**Additional file 1: Detailed methods of Mendelian randomization study**

**Study design**

This Mendelian randomization (MR) study adheres to the STROBE-MR reporting guidelines, fully published in JAMA in 2021[1], thus improving the quality of the study. The exposure and outcome analyzed in this study were derived from distinct populations, ensuring no overlap in samples. This study made use of publicly accessible, de-identified summary data. Informed consent was secured from every participant in the initial research, with each study receiving approval from the local ethics board.

The core assumptions of MR include three key principles[2]: the correlation assumption, which requires a strong association between instrumental variables and exposure factors; the independence assumption, meaning that instrumental variables are uncorrelated with any confounding factors; and the exclusion restriction assumption, which stipulates that instrumental variables affect the outcome solely through the exposure factors, without a direct association with the outcome itself.

In this comprehensive MR analysis, we examined serum γ-glutamyl transferase (GGT) levels as the exposure and breast cancer as the outcome. Initially, we performed a univariate MR analysis to assess the causal effect of GGT on breast cancer. To account for potential confounders and determine the independent causal effect of GGT, we incorporated relevant covariates into a multivariate MR framework.

All analyses were performed using R studio (version 4.3.3) with the TwoSampleMR package (version 0.6.8), MendelianRandomization package (version 0.8.0), RadialMR package (version 1.1), and MVMR package (version 0.4).

**Exposure data**

The genome-wide association studies (GWAS) data for GGT was obtained from a recent study by Raha Pazoki et al.[3], which conducted a genetic analysis of liver enzyme levels in European ancestry individuals. Compared to a previous study investigating serum liver enzyme activity[4], this study identified a greater number of genetic loci, pinpointing 328 SNPs for GGT at a predefined stringent significance threshold of $P < 1 \times 10^{-8}$ among 437,194 participants. The findings underscore the pivotal role of liver-regulated molecular pathways in metabolic disorders. The GGT GWAS dataset is publicly accessible in the GWAS Catalog under the data ID

GCST90013407 (https://www.ebi.ac.uk/gwas/studies/GCST90013407). In the present study, the GGT GWAS data were harmonized with data from the catalog.

**Outcome data**

The FinnGen study is a large-scale genomics initiative that has analyzed over 500,000 Finnish biobank samples and correlated genetic variation with health data to understand disease mechanisms and predispositions[5]. GWAS data on the breast cancer outcome were obtained from the FinnGen R12 database. The breast cancer trait was defined according to the International Classification of Diseases-10 (C50) criteria. The summary statistics included 24,270 cases and 222,078 controls. For further details, please refer to the original source (https://risteys.finngen.fi/endpoints/C3_BREAST).

**Covariates data**

Body mass index (BMI), a widely used measure for assessing obesity levels, has also been implicated in cancer risk. Previous research indicates that prolonged duration and higher severity of overweight and obesity during early adulthood, as well as an earlier onset of elevated BMI, are associated with an increased risk of 18 different types of cancer[6]. Alcohol consumption is another major contributor to the global cancer burden, with strong links to the development of various cancers[7, 8]. In multivariate MR study, BMI and alcohol drinking status were included as covariates. Their GWAS summary data were obtained from the IEU database, with GWAS IDs ebi-a-GCST90029007 and ukb-d-20117_2, respectively.

**Selection of genetic instruments**

Genetic instruments were selected using a stringent genome-wide significance threshold ($P < 5 \times 10^{-8}$)[9]. Samples of European ancestry from the 1000 Genomes Project were evaluated for linkage disequilibrium (LD) using the PLINK clustering method. For quality assurance of the instrument, only standard SNPs that are within 10,000 kb of the index variant and have an $r^2$ value under 0.001 were preserved[10]. To minimize bias from weak instruments, F-statistics were calculated, and SNPs with F-statistics <10 were excluded to ensure that the retained instrument SNPs had sufficient statistical power[11]. Ambiguous effect alleles and palindromic SNPs were also excluded from the analysis.

**Univariate MR analysis**

In the univariate MR analysis, we utilized the inverse variance-weighted (IVW) estimates as primary outcome measure. A radial MR approach was utilized to identify and exclude SNPs that significantly contributed to horizontal pleiotropy and heterogeneity. This process improved the quality of the IVW radial regression plots[12]. Additionally, we performed supplementary analyses using MR-Egger, Weighted median, Simple mode, and Weighted mode methods[10]. Only MR models with significant IVW estimates and consistent effect directions across all methods were considered significant. Heterogeneity was evaluated using Cochran's Q test[13], while potential horizontal pleiotropy was assessed with the Egger intercept test[14]. To ensure the reliability of our findings, a leave-one-out analysis and funnel plots were conducted. Scatter plots were generated to visualize the strength and direction of associations. Additionally, statistical power was evaluated using an online calculator (https://sb452.shinyapps.io/power/)[15].

**Multivariate MR analysis**

To examine the independent effects of GGT levels on breast cancer, we constructed a combined instrumental variable set incorporating GGT data, BMI and alcohol drinking status[16]. The effect sizes of genetic prediction were estimated using both IVW and MR-Egger methods. Heterogeneity was evaluated, and the Egger intercept test was used to assess horizontal pleiotropy. If $P < 0.05$ in the IVW heterogeneity Q test, it indicates the presence of heterogeneity. In such cases, alternative methods, such as the Weighted Median approach, should be considered to address heterogeneity, as they can provide more reliable estimates of causality.

**Reference**

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