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Identification of Clinically Relevant Druggable Targets for Breast Cancer through Mendelian Randomization and Population-Based Analyses

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Abstract

Repurposing existing medications offers a practical and economical way to tackle the demand for new breast cancer prevention and treatment options. Our goal was to pinpoint druggable targets with potential clinical relevance through Mendelian randomization (MR) and confirm promising drug candidates via real-world population data. We selected genetic variants as instruments for 1406 actionable targets of licensed non-cancer drugs, drawing from gene expression (eQTL), DNA methylation (mQTL), and protein expression (pQTL) quantitative trait loci. Summary statistics came from a large breast cancer genome-wide association study (GWAS) by the Breast Cancer Association Consortium (122,977 cases and 105,974 controls). We also performed a nested case-control analysis using Swedish national register data to evaluate the drugs highlighted by the MR results. MR analyses revealed six notable links at the gene expression level (TUBB, MDM2, CSK, ULK3, MC1R, and KCNN4) and two at the DNA methylation level involving 21 CpG sites (RPS23 and MAPT). In the nested case-control study, raloxifene use (which targets MAPT) correlated with a 35% lower breast cancer risk (odds ratio [OR]: 0.65; 95% confidence interval [CI]: 0.51–0.83). Conversely, estradiol, tolterodine, and nitrofurantoin (also linked to MAPT) showed associations with elevated risk, with adjusted ORs (95% CIs) of 1.10 (1.07–1.13), 1.16 (1.09–1.24), and 1.09 (1.05–1.13), respectively. The associations for raloxifene and nitrofurantoin no longer reached significance in sensitivity analyses employing active-comparator and new-user designs. This extensive MR investigation, supported by population-level confirmation, uncovered eight druggable genes linked to breast cancer and highlighted raloxifene as a valuable agent for chemoprevention.

Keywords: Drug repositioning, Breast cancer, Druggable target, Mendelian randomization, Population-based study

Introduction

Among women, breast cancer has become the most common cancer globally (2.3 million new cases, 11.7% of all cancers). It ranks as the fifth leading cause of cancer death, accounting for 685,000 fatalities in 2020 [1]. In high-income nations, incidence rates have stabilized or slowed since the mid-2000s, while mortality has steadily declined thanks to better prevention programs, heightened awareness, earlier diagnosis, and more effective therapies [2]. In lower- and middle-income countries, however, death rates continue to climb, reflecting limited access to screening and treatment resources [3]. Given that prevention represents the most affordable approach to reducing cancer burden and mitigating its wide-ranging consequences, there is an urgent need for drugs capable of lowering breast cancer incidence. Drug repurposing—the use of already approved medications originally developed for non-cancer indications—offers an efficient means to expand prevention and treatment options at reduced cost [4].

The expanding body of human genetic information is increasingly leveraged to guide pharmaceutical

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development. Drug-target-disease relationships backed by genetic evidence can substantially raise success rates in drug pipelines [5]. By employing single-nucleotide polymorphisms (SNPs) as instruments, Mendelian randomization (MR) mimics the random allocation of genotypes (Mendel's second law) to estimate the causal effects of modifying a drug target, much like a randomized controlled trial but without actual drug administration. This framework has proven valuable for exploring the repurposing potential of existing drugs, thereby enhancing development efficiency and minimizing late-stage attrition risks [6].

To discover candidate druggable targets for breast cancer, we performed large-scale MR analyses utilizing genetic data on gene expression, methylation, and protein levels for genes encoding targets of approved drugs. We concentrated on actionable druggable genes—those encoding proteins targeted by licensed medications. We then tested these MR findings in a nested case-control study using comprehensive Swedish national register data to determine whether drugs acting on the identified targets confer protection against breast cancer.

Materials and Methods

Identification of actionable druggable targets

We obtained data on medications and their corresponding biological targets from the ChEMBL database, a comprehensive, publicly available resource for drug discovery [7]. To enable repurposing of non-cancer drugs and subsequent verification with national registry information, we restricted our focus to actionable proteins serving as therapeutic targets or potential targets of FDA-approved non-oncology drugs (excluding oncology agents classified under Anatomical Therapeutic Chemical code "L01"). We derived therapeutic targets from ChEMBL's mechanism-of-action information and mapped them to their constituent proteins. We excluded nonhuman proteins and non-drug-binding components within protein complexes. For approved drugs, we identified additional human target proteins from ChEMBL pharmacological assay records showing strong binding affinity or efficacy (pChEMBL value ≥ 7, corresponding to measurements ≤ 100 nM) [8]. In total, we compiled 1406 distinct human proteins classified as 'actionable', encompassing 660 therapeutic targets and 1074 potential targets bound by FDA-approved drugs. While the clinical connection between some targets in the latter category and the drug's primary indication may not be fully confirmed, the strong affinity/efficacy data suggest these drugs can effectively modulate the proteins. Pathway and process enrichment analysis of these potential targets revealed 177 genes directly implicated in breast cancer pathways.

Selection of instruments for actionable druggable targets

We utilized eQTL summary data from the eQTLGen Consortium (accessible at https://www.eqtlgen.org/cis-eqtls.html) to select genetic variants linked to gene expression in blood, positioned within 1000 kb upstream or downstream of the gene's coding region (cis-eQTL) [9]. These variants served as instruments for the druggable targets. The eQTLGen dataset includes data on 10,317 trait-associated SNPs from 31,684 participants, though details for genes on the X and Y chromosomes and mtDNA were unavailable. From the cis-eQTL data, we extracted 438,534 SNPs related to the expression of 852 druggable target transcripts.

We additionally derived eQTL instruments from GTEx version 8 data in individuals of European ancestry, using 459 breast tissue samples [10]. This yielded 19,852 SNPs linked to the expression of 406 druggable target genes.

For DNA methylation, we obtained instruments from a meta-analysis of genetic variants associated with methylation levels of druggable targets across two cohorts (total n = 1980) [11]. This resulted in 1,092,040 SNPs corresponding to 5592 druggable target-related CpG sites.

We sourced instruments for protein expression from a study measuring 4907 aptamer-based plasma protein levels in 35,559 Icelandic participants [12]. We retrieved 2364 SNPs robustly associated with the levels of 146 druggable proteins.

A standard genome-wide significance threshold of $P < 5 \times 10^{-8}$ was applied to select the strongest associated variants as instruments for the SMR analyses.

Estimates for breast cancer

Summary statistics for breast cancer and its subtypes in individuals of European ancestry were acquired from the Breast Cancer Association Consortium (BCAC), comprising 122,977 cases and 105,974 controls [13]. These data are publicly available via the consortium website.

Mendelian randomization and colocalization

We estimated power for the MR analysis using an online calculator based on a total sample size of 228,951 participants, yielding 89.7% power.

We applied the Summary-based MR (SMR) method (version 1.02) to perform two-sample MR analyses, with breast cancer as the outcome and druggable targets (at gene expression, DNA methylation, and protein expression levels) as exposures. Analyses were performed via command line (code available at https://yanglab.westlake.edu.cn/software/smr/#Overview) [14]. Odds ratios (ORs) estimating the effect of druggable targets on breast cancer risk were obtained by exponentiating the β coefficient. The QTL β value indicates the direction of effect on gene expression, methylation, or protein levels (positive β for increase, negative β for decrease). ORs reflect the change in disease odds per one standard deviation alteration in the exposure. To account for multiple testing, we applied Bonferroni correction by adjusting the significance threshold (α) according to the number of tests performed. To assess potential influence from linkage disequilibrium (LD), we conducted the HEIDI test, which also functions as a colocalization approach [15]. Probes displaying strong LD evidence (HEIDI $P < 0.05$) were excluded.

Furthermore, we performed Bayesian colocalization testing using the "coloc" R package (version 5.1.0; https://chr1swallace.github.io/coloc/) to calculate posterior probabilities of shared causal variants. For every significant MR finding, SNPs within a ±100 kb window around the lead SNP were extracted for colocalization between the QTL and GWAS signals. A posterior probability for hypothesis 4 (PP.H4) ≥ 80% was required as evidence of colocalization. **Figure 1** illustrates the overall analytical workflow.

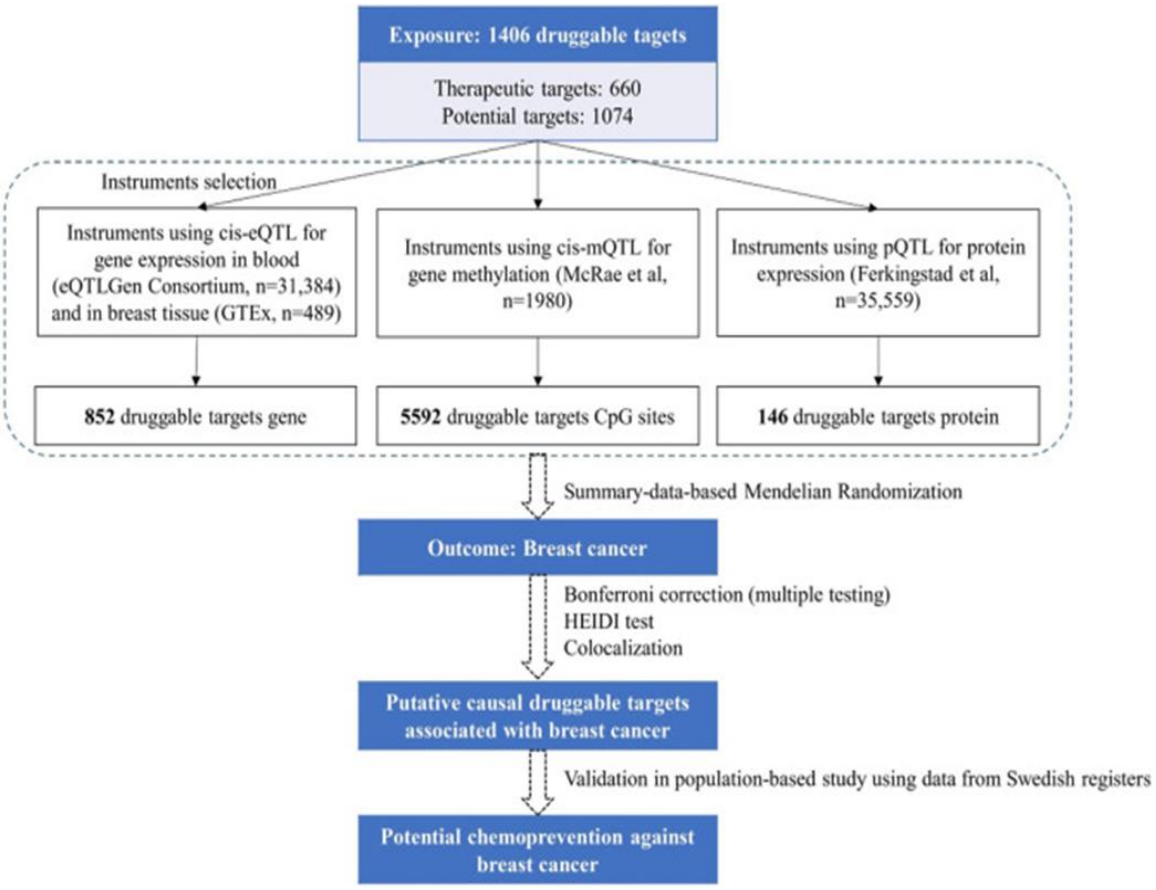

Figure 1. Diagram illustrating the sequence of analyses conducted in the study.

We performed additional sensitivity checks employing the TwoSampleMR R package to confirm the key associations found in the main SMR results. Since horizontal pleiotropy can distort causal inferences in MR studies, we examined this issue with the MR-Egger regression method. This technique accounts for

pleiotropy through an intercept term that captures the average directional pleiotropic effect. We also examined the consistency of estimates by testing for heterogeneity using Cochran's Q statistic.

Validation of candidate drugs through population-based analysis

To assess whether the potential drugs highlighted by MR analyses affect breast cancer risk, we conducted a nested case-control investigation using comprehensive Swedish registry data. The base cohort comprised 4,537,661 women born in Sweden who were listed in the Total Population Register from July 1, 2005, to March 31, 2014 [16]. Because the national Prescribed Drug Register started in July 2005, follow-up began on July 1, 2006. Women with any cancer diagnosis recorded before this entry date were removed from the analysis (n = 190,883). We identified 41,917 women diagnosed with breast cancer between January 1, 2007, and March 31, 2014, using ICD-10 code C50 from the Swedish Cancer Registry [17]. The index date for cases was the breast cancer diagnosis date.

We matched each case to five randomly selected controls from the risk set at the time of case diagnosis via incidence-density sampling. Controls had no cancer history before their selection date and were matched on a propensity score. This score was calculated using age, highest educational attainment (1–9, 10–11, or ≥ 12 years), income quartile (lowest, middle-low, middle-high, highest), area of residence (large cities, southern Sweden, northern Sweden), number of children (0, 1, 2, 3, ≥ 4), presence of obesity (yes/no), COPD diagnosis (yes/no, serving as a proxy for smoking), and Charlson Comorbidity Index (0, 1, 2, ≥ 3). Controls received the identical index date as their matched case.

Exposure status was defined as having filled at least one prescription for the candidate drugs between July 2005 and March 2014, identified through ATC codes in the Swedish Prescribed Drug Register.

We applied conditional logistic regression to compute odds ratios and 95% confidence intervals linking candidate drug use to breast cancer occurrence within the matched sets. We used Bonferroni adjustment to control for multiple comparisons and limit false positives.

To minimize confounding by indication, we ran further sensitivity analyses adopting an active-comparator approach and a new-user design to verify the primary case-control findings.

We conducted these register-based analyses using SAS software version 9.4 (SAS Institute, Cary, NC). The study received ethical clearance from the Regional Ethical Review Board in Lund.

Results and Discussion

MR analysis using cis-eQTLs for breast cancer risk

We applied a Bonferroni correction to control the overall type I error rate across multiple tests. Thresholds were set at $P < 5.87 \times 10^{-5}$ (0.05 divided by 852 genes) for blood gene expression and $P < 1.23 \times 10^{-4}$ (0.05 divided by 406 genes) for breast tissue expression. We then applied HEIDI testing (requiring P_HEIDI > 0.05). This process yielded seven independent signals at seven separate loci in blood and one locus in breast tissue. We then conducted colocalization testing to exclude confounding due to shared variants or pleiotropy, applying a threshold of PP.H4 > 0.80 (**Figure 2**).

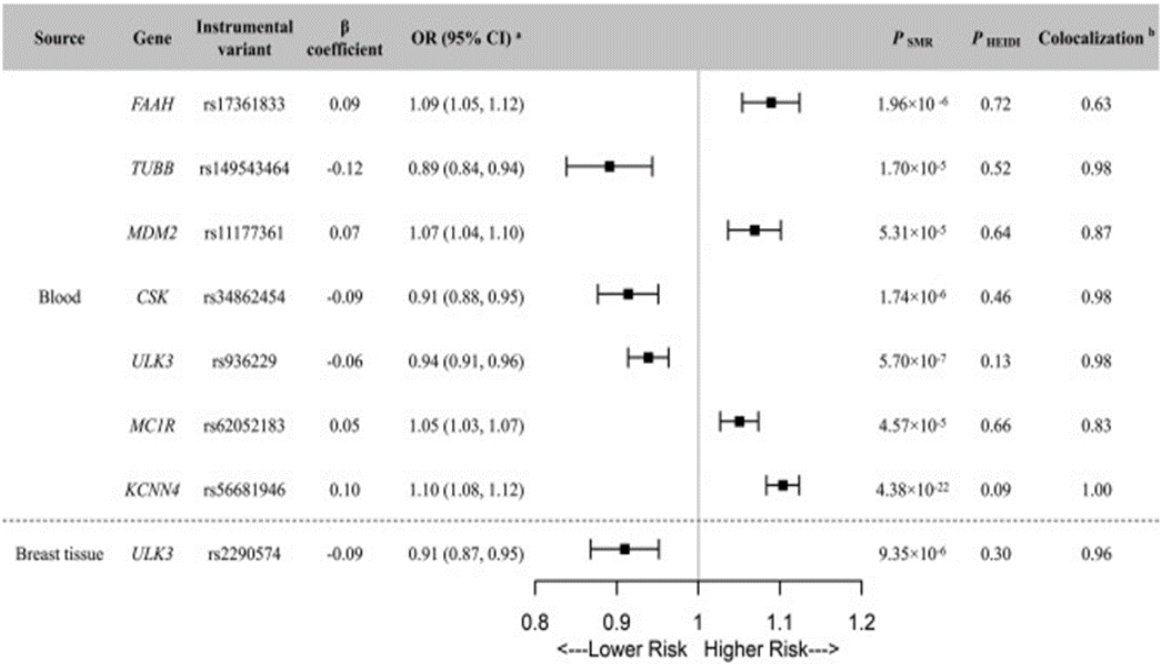

Figure 2. Summary of Mendelian randomization findings showing links between druggable target gene expression and breast cancer susceptibility.

[a] Odds ratios derived by exponentiating the causal β estimate.

[b] 'Colocalization' refers to the posterior probability PP.H4 for shared causal variants between eQTL and GWAS data. A PP.H4 exceeding 0.8 is the standard threshold indicating strong colocalization evidence.

In analyses utilizing instruments from blood-derived eQTL data, higher TUBB expression (per 1-SD increase) correlated with an 11% lower breast cancer risk (OR: 0.89; 95% CI: 0.84–0.94; $P_{SMR} = 1.70 \times 10^{-5}$). Likewise, elevated expression of CSK and ULK3 in blood was associated with 9% (OR: 0.91; 95% CI: 0.88–0.95; $P_{SMR} = 1.74 \times 10^{-6}$) and 6% (OR: 0.94; 95% CI: 0.91–0.96; $P_{SMR} = 5.70 \times 10^{-7}$) reductions in risk, respectively. In contrast, greater expression of MDM2, MC1R, and KCNN4 in blood was tied to higher breast cancer risk, with corresponding ORs (95% CIs) and P_{SMR} values of 1.07 (1.04–1.10; $P_{SMR} = 5.31 \times 10^{-5}$), 1.05 (1.03–1.07; $P_{SMR} = 4.57 \times 10^{-5}$), and 1.10 (1.08–1.12; $P_{SMR} = 4.38 \times 10^{-22}$).

Notably, increased ULK3 expression specifically in breast tissue samples also showed a protective association (OR per SD: 0.91; 95% CI: 0.87–0.95; $P_{SMR} = 9.35 \times 10^{-6}$), aligning closely with the blood-based findings.

MR analysis of cis-mQTL and breast cancer outcomes

We applied a Bonferroni correction ($P_{SMR} < 8.94 \times 10^{-6}$) along with the HEIDI test to filter reliable SMR associations between DNA methylation at druggable target sites and breast cancer. This process uncovered 33 significant signals distributed across 28 distinct genetic loci (**Figure 3**). Methylation at RPS23 displayed a positive relationship with breast cancer risk (OR: 1.08; 95% CI: 1.06–1.13; $P_{SMR} = 5.03 \times 10^{-10}$). Additionally, we detected 15 separate loci influencing methylation at 20 different CpG sites within MAPT, each showing an inverse association with breast cancer risk.

Gene	Methylation prob	Instrumental variant	β coefficient	OR (95% CI) [a]	P_{SMR}	P_{HEIDI}	Colocalization [b]
SV2A	cg11085602	rs11205303	0.12	1.12 (1.09, 1.16)	3.93×10^{-12}	0.87	1.3×10^{-11}
DCLK2	cg02587405	rs7685302	0.09	1.09 (1.06, 1.13)	5.13×10^{-6}	0.71	4.27×10^{-4}
RPS23	cg03785828	rs226205	0.08	1.08 (1.06, 1.11)	5.03×10^{-10}	0.11	0.99
CYP51A1	cg01341755	rs10231350	0.18	1.20 (1.12, 1.28)	6.43×10^{-6}	0.15	2.33×10^{-5}
	cg22117172	rs6972027	0.09	1.10 (1.07, 1.13)	1.27×10^{-8}	0.45	1.95×10^{-7}
	cg03714773	rs9691343	0.11	1.11 (1.07, 1.15)	8.09×10^{-8}	0.95	1.4×10^{-7}
	cg01689657	rs6953816	0.10	1.10 (1.07, 1.14)	1.64×10^{-8}	0.55	1.76×10^{-7}
COL1A2	cg20943251	rs9649190	-0.08	0.93 (0.9, 0.95)	2.08×10^{-10}	0.05	2.34×10^{-8}
FGFR2	cg07250222	rs1649155	-0.46	0.63 (0.48, 0.78)	1.32×10^{-8}	0.39	3.42×10^{-127}
CYP1A2	cg01359532	rs11072508	-0.09	0.92 (0.88, 0.95)	4.84×10^{-6}	0.45	0.01
ULK3	cg04877966	rs9210	0.06	1.06 (1.04, 1.08)	1.15×10^{-6}	0.61	0.02
MAPT	cg05301556	rs10445363	0.08	1.08 (1.05, 1.11)	2.96×10^{-8}	0.10	0.86
	cg11117266	rs144416125	0.17	1.18 (1.11, 1.25)	7.81×10^{-6}	0.12	0.96
	cg16520312	rs113790915	0.08	1.08 (1.05, 1.10)	1.82×10^{-8}	0.17	0.88
	cg23202277	rs112995313	-0.11	0.90 (0.86, 0.94)	1.43×10^{-7}	0.18	0.94
	cg00891649	rs112003140	0.06	1.06 (1.04, 1.08)	9.82×10^{-8}	0.46	0.86
	cg11489262	rs112995313	-0.12	0.89 (0.84, 0.93)	2.94×10^{-7}	0.62	0.94
	cg18878992	rs112155389	-0.06	0.94 (0.93, 0.96)	6.92×10^{-8}	0.45	0.96
	cg14202850	rs112746008	-0.11	0.90 (0.86, 0.94)	1.61×10^{-7}	0.29	0.96
	cg10224600	rs112197756	-0.15	0.86 (0.8, 0.92)	2.14×10^{-6}	0.59	0.97
	cg24801230	rs111751251	0.03	1.03 (1.02, 1.04)	5.13×10^{-10}	0.22	0.94
	cg18228076	rs111751251	0.03	1.03 (1.02, 1.04)	5.62×10^{-10}	0.21	0.94
	cg05772917	rs112155389	-0.15	0.86 (0.80, 0.92)	2.13×10^{-6}	0.15	0.96
	cg02228913	rs112572874	0.03	1.03 (1.02, 1.04)	1.5×10^{-8}	0.17	0.93
	cg21705961	rs17689882	0.06	1.06 (1.04, 1.08)	9.84×10^{-8}	0.55	0.85
	cg01934064	rs2668668	-0.08	0.93 (0.90, 0.95)	7.08×10^{-8}	0.18	0.95
	cg00480298	rs112746008	-0.13	0.88 (0.82, 0.93)	7.79×10^{-7}	0.08	0.96
	cg05721485	rs111970616	-0.12	0.89 (0.84, 0.93)	$3.29\times10^{-}$	0.21	0.97
	cg12604352	rs112310745	0.13	1.14 (1.09, 1.20)	8.36×10^{-7}	0.17	0.94
	cg07368061	rs112003140	0.05	1.05 (1.04, 1.07)	6.27×10^{-8}	0.27	0.86
	cg09764761	rs145848989	0.06	1.06 (1.04, 1.08)	1.44×10^{-8}	0.55	0.96
KCNN4	cg11624345	rs62116961	-0.29	0.75 (0.65, 0.85)	6.01×10^{-8}	0.56	1.84×10^{-18}
GIPR	cg17351154	rs10423928	-0.10	0.90 (0.87, 0.94)	2.9×10^{-7}	0.06	2.35×10^{-6}

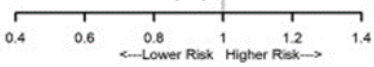

Figure 3. Mendelian randomization findings on the relationship between methylation of druggable target genes and breast cancer risk.

[a] Odds ratios were obtained by exponentiating the causal β coefficient.

[b] 'Colocalization' denotes the posterior probability PP.H4 for shared causal variants between mQTL and GWAS signals. A PP.H4 value above 0.8 serves as the conventional threshold supporting colocalization.

We additionally performed SMR analyses to explore potential causal effects of DNA methylation at druggable target genes on their corresponding expression levels, leveraging shared genetic instruments to connect methylation with expression.

MR analysis of pQTL and breast cancer outcomes

From the available pQTL data, only 146 suitable instruments could be derived. Protein expression levels of COL6A3, EGFR, and ULK3 showed associations with breast cancer risk. However, the HEIDI test could not be performed for these signals, most likely because of insufficient linkage disequilibrium reference data at those genomic regions.

Sensitivity analysis using the TwoSampleMR package

Sensitivity analyses with the TwoSampleMR package further supported the main findings, confirming significant associations between breast cancer risk and gene expression levels of TUBB, MDM2, CSK, ULK3, and MC1R, as well as methylation at RPS23 and MAPT.

Validation by a population-based study

We performed a nested case–control study using Swedish national register data to examine whether the candidate drugs influence breast cancer development. Over a mean follow-up period of 7.1 years, 41,917 breast cancer cases were identified within the source population, corresponding to an incidence rate of 4.34 per 10,000 person-years. **Table 1** summarizes baseline characteristics for the cases and matched controls.

Table 1. Demographic and clinical characteristics among breast cancer patients and matched controls.

	Cases (n = 41,917)		**Controls (n = 209,583)**		**SMD**
	Mean	**SD**	**Mean**	**SD**	
Age	63.96	13.70	63.96	13.70	< 0.001
	No.	%	No.	%	
Highest education level, *year*					< 0.001
1–9	10,912	26.0	54,499	26.0	
10–11	16,922	40.4	84,846	40.5	
≥ 12	14,083	33.6	70,238	33.5	
Income					0.002
Lowest	9964	23.8	49,875	23.8	
Middle-low	10,522	25.1	52,817	25.2	
Middle-high	10,665	25.4	53,206	25.4	
Highest	10,766	25.7	53,685	25.6	
Residence area					0.006
Big cities	6518	15.5	32,131	15.3	
South Sweden	27,379	65.3	137,271	65.5	
North Sweden	8020	19.1	40,181	19.2	
Obesity					0.030
No	41,330	98.6	207,339	98.9	
Yes	587	1.4	2244	1.1	
COPD					0.011
No	38,820	92.6	194,696	92.9	
Yes	3097	7.4	14,887	7.1	
Parity					0.002
0	5794	13.8	28,717	13.7	
1	6931	16.5	34,592	16.5	
2	17,818	42.5	89,409	42.7	
3	8311	19.8	41,708	19.9	
≥ 4	3063	7.3	15,157	7.2	
CCI					0.017
0	31,654	75.5	159,180	76.0	
1	6876	16.4	34,559	16.5	
2	2102	5.0	9960	4.8	
≥ 3	1285	3.1	5884	2.8	

SMD = standardized mean difference.

A total of 319 potential medications emerged from the Mendelian randomization (MR) evaluations that displayed notable colocalization signals. After excluding non-oral agents and those with rare exposure (below 0.1% prevalence in the cohort), 36 medications advanced to evaluation within the nested case-control investigation. **Figure 4** illustrates the usage rates and odds ratios (ORs) of these candidate medications among cases versus controls. To minimize false positives, we applied a Bonferroni adjustment ($P < 0.0014$, based on 0.05/36).

Results indicated that raloxifene intake correlated with a 35% lower breast cancer risk (95% CI, 0.51–0.83; P = 0.0004). Conversely, exposure to estradiol, tolterodine, and nitrofurantoin linked to elevated breast cancer risk, showing adjusted ORs of 1.10 (95% CI, 1.07–1.13; $P < 0.0001$), 1.16 (95% CI, 1.09–1.24; $P < 0.0001$), and 1.09 (95% CI, 1.05–1.13; $P < 0.0001$), respectively. Outcomes from the sensitivity analyses 9, which applied active-comparator and new-user frameworks, largely aligned with the main findings for estradiol and nitrofurantoin. That said, raloxifene's protective association became only marginally non-significant owing to limited statistical power (HR, 0.50; 95% CI, 0.24–1.04; P = 0.064), while tolterodine showed no meaningful link to breast cancer risk (HR, 1.02; 95% CI, 0.92–1.13; P = 0.711).

Drug	ATC	No. (%) of users in cases	No. (%) of users in controls	Adjusted HR[a]	95% CI	P
Pantoprazole	A02BC02	8849 (21.11)	43958 (20.97)	1.06	0.97-1.16	0.1736
Lansoprazole	A02BC03	612 (1.46)	2829 (1.35)	1.00	0.94-1.07	0.9598
Rabeprazole	A02BC04	1170 (2.79)	5807 (2.77)	0.94	0.70-1.28	0.7117
Sulfasalazine	A07EC01	224 (0.53)	1168 (0.56)	0.96	0.83-1.11	0.5532
Orlistat	A08AB01	420 (1.00)	1837 (0.88)	1.09	0.98-1.21	0.1280
Glibenclamide	A10BB01	428 (1.02)	2054 (0.98)	1.00	0.90-1.11	0.9591
Glipizide	A10BB07	234 (0.56)	1040 (0.50)	1.11	0.96-1.28	0.1681
Dipyridamole	B01AC07	307 (0.73)	1391 (0.66)	1.09	0.96-1.23	0.2059
Carvedilol	C07AG02	177 (0.42)	746 (0.36)	1.18	1.00-1.39	0.0482
Felodipine	C08CA02	3023 (7.21)	14412 (6.88)	1.05	1.00-1.09	0.0309
Nifedipine	C08CA05	199 (0.47)	1111 (0.53)	0.89	0.77-1.04	0.1415
Captopril	C09AA01	78 (0.19)	333 (0.16)	1.15	0.90-1.47	0.2767
Bromocriptine	G02CB01	47 (0.11)	212 (0.10)	1.10	0.80-1.51	0.5513
Estradiol	G03CA03	8803 (21.00)	41141 (19.63)	1.10	1.07-1.13	<.0001
Progesterone	G03DA04	123 (0.29)	679 (0.32)	0.89	0.73-1.09	0.2609
Clomifene	G03GB02	91 (0.22)	455 (0.22)	1.00	0.79-1.25	0.9745
Raloxifene	G03XC01	76 (0.18)	585 (0.28)	0.65	0.51-0.83	0.0004
Tolterodine	G04BD07	1090 (2.60)	4701 (2.24)	1.16	1.09-1.24	<.0001
Liothyronine sodium	H03AA02	43 (0.10)	215 (0.10)	0.98	0.71-1.36	0.9087
Nitrofurantoin	J01XE01	3752 (8.95)	17407 (8.31)	1.09	1.05-1.13	<.0001
Itraconazole	J02AC02	120 (0.29)	544 (0.26)	1.10	0.90-1.34	0.3658
Indometacin	M01AB01	161 (0.38)	872 (0.42)	0.91	0.77-1.08	0.2967
Ketoprofen	M01AE03	1363 (3.25)	6435 (3.07)	1.06	1.00-1.13	0.0555
Chlorzoxazone	M03BB03	1223 (2.92)	5538 (2.64)	1.11	1.04-1.18	0.0019
Paracetamol	N02BE01	14129 (33.71)	69789 (33.3)	1.01	0.99-1.04	0.2780
Dihydroergotamine	N02CA01	79 (0.19)	498 (0.24)	0.79	0.62-1.00	0.0491
Phenytoin	N03AB02	64 (0.15)	386 (0.18)	0.83	0.64-1.08	0.1696
Perphenazine	N05AB03	91 (0.22)	399 (0.19)	1.14	0.91-1.44	0.2544
Prochlorperazine	N05AB04	74 (0.18)	405 (0.19)	0.88	0.68-1.13	0.2965
Flupentixol	N05AF01	123 (0.29)	644 (0.31)	0.95	0.78-1.15	0.5821
Aripiprazole	N05AX12	73 (0.17)	316 (0.15)	1.13	0.87-1.46	0.3697
Zopiclone	N05CF01	5689 (13.57)	27266 (13.01)	1.05	1.01-1.08	0.0054
Disulfiram	N07BB01	168 (0.40)	656 (0.31)	1.29	1.08-1.53	0.0038
Acetylcysteine	R05CB01	5549 (13.24)	26646 (12.71)	1.04	1.01-1.07	0.0251
Noscapine	R05DA07	96 (0.23)	431 (0.21)	1.10	0.88-1.37	0.4193
Loratadine	R06AX13	1529 (3.65)	7067 (3.37)	1.08	1.02-1.14	0.0107

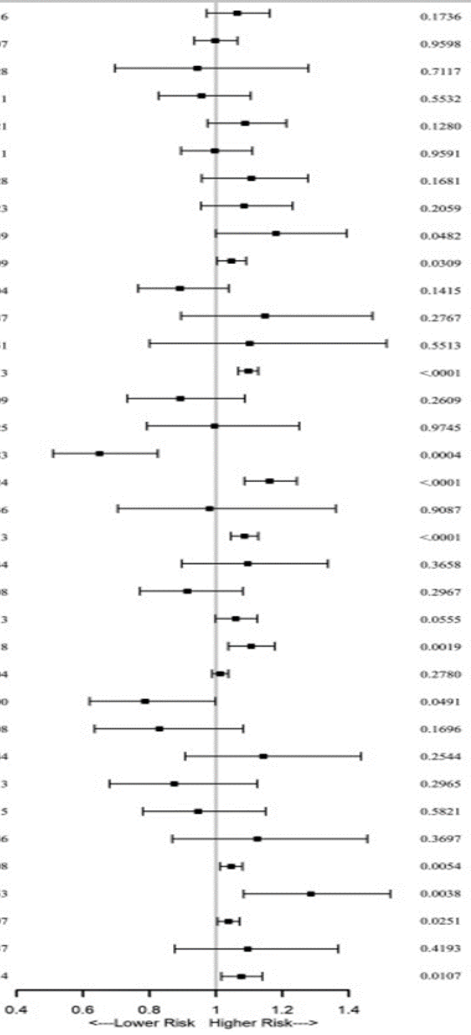

Figure 4. Odds ratios along with 95% confidence intervals for breast cancer risk linked to medication exposure in the Swedish population. [a]: Adjusted for age, education, income, area of residence, parity, obesity, chronic obstructive pulmonary disease, and Charlson Comorbidity Index score.

To explore drug-repurposing prospects for breast cancer prevention, we conducted an extensive Mendelian randomization (MR) investigation grounded in strong mechanistic evidence linking genetic variants, gene expression, and methylation patterns. This effort pinpointed key potentially causal, druggable genes for breast cancer: TUBB, MDM2, CSK, ULK3, MC1R, KCNN4, RPS23, and MAPT. We subsequently confirmed the drugs targeting these targets identified in the MR results. We determined that raloxifene use corresponded to a 35% decrease in breast cancer risk, thereby reinforcing its value as a chemopreventive agent. This aligns with findings from the STAR trial [18] and the USPSTF recommendation [19]. Nonetheless, raloxifene's benefit appeared only marginally non-significant in additional checks using active-comparator and new-user designs, primarily because of limited sample size. On the other hand, estradiol and nitrofurantoin exposure appeared to raise the likelihood of developing breast cancer. The association with nitrofurantoin lost statistical significance in sensitivity analyses that incorporated active-comparator and new-user approaches.

TUBB has been documented as overexpressed across multiple cancer types, including breast cancer [20-22]. More recent research links elevated TUBB levels to chemotherapy resistance in breast cancer patients [20, 23]. MDM2 functions as a proto-oncogene situated at chromosome 12q15. As a transcriptional target of p53, it plays a central part in reducing p53 protein levels. Consequently, MDM2 inhibitors represent a hopeful approach for managing p53-wild-type malignancies [24]. Beyond p53 suppression, excessive MDM2 activity influences diverse cellular processes and promotes tumor growth and advancement through pathways involving the cell cycle, apoptosis, DNA repair, migration, invasion, angiogenesis, and chemoresistance [25]. CSK codes for protein kinases that participate in various signaling routes, notably the suppression of Src family kinases [26]. Src interacts with EGFR (ErbB1) and ErbB2—receptors often overexpressed in breast cancer and already addressed by several FDA-approved anticancer therapies [27]. ULK3 produces a protein that can trigger autophagy in human fibroblasts [28]. Its expression and related gene signatures connect to keratinocyte proliferation and cancer development, with clinical observations indicating elevated ULK3 in squamous cell carcinomas of the lung, head and neck, and cervix [29]. MC1R encodes the receptor for melanocyte-stimulating hormone and strongly influences pigmentation. MC1R stimulation enhances cAMP signaling, increases melanin synthesis, and supports DNA repair after UV exposure [30]. KCNN4 forms an intermediate-conductance calcium-activated potassium channel whose overexpression occurs in breast cancer and contributes to various forms of chemoresistance [31]. RPS23 serves as a direct target of miR-542-3p and helps regulate p53. Reduced levels of certain ribosomal proteins such as RPS23, mediated by miR-542-3p, elevate RPL11 and thereby stabilize p53 [32].

All four medications verified in our cohort targeted methylation changes involving MAPT. MAPT encodes the tau protein, which is predominantly expressed in neurons but also appears in other tissues such as skeletal muscle and breast [33]. Dysregulated and mismatched tau expression has been noted in several neurodegenerative conditions [34-36]. Growing evidence points to tau's involvement in cancer biology. Primarily bound to microtubules—which are vital for mitosis during oncogenesis [37]—MAPT expression correlates strongly with estrogen receptor status in breast cancer, likely because of an imperfect estrogen-response element in its promoter region [38]. Nuclear tau may further influence cancer progression by modulating p53, a critical tumor suppressor. p53 forms part of a complex with nuclear tau, Pin1, and poly(A)-specific ribonuclease that can alter transcript expression patterns disrupted in malignancy [39]. Tau may also affect breast cancer via BRCA1 and BRCA2, key tumor suppressors that preserve genomic stability through double-strand break repair [40-42].

Multiple elements elevate breast cancer susceptibility, such as sex, advancing age, estrogen exposure, hereditary factors, genetic mutations, and adverse lifestyle habits. Both internally produced and externally supplied estrogen elevations are tied to higher breast cancer incidence [43]. This matches our observations, as numerous investigations have linked hormone replacement therapy—particularly regimens combining exogenous estrogen and progesterone—to increased breast cancer probability [44].

Raloxifene, classified as a second-generation selective estrogen-receptor modulator (SERM), binds to intracellular estrogen receptors (ERs) in various tissues, acting as either an agonist or antagonist. It is thus suitable for managing conditions stemming from estrogen

shortage, including postmenopausal osteoporosis. Preclinical and clinical data support its ability to prevent invasive breast cancer [45]. A systematic review of placebo-controlled trials conducted for the US Preventive Services Task Force reported that raloxifene lowered invasive breast cancer risk by 56% [46]. Despite these advantages, raloxifene carries infrequent side effects, notably thromboembolic complications (RR: 1.56; 95% CI, 1.11–2.60).

Tolterodine remains the leading prescription for overactive bladder management in Sweden [47]. Earlier research noted elevated rates of bladder and prostate cancer following the start of overactive bladder treatments. However, protopathic bias may explain this, whereby early cancer symptoms drive medication use and prompt subsequent diagnosis [48, 49]. A separate Swedish investigation found an inverse relationship between overactive bladder drugs and risks of colon or lung cancer [50]. To date, no dedicated study has examined tolterodine specifically in relation to breast cancer, underscoring the need for future preclinical and clinical research.

Nitrofurantoin is an antibiotic used to treat and prevent urinary tract infections. Currently, data on its connection to cancer remain scarce [51]. Just two sizable population-based Nordic studies indicated that prenatal nitrofurantoin exposure might elevate childhood leukemia risk [52, 53]. Confirmation in additional populations and cancer categories is required.

The primary advantage of this investigation lies in its thorough MR examination of druggable targets drawn from multiple data sources, followed by real-world population validation. Leveraging nationwide registry data allowed us to substantially limit selection and recall biases. Additionally, the exceptionally large cohorts for both MR and epidemiological components granted robust statistical power to clarify potential causal links. Beyond the core SMR method, we applied five supplementary MR techniques to explore MAPT expression and breast cancer. We also employed the HEIDI test and colocalization analyses to reduce false positives arising from linkage disequilibrium or horizontal pleiotropy. To address population stratification, we confined analyses to individuals of European ancestry. Lastly, by integrating comprehensive gene expression, DNA methylation, and plasma protein datasets, we applied stringent criteria to evaluate roughly 1400 druggable targets. We compiled an expanded inventory of causal targets and medications that can be extended to other disease outcomes.

Nevertheless, certain limitations remain. First, despite sourcing instrumental variables from diverse datasets, coverage of the complete druggable genome remains incomplete. Existing eQTL and mQTL resources lack variants related to gene expression or methylation on the X and Y chromosomes. Second, restricting instruments to cis-acting variants helped curb horizontal pleiotropy, yet this may have overlooked isoform-specific effects. Third, while MR analyses highlighted eight causal druggable targets corresponding to 319 candidate drugs, only 36 met criteria for population validation with adequate power; the complete list is available for subsequent experimental and epidemiological confirmation. Fourth, nationwide registers lacked detailed data on BMI, smoking, alcohol consumption, physical activity, and diet. We addressed smoking via COPD as a proxy—though imperfect—and adjusted for education and income to partially account for lifestyle-related confounding. Finally, the Swedish Drug Register does not capture medication non-adherence, which could underestimate effects, though such bias is expected to be modest and primarily affect the exposed group.

Conclusion

In summary, this extensive MR study, supported by population-based confirmation, found that raloxifene use is linked to lower breast cancer risk, consistent with the STAR trial and supporting the utility of MR for identifying causal genes in breast cancer. In contrast, estradiol, tolterodine, and nitrofurantoin use may heighten breast cancer risk, highlighting the importance of clinical vigilance and additional validation.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence

and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49.
2. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. Lancet. 2021;397(10286):1750-69.
3. Denny L, de Sanjose S, Mutebi M, Anderson BO, Kim J, Jeronimo J, et al. Interventions to close the divide for women with breast and cervical cancer between low-income and middle-income countries and high-income countries. Lancet. 2017;389(10071):861-70.
4. Bertolini F, Sukhatme VP, Bouche G. Drug repurposing in oncology--patient and health systems opportunities. Nat Rev Clin Oncol. 2015;12(12):732-42.
5. Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, et al. The support of human genetic evidence for approved drug indications. Nat Genet. 2015;47(8):856-60.
6. Finan C, Gaulton A, Kruger FA, Lumbers RT, Shah T, Engmann J, et al. The druggable genome and support for target identification and validation in drug development. Sci Transl Med. 2017;9(383):eaag1166.
7. Mendez D, Gaulton A, Bento AP, Chambers J, De Veij M, Félix E, et al. ChEMBL: towards direct deposition of bioassay data. Nucleic Acids Res. 2019;47(D1):D930-40.
8. Gaziano L, Giambartolomei C, Pereira AC, Gaulton A, Posner DC, Swanson SA, et al. Actionable druggable genome-wide Mendelian randomization identifies repurposing opportunities for COVID-19. Nat Med. 2021;27(4):668-76.
9. Vosa U, Claringbould A, Westra HJ, Bonder MJ, Deelen P, Zeng B, et al. Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. Nat Genet. 2021;53(9):1300-10.
10. Consortium GT. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science. 2020;369(6509):1318-30.
11. McRae AF, Marioni RE, Shah S, Yang J, Powell JE, Harris SE, et al. Identification of 55,000 replicated DNA methylation QTL. Sci Rep. 2018;8(1):17605.
12. Ferkingstad E, Sulem P, Atlason BA, Sveinbjornsson G, Magnusson MI, Styrmisdottir EL, et al. Large-scale integration of the plasma proteome with genetics and disease. Nat Genet. 2021;53(12):1712-21.
13. Zhang H, Ahearn TU, Lecarpentier J, Barnes D, Beesley J, Qi G, et al. Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses. Nat Genet. 2020;52(6):572-81.
14. Wu Y, Zeng J, Zhang F, Zhu Z, Qi T, Zheng Z, et al. Integrative analysis of omics summary data reveals putative mechanisms underlying complex traits. Nat Commun. 2018;9(1):918.
15. Giambartolomei C, Vukcevic D, Schadt EE, Franke L, Hingorani AD, Wallace C, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. PLoS Genet. 2014;10(5):e1004383.
16. Hemminki K, Ji J, Forsti A. Risks for familial and contralateral breast cancer interact multiplicatively and cause a high risk. Cancer Res. 2007;67(3):868-70.
17. Ji J, Sundquist K, Sundquist J, Hemminki K. Comparability of cancer identification among death registry, cancer registry and hospital discharge registry. Int J Cancer. 2012;131(9):2085-93.
18. Bevers TB. The STAR trial: evidence for raloxifene as a breast cancer risk reduction agent for postmenopausal women. J Natl Compr Canc Netw. 2007;5(8):719-24.
19. US Preventive Services Task Force, Owens DK, Davidson KW, Krist AH, Barry MJ, Cabana M, et al. Medication use to reduce risk of breast cancer: US preventive Services Task Force recommendation statement. JAMA. 2019;322(9):857-67.
20. Nami B, Wang Z. Genetics and expression profile of the tubulin gene superfamily in breast cancer subtypes and its relation to taxane resistance. Cancers (Basel). 2018;10(8):274.
21. Yu X, Zhang Y, Wu B, Kurie JM, Pertsemlidis A. The miR-195 Axis regulates chemoresistance through TUBB and lung cancer progression through BIRC5. Mol Ther Oncolytics. 2019;14:288-98.
22. Bakker EY, Fujii M, Krstic-Demonacos M, Demonacos C, Alhammad R. Protein disulfide isomerase A1-associated pathways in the development of stratified breast cancer therapies. Int J Oncol. 2022;60(2):16.
23. Barron-Gallardo CA, Garcia-Chagollan M, Moran-Mendoza AJ, Delgadillo-Gutierrez Z, Villanueva-Castro E, Robles-Espinoza CD, et al. Transcriptomic analysis of breast cancer patients sensitive and resistant to chemotherapy: looking for overall

survival and drug resistance biomarkers. Technol Cancer Res Treat. 2022;21:15330338211068965.
24. Konopleva M, Martinelli G, Daver N, Papayannidis C, Wei A, Higgins B, et al. MDM2 inhibition: an important step forward in cancer therapy. Leukemia. 2020;34(11):2858-74.
25. Nag S, Zhang X, Srivenugopal KS, Wang MH, Wang W, Zhang R. Targeting MDM2-p53 interaction for cancer therapy: are we there yet? Curr Med Chem. 2014;21(5):553-74.
26. Di Stefano P, Damiano L, Cabodi S, Aramu S, Tordella L, Pradelli LA, et al. p140Cap protein suppresses tumour cell properties, regulating Csk and Src kinase activity. EMBO J. 2007;26(12):2843-55.
27. Tebbutt N, Pedersen MW, Johns TG. Targeting the ERBB family in cancer: couples therapy. Nat Rev Cancer. 2013;13(9):663-73.
28. Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JFJ, et al. Autophagy mediates the mitotic senescence transition. Genes Dev. 2009;23(7):798-803.
29. Goruppi S, Clocchiatti A, Bottoni G, Botti V, Civenni G, Dotto GP, et al. The ULK3 kinase is a determinant of keratinocyte self-renewal and tumorigenesis targeting the arginine methylome. Nat Commun. 2023;14(1):887.
30. Chen S, Zhu B, Yin C, Liu W, Han C, Chen B, et al. Palmitoylation-dependent activation of MC1R prevents melanomagenesis. Nature. 2017;549(7672):399-403.
31. Lin P, Li J, Ye F, Yu Y, Shao Z, Ou Z, et al. KCNN4 induces multiple chemoresistance in breast cancer by regulating BCL2A1. Am J Cancer Res. 2020;10(10):3302-15.
32. Wang Y, Huang JW, Castella M, Huntsman DG, Taniguchi T. p53 is positively regulated by miR-542-3p. Cancer Res. 2014;74(12):3218-27.
33. Papin S, Paganetti P. Emerging evidences for an implication of the neurodegeneration-associated protein TAU in cancer. Brain Sci. 2020;10(11):862.
34. Strang KH, Golde TE, Giasson BI. MAPT mutations, tauopathy, and mechanisms of neurodegeneration. Lab Invest. 2019;99(7):912-28.
35. Pan L, Meng L, He M, Zhang Z. Tau in the pathophysiology of Parkinson's disease. J Mol Neurosci. 2021;71(11):2179-91.
36. Greaves CV, Rohrer JD. An update on genetic frontotemporal dementia. J Neurol. 2019;266(8):2075-86.
37. Bonneau C, Gurard-Levin ZA, Andre F, Pusztai L, Rouzier R. Predictive and prognostic value of the TauProtein in breast cancer. Anticancer Res. 2015;35(10):5179-84.
38. Ikeda H, Taira N, Hara F, Fujita T, Yamamoto H, Soh J, et al. The estrogen receptor influences microtubule-associated protein tau (MAPT) expression and the selective estrogen receptor inhibitor fulvestrant downregulates MAPT and increases the sensitivity to taxane in breast cancer cells. Breast Cancer Res. 2010;12(3):R43.
39. Baquero J, Varriano S, Ordonez M, Jiao W, Wang R, Abisambra JF, et al. Nuclear tau, p53 and Pin1 regulate PARN-mediated deadenylation and gene expression. Front Mol Neurosci. 2019;12:242.
40. Lee A, Moon BI, Kim TH. BRCA1/BRCA2 pathogenic variant breast cancer: treatment and prevention strategies. Ann Lab Med. 2020;40(2):114-21.
41. Mano T, Nagata K, Nonaka T, Tarutani A, Imoto S, Hashimoto T, et al. Neuron-specific methylome analysis reveals epigenetic regulation and tau-related dysfunction of BRCA1 in Alzheimer's disease. Proc Natl Acad Sci U S A. 2017;114(45):E9645-54.
42. Kurihara M, Mano T, Saito Y, Murayama S, Toda T, Iwata A. Colocalization of BRCA1 with tau aggregates in human tauopathies. Brain Sci. 2019;10(1):7.
43. Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. N Engl J Med. 2006;354(3):270-82.
44. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, et al. Risk factors and preventions of breast cancer. Int J Biol Sci. 2017;13(11):1387-97.
45. Gennari L, Merlotti D, Paola VD, Nuti R. Raloxifene in breast cancer prevention. Expert Opin Drug Saf. 2008;7(3):259-70.
46. Nelson HD, Fu R, Zakher B, McDonagh M, Pappas M, Stillman L. Medication use for the risk reduction of primary breast cancer in women: a systematic review for the US preventive Services Task Force. JAMA. 2019;322(9):868-86.
47. Margulis AV, Linder M, Arana A, Munk H, de Germay S, Gislason G, et al. Patterns of use of antimuscarinic drugs to treat overactive bladder in Denmark, Sweden, and the United Kingdom. PLoS One. 2018;13(9):e0204456.

48. Hallas J, Margulis AV, Pottegard A, Munk H, Linder M, Gislason G, et al. Incidence of common cancers in users of antimuscarinic medications for overactive bladder: a Danish nationwide cohort study. Basic Clin Pharmacol Toxicol. 2018;122(6):612-9.
49. Kaye JA, Margulis AV, Fortuny J, Munk H, Linder M, Gislason G, et al. Cancer incidence after initiation of antimuscarinic medications for overactive bladder in the United Kingdom: evidence for protopathic bias. Pharmacotherapy. 2017;37(6):673-83.
50. Lofling L, Sundstrom A, Kieler H, Bahmanyar S, Linder M. Exposure to antimuscarinic medications for treatment of overactive bladder and risk of lung cancer and colon cancer. Clin Epidemiol. 2019;11:133-43.
51. Kaye JA, Jick H. Antibiotics and the risk of breast cancer. Epidemiology. 2005;16(5):688-90.
52. Hjorth S, Pottegard A, Broe A, Munk H, Linder M, Gislason G, et al. Prenatal exposure to nitrofurantoin and risk of childhood leukaemia: a registry-based cohort study in four Nordic countries. Int J Epidemiol. 2022;51(3):778-88.
53. Momen NC, Olsen J, Gissler M, Kieler H, Haglund B, Li J. Exposure to systemic antibacterial medications during pregnancy and risk of childhood cancer. Pharmacoepidemiol Drug Saf. 2015;24(8):821-9.