P = 1.98 \times 10^{-2}$). On the contrary, an independent protective effect of childhood BMI was observed in breast cancer overall, irrespective of adult measures (adjusted for adult BMI: OR = 0.84, 95% CI = 0.77–0.93, $P = 3.93 \times 10^{-4}$; adjusted for adult WHR: OR = 0.84, 95% CI = 0.76–0.91, $P = 6.57 \times 10^{-5}$; adjusted for adult WHR$_{adj}$BMI: OR = 0.80, 95% CI = 0.74–0.87, $P = 1.24 \times 10^{-7}$).

Conclusion: Although successfully replicating the inverse causal relationship between adult obesity-related exposures and risk of breast cancer, our study demonstrated such effects to be largely (adult BMI) or partly (adult WHR or WHR$_{adj}$BMI) attributed to childhood obesity. Our findings highlighted an independent role of childhood obesity in affecting the risk of breast cancer as well as the importance of taking into account the complex interplay underlying correlated exposures.

Key words: Childhood adiposity, general obesity, abdominal obesity, Mendelian randomization, breast cancer

Key Messages

- In this study, we conducted a comprehensive multivariable Mendelian randomization to revisit the causal role of multiple obesity-related traits on breast cancer susceptibility, considering the distribution of fat, covering both early and late life.
- Using data from the hitherto largest genome-wide association studies, we successfully replicated the total effect of both adult and childhood obesity on lowering the risk of breast cancer.
- Conditional on childhood body mass index (BMI), we further identified that the total effect of adult general obesity (adult BMI) on breast cancer was largely attributed to childhood obesity, whereas that of adult abdominal obesity [waist-to-hip ratio (WHR) with and without adjustment for BMI, WHR and WHR$_{adj}$BMI] was at least partly attributed to childhood obesity. Biological mechanisms underlying such findings warrant attention in future research.
- These findings indicate a predominantly independent effect of childhood BMI in affecting breast cancer onset and highlight the importance of taking into account the complex interplay underlying correlated exposures.

Introduction

Obesity, a widely recognized public health challenge, plays a complex role in the development of female breast cancer,[1] with associations differing depending on their distribution (e.g. general vs abdominal) and timing (e.g. childhood vs adulthood). Traditional epidemiological studies have consistently observed an increased risk of post-menopausal breast cancer and a decreased risk of pre-menopausal breast cancer to be associated with adult body mass index [body mass index (BMI), a measure of adult general obesity], whereas the results for waist-to-hip ratio (WHR, a measure of adult abdominal obesity) and

childhood BMI remain conflicting.[2–4] These intricate associations motivate the need for understanding the causality and interaction of multiple obesity-related traits on breast cancer risk.

Mendelian randomization (MR) is a powerful tool that uses genetic variants [single-nucleotide polymorphisms (SNPs)] as instrumental variables (IVs) to make causal inference[5] and has been widely applied to determine the causal association between obesity and breast cancer risk.[6–10] Using 15 748 breast cancer cases and a limited number of IVs (77 SNPs for adult BMI; 15 SNPs for childhood BMI), Gao *et al.* conducted a univariable MR, which found a suggestive protective effect of general obesity (adult BMI: OR = 0.66, 95% CI = 0.57–0.77; childhood BMI: OR = 0.71, 95% CI = 0.60–0.80) on breast cancer overall.[7] In an enlarged MR study conducted by Ooi *et al.* using 122 977 breast cancer cases and the same number of IVs (77 SNPs for adult BMI as in Gao *et al.*), a consistent protective effect of BMI on breast cancer overall was identified (OR = 0.81, 95% CI = 0.74–0.89). Such an effect remained in subtypes defined by oestrogen receptor (ER) status (ER+: OR = 0.81, 95% CI = 0.74–0.89; ER−: OR = 0.78, 95% CI = 0.67–0.91).[10] Expanding the number of IVs into 166 or ∼700 BMI-associated SNPs, another two studies drew similar conclusions.[6,8] Opposite to general obesity, MR studies on abdominal obesity remain sparse. A null association was detected for WHR,[7] whereas a decreased risk of breast cancer (OR = 0.85, 95% CI = 0. 79–0.91) was reported by Shu *et al.* for BMI-adjusted WHR (WHR$_{adj}$BMI, representing abdominal body fat independent of general body fat) using 54 WHR$_{adj}$BMI-associated SNPs.[6] Furthermore, to understand the independent effect of correlated exposures on outcome, multivariable MR[11] (an extension to univariable MR) has been developed. In the hitherto only available multivariable MR study,[9] which modelled simultaneously adult and childhood body size using composite IVs (191 SNPs for adult body size plus 124 SNPs for childhood body size), a protective effect of childhood body size with breast cancer was observed conditioning on adult body size, whereas the protective effect of adult body size turned to null conditioning on childhood body size, highlighting the importance of taking into account multiple obesity-related traits over the life course simultaneously.

Despite existing MR studies having advanced our knowledge on an intrinsic link underlying obesity and breast cancer, a few gaps need to be filled. First, most studies did not use female-specific IVs to match with a female disease—heterogeneity derived from sex-combined IVs would lead to a biased MR estimate.[12] Second, existing studies using a handful of IVs were of poor statistical power—the most updated genome-wide association study

(GWAS) of BMI and WHR has identified a 4-fold enlarged number of female-specific IVs, which would greatly improve the statistical power and the accuracy of estimates. Third, the only available multivariable MR study to date used retrospective questionnaire-based categorized data for perceived childhood obesity, yielding potentially to measurement error. Last but not least, despite breast cancer being a complex disease with distinct subtypes, most studies did not phenotype the disease by ER status.

As the sample size of GWAS(s) continues to grow and the data continue to accumulate,[13,14] it is timely to conduct a comprehensive reassessment of the causal role of obesity in breast cancer through an MR design. Therefore, in this study, we used a largely increased set of sex-specific IVs derived from the hitherto largest GWAS(s) conducted for exposure and outcome[13–16] to (i) re-evaluate the total effect of obesity-related traits (general and abdominal obesity, adult and childhood obesity) on breast cancer overall and its ER-defined subtypes; (ii) estimate the independent effect of each obesity-related trait, accounting for the confounding effects from four major risk factors, including smoking, drinking, age at menarche and age at natural menopause; (iii) investigate the independent effect of adult and childhood obesity taking into account their intercorrelation.

Methods

Data sources

Exposure GWAS(s)
The hitherto largest GWAS(s)[14] of general obesity (BMI) and abdominal obesity (WHR and WHR$_{adj}$BMI) in adults were conducted via a collaboration of UK Biobank and the Genetic Investigation of Anthropometric Traits consortium in 2019, including ∼700 000 individuals of European ancestry. Anthropometric parameters, including height, weight, waist and hip circumferences, were measured according to standard protocols. BMI was calculated dividing weight by height squared and WHR was calculated by dividing waist circumference by hip circumference. WHR$_{adj}$BMI was generated from the regression of WHR on BMI by including BMI as an additional independent variable. Due to the large sample size, sex-specific analysis was performed based on 434 794 women for BMI, 381 152 women for WHR and 379 501 women for WHR$_{adj}$BMI.

As for childhood BMI, the latest and the largest GWAS was conducted by the Early Growth Genetics consortium in 2020[13] combining data of 41 studies involving 55 354 children aged 6–10 years and of European ancestry. Unfortunately, sex-specific results of childhood BMI were not available due to data restrictions.

Outcome GWAS(s)

Summary-level data were available for three phenotypes—the overall breast cancer, ER+ and ER− subtypes. For breast cancer overall, we retrieved data from the most updated GWAS conducted in 2020 involving 133 384 cases and 113 789 controls of European ancestry combining results from 82 studies participating in the Breast Cancer Association Consortium and 11 other breast cancer genetic studies.[15] This GWAS expanded upon a previous GWAS from the Breast Cancer Association Consortium[16] (2017) with an additional 10 407 cases and 7815 controls (10% increase) and identified 32 novel susceptibility loci upon the previously detected 153 loci.

For breast cancer subtypes, we used data from a previous GWAS from the Breast Cancer Association Consortium[16] (2017) including 69 501 ER+ cases, 21 468 ER− cases and 105 974 controls, which is the hitherto largest GWAS performed for ER subtypes.

Other GWAS(s)

We included four risk factors (age at menarche, age at natural menopause, smoking and drinking) as potential confounders to be controlled for in our MR study. For age at menarche, we used the GWAS from the Reproductive Genetics Consortium published in 2017 comprising 329 345 European women from 40 participating studies ($N = 179\,117$), 23andMe ($N = 76\,831$) and UK Biobank ($N = 73\,397$).[17] For age at natural menopause, we used the most updated GWAS from the Reproductive Genetics Consortium in 2021 involving data of 201 323 European women.[18] For smoking and drinking, we used data published in 2019 from the GWAS & Sequencing Consortium of Alcohol and Nicotine use, with 1 232 091 participants for smoking initiation and 941 280 participants for drinks per week,[19] all of European ancestry.

Instrument selection

We extracted genome-wide significant and uncorrelated (i.e. not in linkage disequilibrium) genetic variants from the original GWASs as our IVs for each exposure. Specifically, this yielded 281 independent top-associated SNPs for BMI, 203 independent top-associated SNPs for WHR, 266 independent top-associated SNPs for WHR$_{adj}$BMI (all restricted to females, $P < 5 \times 10^{-9}$, $r^2 < 0.05$) and 25 independent top-associated SNPs for childhood BMI (both sexes combined, $P < 5 \times 10^{-8}$, $r^2 < 0.20$). In addition, we also extracted independent top-associated SNPs for the confounders, resulting in 375 SNPs for age at menarche, 290 SNPs for age at natural menopause ($P < 5 \times 10^{-8}$, $r^2 < 0.05$), as well as 378 and 99 SNPs for smoking and drinking ($P < 5 \times 10^{-9}$, $r^2 < 0.10$). We then matched and harmonized these SNPs

with the outcome GWAS. For details, please also see Supplementary Table S1 (available as Supplementary data at *IJE* online).

Bias from weak instruments can result in seriously misleading estimates of causal effects. We therefore calculated F-statistics to measure the strength of instruments, using the proportion of variance in the phenotype explained by genetic variants (R^2), sample size (N) and number of instruments (K) via the formula $F = \left(\frac{N-K-1}{K}\right)\left(\frac{R^2}{1-R^2}\right)$. An F-statistic of >10 indicates a strong instrument and sufficient strength to ensure the validity of instrumental variable methods. We extracted R^2, N and K from the original GWASs. When not reported by the original GWAS, R^2 was calculated using $\hat{\beta}$ (estimated genetic association of each SNP with the trait) and MAF (minor allele frequency) via the formula $R^2 = \sum 2 \times \hat{\beta}^2 \times MAF \times (1 - MAF)$.

Statistical analysis

A comprehensive two-sample MR analysis was performed to evaluate a putative causal relationship between exposures (BMI, WHR, WHR$_{adj}$BMI, childhood BMI) and outcomes (breast cancer overall, ER+ and ER− subtypes), with an analytical schematic diagram presented in Figure 1.

Univariable MR analysis

To investigate the total effect of each obesity-related trait on breast cancer, univariable MR was conducted as our primary analysis. We first employed an inverse-variance weighted (IVW) approach to estimate the causal effect by regressing the outcome effect coefficient on the exposure effect coefficient with no intercept term.[20] Considering the potential bias derived from horizontal pleiotropy of instruments, we complemented IVW with MR–Egger regression[21] and the weighted-median approach.[22] MR–Egger regression is largely similar to IVW except its regression model contains an intercept to reflect directional pleiotropy. The weighted-median approach is more robust to invalid IVs compared with IVW and MR–Egger regression. Moreover, we also implemented MR-PRESSO (Mendelian Randomization Pleiotropy Residual Sum and Outlier) to evaluate the presence of horizontal pleiotropy and to re-evaluate the causal effect after removing the detected outlying SNPs.[23] A putative total causal effect was considered if the exact *P*-value was <0.05 in any of these four methods and the estimates maintained directional consistency across methods.

Several sensitivity analyses were conducted to assess the robustness of results, including (i) analysis using IVs excluding palindromic SNPs with strand ambiguity; (ii) analysis using IVs excluding pleiotropic SNPs that were associated with the potential confounding traits (age at menarche, age at natural menopause, smoking and drinking)
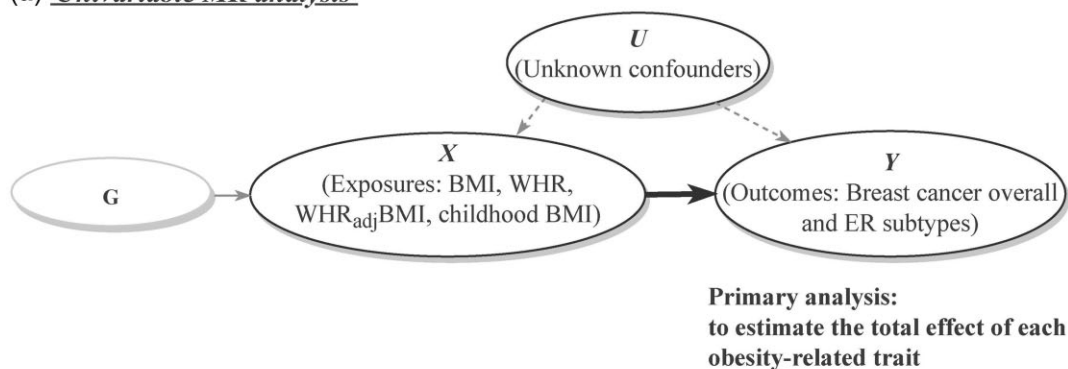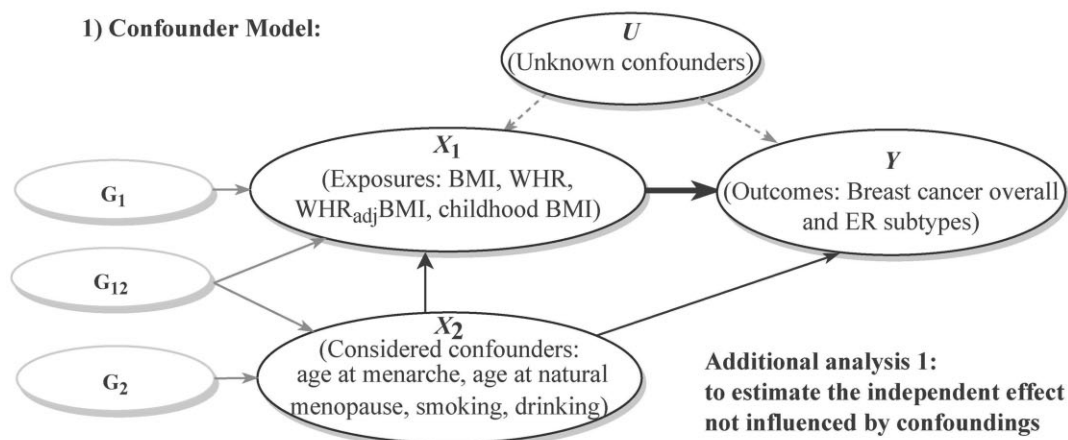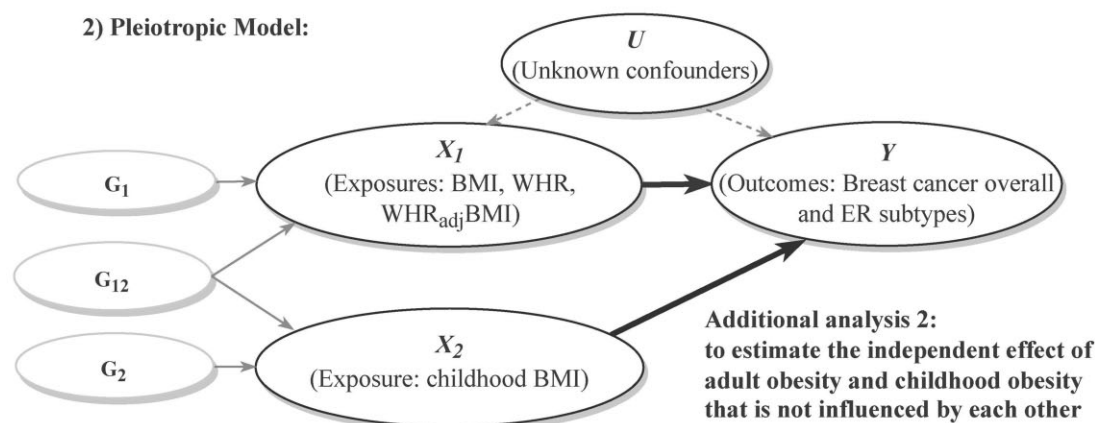
(a) *Univariable MR analysis*

(b) *Multivariable MR analysis*

1) Confounder Model:

2) Pleiotropic Model:

Figure 1 Analytical schematic diagram of the Mendelian randomization (MR) analysis implemented in this study

(a) Univariable MR analysis; (b) multivariable MR analysis, including two models: (i) confounder model; (ii) pleiotropic model.

G represents genetic variants (single-nucleotide polymorphisms, SNPs) that reliably predict the exposure variable (*X*) and are used as instrumental variables to represent exposure. G_1 and G_2 represent SNPs that specifically affect X_1 and X_2, respectively, whereas G_{12} represents SNPs that affect both X_1 and X_2 simultaneously. Thick lines illustrate the causal effect confirmed by the current analysis.

BMI, body mass index; WHR, waist-to-hip ratio; WHR$_{adj}$BMI, waist-to-hip ratio adjusted for body mass index; ER, oestrogen receptor.

according to GWAS Catalog; (iii) leave-one-out analysis in which each SNP was removed sequentially to identify outliers that might bias the MR estimates.[24]

In addition, a bidirectional MR analysis was also performed to evaluate whether genetic predisposition to breast cancer would influence obesity. We collected all

previously reported IVs reaching genome-wide significance ($P < 5 \times 10^{-8}$) in the breast cancer GWAS.[16]

Multivariable MR analysis

To further evaluate whether the causal effects of obesity on breast cancer are affected by major confounders and whether the casual effects of childhood and adult obesity on breast cancer are independent of each other, we conducted two additional analyses in the framework of multivariable MR.[11,25]

i. Four risk factors (age at menarche, age at natural menopause, smoking and drinking), believed as important confounders of the obesity–breast cancer association, were incorporated together with the exposures, one at a time as well as simultaneously to estimate the independent effect of each exposure after accounting for the confounding effects. Considering that there might be overlapping or correlated SNPs in composite IVs (the sum of IVs from different traits or exposures), we thus removed SNPs in linkage disequilibrium ($r^2 > 0.001$) to obtain a list of independent SNPs, by applying the 'clump_data' function of the 'TwoSampleMR' package (clump_r2 = 0.001, clump_kb = 500). Stratified analysis on ER subtypes was performed following the same procedure.

ii. Considering the inter-correlation between adult and childhood obesity, childhood BMI was incorporated with each adult obesity trait (BMI, WHR, WHR$_{adj}$BMI) to examine their independent effect on breast cancer. Three sets of composite IVs after a linkage disequilibrium clumping with $r^2 > 0.001$ were used.[26] These included composite IVs involving 270 SNPs for BMI and childhood BMI, 208 SNPs for WHR and childhood BMI and 266 SNPs for WHR$_{adj}$BMI and childhood BMI. Stratified analysis on ER subtypes was performed following the same procedure.

In our MR analysis, *P*-values were transformed to *q*-values to account for the false discovery rate in multiple tests. We conducted univariable MR using the package 'TwoSampleMR' (version 0.5.6) and multivariable MR using the package 'MendelianRandomization' (version 0.5.1) in software R (version 4.1.0).

Genetic correlation analysis

To understand the shared genetic basis between exposures and outcomes, a genome-wide genetic correlation analysis was further conducted. Full-set GWAS summary data were used to estimate genome-wide genetic correlations (r_g), which quantifies the intrinsic average sharing of the genetic effect between pairs of traits that is independent of environmental factors.[27] An algorithm implemented in software linkage disequilibrium score regression (LDSC) was adopted to perform regression on the product of z-scores across any two traits leveraging SNPs across the whole genome.[28]

Results

The basic characteristics of each GWAS data set and IVs are shown in Table 1. Current IVs explained ∼4% of the phenotypic variance of each exposure (4.0% for adult BMI with 281 index SNPs; 4% for WHR with 203 index SNPs; 3.6% for WHR$_{adj}$BMI with 266 index SNPs; 3.6% for childhood BMI with 25 index SNPs). F-statistics for these IVs ranged from 53 to 78, suggesting strong instruments.

The intrinsic average genome-wide sharing between pairs of exposure and outcome was evaluated. The genetic correlation analysis showed a negative genetic correlation between breast cancer and childhood BMI (overall: $r_g = -0.06$, $P = 4.98 \times 10^{-2}$; ER+: $r_g = -0.08$, $P = 1.82 \times 10^{-2}$) but not for BMI, WHR and WHR$_{adj}$BMI (Supplementary Figure S1 and Supplementary Table S2, available as Supplementary data at *IJE* online).

Motivated by these findings, we continued to investigate the putative causal relationship between exposures and outcomes. As shown in Figure 2, using univariable MR, genetically predicted female-specific BMI presented an inverse association with the risk of breast cancer overall (OR = 0.89, 95% CI = 0.83–0.96, $P = 2.06 \times 10^{-3}$). Such a relationship remained consistent in both ER+ (OR = 0.90, 95% CI = 0.83–0.97, $P = 6.37 \times 10^{-3}$) and ER− subtypes (OR = 0.85, 95% CI = 0.76–0.95, $P = 4.76 \times 10^{-3}$) and all survived the false discovery rate correction. Similar findings were observed for genetically predicted female-specific WHR on a decreased risk of breast cancer overall (OR = 0.87, 95% CI = 0.80–0.96, $P = 3.77 \times 10^{-3}$), as well as for ER+ subtype (OR = 0.88, 95% CI = 0.80–0.98, $P = 1.84 \times 10^{-2}$), but not for ER− subtype. When the effect of BMI was removed from WHR (WHR$_{adj}$BMI), the observed inverse association of WHR reduced to some extent in breast cancer overall (OR = 0.94, 95% CI = 0.88–1.00, $P = 0.06$) and in ER+ subtype (OR = 0.92, 95% CI = 0.86–1.00, $P = 4.38 \times 10^{-2}$). As for childhood BMI, strong evidence of a protective effect was observed consistently across all breast cancer phenotypes (overall: OR = 0.78, 95% CI = 0.70–0.87, $P = 4.58 \times 10^{-6}$; ER+: OR = 0.80, 95% CI = 0.72–0.89, $P = 4.53 \times 10^{-5}$; ER−: OR = 0.71, 95% CI = 0.60–0.85, $P = 1.40 \times 10^{-4}$). All these aforementioned results derived from IVW were further supported by the weighed-median approach and the MR–Egger regression, with estimates consistent in direction and without apparent sign of horizontal pleiotropy (Supplementary Table S3,

Table 1 Description of the genome-wide association study data sets and instrumental variables used in our study

Phenotype	IV	Sample size	Ethnicity	Consortium	R^2	F-statistic	Author, year
Exposures							
BMI	281	434 794 females	European	Genetic Investigation of ANthropometric Traits (GIANT) and UK Biobank	0.040	63.799	Pulit, 2019
WHR	203	381 152 females	European	Genetic Investigation of ANthropometric Traits (GIANT) and UK Biobank	0.040	78.191	Pulit, 2019
WHR$_{adj}$BMI	266	379 501 females	European	Genetic Investigation of ANthropometric Traits (GIANT) and UK Biobank	0.036	53.242	Pulit, 2019
Childhood BMI	25	55 354	European	Early Growth Genetics (EGG)	0.036	58.975	Vogelezang, 2020
Outcomes							
BC overall	170	133 384 cases/ 113 789 controls	European	Breast Cancer Association Consortium (BCAC)	0.302	608.642	Zhang, 2020
ER+	NA	69 501 cases/ 105 974 controls	European	Breast Cancer Association Consortium (BCAC)	NA	NA	Michailidou, 2017
ER−	NA	21 468 cases/ 105 974 controls	European	Breast Cancer Association Consortium (BCAC)	NA	NA	Michailidou, 2017
Confounders							
AAM	375	329 345	European	Reproductive Genetics (ReproGen)	0.074	67.656	Day, 2017
ANM	290	201 323	European	Reproductive Genetics (ReproGen)	0.130	103.187	Ruth, 2021
Smoking	378	1 232 091	European	GWAS & Sequencing Consortium of Alcohol and Nicotine (GSCAN)	0.023	77.222	Liu, 2019
Drinking	99	941 280	European	GWAS & Sequencing Consortium of Alcohol and Nicotine (GSCAN)	0.002	17.811	Liu, 2019

GWAS, genome-wide association study; IV, instrumental variable; BMI, body mass index; WHR, waist-to-hip ratio; WHR$_{adj}$BMI, waist-to-hip ratio adjusted for body mass index; BC, breast cancer; ER, oestrogen receptor; AAM, age at menarche; ANM, age at natural menopause.
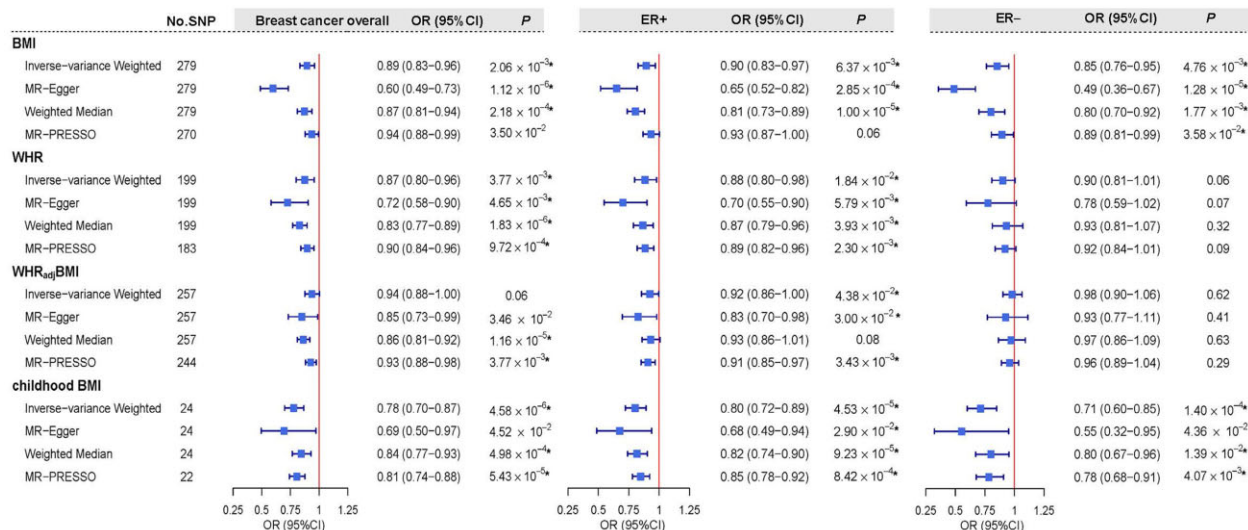
Figure 2 Estimated total effects of obesity-related traits on the risk of breast cancer using univariable Mendelian randomization

Boxes denote the point estimates of causal effects, and error bars denote 95% confidence intervals. Asterisks (*) denote the tests survived false discovery rate (FDR) correction ($P_{FDR} < 0.05$). Inverse-variance weighted approach was used as primary analysis; MR–Egger, weighted-median and MR-PRESSO were used as sensitivity analyses.

BMI, body mass index; WHR, waist-to-hip ratio; WHR$_{adj}$BMI, waist-to-hip ratio adjusted for body mass index; ER, oestrogen receptor; No. SNP, number of instrumental variables; OR, odds ratio.

available as Supplementary data at *IJE* online). Results from MR-PRESSO using the outlier-corrected method were also highly consistent with those from IVW.

Sensitivity analyses excluding pleiotropic SNPs or palindromic SNPs, as well as the leave-one-out analysis, showed similar findings, demonstrating the robustness of the results (Supplementary Figures S2 and S3, available as Supplementary data at *IJE* online). Additionally, bidirectional MR did not find a genetic predisposition to breast cancer overall to affect any obesity trait (Supplementary Table S4, available as Supplementary data at *IJE* online).

Considering important mediatory phenotypes or risk factors that may affect the relationship between obesity and breast cancer, we performed multivariable MR by incorporating each exposure with confounders (age at menarche, age at natural menopause, smoking and drinking), separately and together. The effect of each obesity trait on breast cancer remained consistent in both direction and magnitude after adjusting for confounders and all survived multiple testing corrections (Figure 3).

Despite our prior results providing evidence that both childhood and adult obesity contribute to a decreased risk of breast cancer, their independent effects remain unclear. We conducted a series of multivariable MR to examine whether the casual effects of childhood and adult obesity on breast cancer are independent of each other (Table 2). Notably, the effect of adult BMI on breast cancer overall attenuated to null in multivariable MR after adjusting for

childhood BMI (OR = 1.00, 95% CI = 0.90–1.10, $P = 0.96$), suggesting the effect of adult general obesity on breast cancer is influenced by childhood obesity. On the contrary, the decreased risk of adult abdominal obesity (WHR and WHR$_{adj}$BMI) with breast cancer overall attenuated slightly when conditional on childhood BMI (WHR: OR = 0.90, 95% CI = 0.82–0.98, $P = 1.49 \times 10^{-2}$; WHR$_{adj}$BMI: OR = 0.92, 95% CI = 0.86–0.99, $P = 1.98 \times 10^{-2}$). Similar results were also identified in ER+ (WHR: OR = 0.90, 95% CI = 0.81–1.00, $P = 4.29 \times 10^{-2}$; WHR$_{adj}$BMI: OR = 0.91, 95% CI = 0.84–0.98, $P = 1.92 \times 10^{-2}$), but not in ER− subtype. These results indicated the effect of adult abdominal obesity on breast cancer to be partially independent of childhood obesity, suggesting multiple distinct pathways influencing breast cancer susceptibility. On the contrary, a strong independent effect of childhood BMI was consistently observed in breast cancer overall when conditional on each adult obesity trait (adjusted for adult BMI: OR = 0.84, 95% CI = 0.77–0.93, $P = 3.93 \times 10^{-4}$; adjusted for adult WHR: OR = 0.84, 95% CI = 0.76–0.91, $P = 6.57 \times 10^{-5}$; adjusted for adult WHR$_{adj}$BMI: OR = 0.80, 95% CI = 0.74–0.87, $P = 1.24 \times 10^{-7}$) and the effect remained across both ER+ subtype (adjusted for adult BMI: OR = 0.86, 95% CI = 0.77–0.95, $P = 4.05 \times 10^{-3}$; adjusted for adult WHR: OR = 0.86, 95% CI = 0.78–0.95, $P = 2.87 \times 10^{-3}$; adjusted for adult WHR$_{adj}$BMI: OR = 0.82, 95% CI = 0.75–0.90,
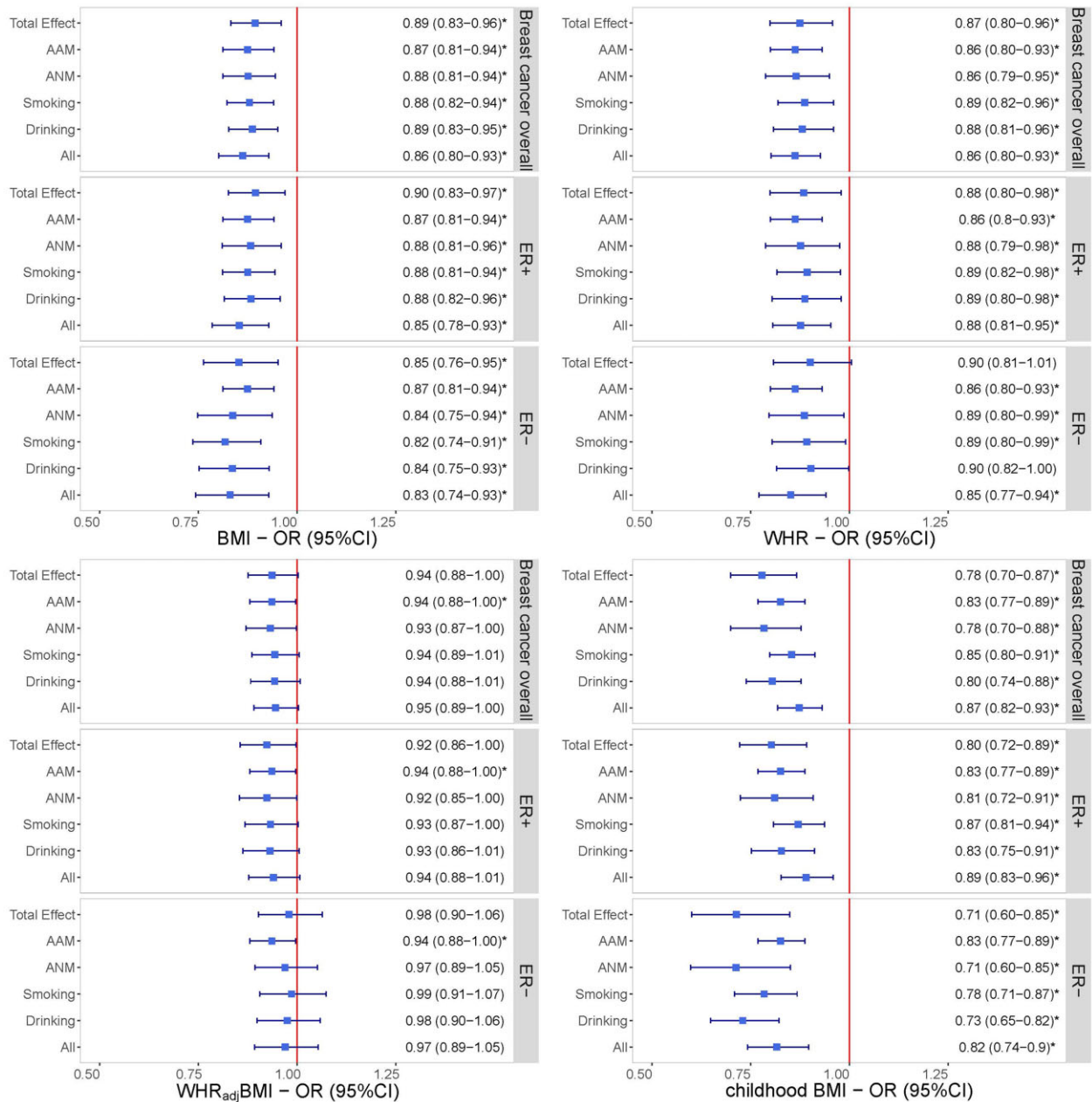
Figure 3 Independent effects of genetically predicted obesity-related traits on the risk of breast cancer after adjusting for each confounder separately and together using multivariable Mendelian randomization

The *y*-axis details the genetically predicted confounder(s) for which adjustment was made and the *x*-axis details the odds ratios and 95% confidence intervals per 1-standard deviation increase in exposure. Asterisks (*) denote the tests survived false discovery rate (FDR) correction ($P_{FDR} < 0.05$). Total effect refers to the estimate derived from univariable Mendelian randomization.

BMI, body mass index; WHR, waist-to-hip ratio; WHR$_{adj}$BMI, waist-to-hip ratio adjusted for body mass index; ER, oestrogen receptor; AAM, age at menarche; ANM, age at natural menopause; OR, odds ratio.

$P = 3.40 \times 10^{-5}$) and ER− subtype (adjusted for adult BMI: OR = 0.83, 95% CI = 0.72–0.96, $P = 1.43 \times 10^{-2}$; adjusted for adult WHR: OR = 0.76, 95% CI = 0.68–0.85, $P = 6.10 \times 10^{-7}$; adjusted for adult WHR$_{adj}$BMI: OR = 0.74, 95% CI = 0.67–0.82, $P = 6.09 \times 10^{-9}$).

Discussion

Our MR study revisited the causal role of multiple obesity-related traits in the development of breast cancer overall as well as its ER-defined subtypes, utilizing data from the hitherto largest GWAS(s) conducted for each trait. By

Table 2 Independent effect of adult obesity and childhood obesity on the risk of breast cancer using multivariable Mendelian randomization analysis

	BC overall		ER+		ER−	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Model 1						
BMI	1.00 (0.90–1.10)	0.96	0.99 (0.88–1.11)	0.88	0.96 (0.82–1.12)	0.58
Childhood BMI	0.84 (0.77–0.93)	3.93×10^{-4}*	0.86 (0.77–0.95)	4.05×10^{-3}*	0.83 (0.72–0.96)	1.43×10^{-2}*
Model 2						
WHR	0.90 (0.82–0.98)	1.49×10^{-2}*	0.90 (0.81–1.00)	4.29×10^{-2}	0.93 (0.84–1.04)	0.20
Childhood BMI	0.84 (0.76–0.91)	6.57×10^{-5}*	0.86 (0.78–0.95)	2.87×10^{-3}*	0.76 (0.68–0.85)	6.10×10^{-7}*
Model 3						
WHR$_{adj}$BMI	0.92 (0.86–0.99)	1.98×10^{-2}*	0.91 (0.84–0.98)	1.92×10^{-2}*	0.96 (0.88–1.05)	0.35
Childhood BMI	0.80 (0.74–0.87)	1.24×10^{-7}*	0.82 (0.75–0.90)	3.40×10^{-5}*	0.74 (0.67–0.82)	6.09×10^{-9}*

Model 1: independent effect of adult BMI and childhood BMI on BC; Model 2: independent effect of adult WHR and childhood BMI on BC; Model 3: independent effect of adult WHR$_{adj}$BMI and childhood BMI on BC. Asterisks (*) denote the tests survived false discovery rate (FDR) correction ($P_{FDR} < 0.05$).

BMI, body mass index; WHR, waist-to-hip ratio; WHR$_{adj}$BMI, waist-to-hip ratio adjusted for body mass index; BC, breast cancer; ER, oestrogen receptor; OR, odds ratio.

incorporating a set of 4-fold enlarged female-specific IVs, both the precision and the accuracy of our MR estimates were substantially improved. We successfully replicated the protective effects of genetically predicted adult BMI, adult WHR, adult WHR$_{adj}$BMI and childhood BMI on breast cancer. Integrating obesity-related traits together, we further found the effect of adult BMI on breast cancer was largely attributed to childhood BMI, whereas the effect of adult WHR (or WHR$_{adj}$BMI) was partly dependent on childhood BMI. On the contrary, childhood BMI consistently showed an independent protective effect on breast cancer irrespective of adult measures. Additionally, subtype-specific analyses suggested that the protective effects of adult and childhood BMI held true for both ER+ and ER− subtypes, whereas the effects of WHR and WHR$_{adj}$BMI were only restricted to ER+ subtypes.

Despite several studies that have applied an MR approach to discover associations between genetically predicted general obesity and breast cancer,[6–10] our work presents a comprehensive reconsideration of these associations. First, compared with previous MR, we used an enlarged set of female-specific instruments involving 281 adult BMI-associated variants explaining 4.0% of the phenotypic variance, greatly enhancing the statistical power. Second, we considered potential influence from important confounders, which previous MR did not have the opportunity for. The consistent protective effect of adult BMI on breast cancer overall with and without conditioning on confounders provided convincing evidence of a putative causal relationship. Third, using multivariable MR, we further controlled for the effect of childhood BMI and found mitigation on the effect of adult BMI, indicating the identified putative causal relationship to be largely attributed to

a high childhood BMI. These findings were supported by a previous multivariable MR[9] conducted based on data from UK Biobank and the Breast Cancer Association Consortium (adult body size: OR$_{univariable}$ = 0.82, $P = 8.04 \times 10^{-4}$ vs OR$_{multivariable}$ = 1.08, $P = 0.32$)—whereas they used questionnaire-based perceived obesity at age 10 years, we used actual measured obesity among children, minimizing the likelihood of misclassification. Collectively, these findings suggest a complex interplay underlying multiple obesity-related traits over the life course, highlighting the importance of taking multiple traits into consideration simultaneously.

Two previous univariable MRs attempted to examine the role of genetically predicted abdominal obesity in breast cancer. One used 14 sex-combined instruments of WHR and concluded a null association,[7] whereas the other used 54 sex-combined instruments of WHR$_{adj}$BMI and reported a decreased effect on breast cancer overall.[6] Our univariable MR, using an expanded set of IVs involving 203 WHR-associated female-specific SNPs, confirmed a protective effect on breast cancer overall. This protective effect of WHR remained even after adjusting for confounders and adult BMI (WHR$_{adj}$BMI), whereas it reduced to some extent after adjusting for childhood BMI. Notably, such a relationship—increased abdominal obesity associated with a decreased risk of breast cancer—conflicts with observational studies that identified a positive association for post-menopausal breast cancer[29,30] and an inconsistent association for pre-menopausal breast cancer.[31–33] One potential interpretation could be that genetically predicted WHR and WHR$_{adj}$BMI primarily reflect excessive visceral adipose tissue deposition by affecting genetic predisposition in early life, rather than in late adulthood. To the best

of our knowledge, the effect of adult abdominal obesity on breast cancer in observational studies was often modified by the obesogenic environment,[34] such as sugar-sweetened beverages, fried foods and physical inactivity, the majority of which were unlikely to be captured by our study using genetic instruments as proxies. Further experimental studies are warranted to clarify the precise molecular mechanism underlying this finding.

Our study highlights a non-trivial role of childhood BMI in the development of breast cancer. The protective effect from univariable MR was largely in line with previous work,[7,9] whereas results of multivariable MR provided strong evidence for an independent causal association of childhood obesity with breast cancer overall irrespective of adult measures. Furthermore, genetic correlation analysis confirmed a negative shared genetic basis, indicating a higher genetically predicted childhood BMI to correlate with a decreased susceptibility to breast cancer carcinogenesis. Our results corroborate findings of prospective cohort studies showing an inverse relationship between childhood BMI and breast cancer.[35,36] Potential mechanisms include a decreased frequency of ovulatory cycles[37] and earlier breast differentiation due to higher levels of oestrogens derived from adipose tissues in obese children,[38] terminally decreasing the susceptibility to malignant transformation.

Subtype-specific analyses provide implications for understanding the biological mechanisms linking genetically predicted obesity with breast cancer risk. In our study, although the effect of BMI in both childhood and adulthood on breast cancer did not differ across ER-defined subtypes (regardless of conditional analysis), the protective effects of genetically predicted adult WHR and WHR$_{adj}$BMI were restricted to ER+ subtype (consistent across all conditional analysis adjusting for childhood obesity and confounders). Obesity is known to profoundly affect oestrogen metabolism and fat-derived oestrogens are considered a principal biological mechanism through which abdominal obesity mainly impacts the risk of ER+ but not ER− subtype.[39]

This is a comprehensive MR conducted to interrogate the independent role of multiple correlated obesity traits in breast cancer using the hitherto largest female-specific data with an almost four-times increased number of instruments and a doubled phenotypic variance explained compared with previous studies, substantially improving statistical power.[40] Although reverse causality is always a potential concern for MR, our findings from bidirectional MR indicated that the obesity–breast cancer causal effect was unlikely to be biased by reverse causation. Nevertheless, we also need to acknowledge several limitations. First, although we adopted female-specific instruments for each adult obesity-related trait to match with female-specific

cancer, we were unable to estimate the sex-specific effect of childhood obesity due to data restrictions. Given that the sex instrumental heterogeneity has been recently confirmed to have a non-ignorable impact on the estimates of two-sample MR,[12] future investigations would benefit from developing girl-specific IVs of childhood obesity. Second, pleiotropy derived from undetected confounders might bias the causal estimates. However, we tried to reduce such bias to the best of our ability. The directional consistent results derived from multiple 'pleiotropy-robust' methods[41] supported the validity of our MR results. Lastly, as the two-sample MR approach is typically based on linear assumption, we could not examine the nonlinear relationship that has been extensively evaluated in the traditional epidemiological studies.[42,43] Future one-sample MR studies using semi-parametric methods[44] are perhaps warranted.

To conclude, our comprehensive MR study with an enlarged sample size successfully replicated the inverse relationship of obesity with the risk of breast cancer. We further identified that the total effect of adult general obesity on breast cancer was largely attributed to childhood obesity, whereas that of adult abdominal obesity was at least partly attributed to childhood obesity. Finally, we demonstrated a predominantly independent effect of childhood BMI in affecting breast cancer onset, irrespective of adult measures. Nevertheless, our results do not advocate weight gain as a preventative intervention against breast cancer, but rather highlight the importance of considering together multiple exposures of obesity at different time points across the life course. We anticipate that understanding the biological mechanisms underlying the inverse association of early-life obesity with breast cancer might motivate the identification of additional modifiable risk factors.

Ethics approval

All analyses were conducted using summary-level data generated by previous studies that have described their relevant ethical approvals.

Data availability

Publicly available GWAS data on breast cancer were obtained from https://bcac.ccge.medschl.cam.ac.uk/bcacdata/. Adult BMI and WHR GWAS data are publicly available at https://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files. Childhood BMI GWAS data are publicly available at https://egg-consortium.org/. Age at menarche and age at natural menopause GWAS data are publicly available at https://www.reprogen.org/data_download.html. Smoking and drinking data are publicly available at https://genome.psych.umn.edu/index.php/GSCAN.

Supplementary data

Supplementary data are available at *IJE* online.

Author contributions

Y.H., J.X., X.J. and J.L. designed the study; Y.L., X.W., H.Z., C.X. and L.Z. provided code and analytical advice; S.B., N.W., X.Z. and P.K. provided analytical advice and critical interpretation of findings; Y.H. and J.X. conducted all analyses and drafted the manuscript; all authors edited and approved the manuscript.

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Conflict of interest

None declared.

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