**Causal associations of thyroid function and dysfunction with overall, breast and thyroid cancer: a two-sample Mendelian randomization study**

**Running head:** Thyroid dysfunctionand cancer

Shuai Yuan1,2, Siddhartha Kar3,4, Mathew Vithayathil5, Paul Carter3, Amy M. Mason5, Stephen Burgess5, 6, Susanna C. Larsson1,2

1Unit of Cardiovascular and Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

2 Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

3 Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK

4 MRC Integrative Epidemiology Unit, Bristol Medical School, University of Bristol, Bristol, UK

5 MRC Cancer Unit, University of Cambridge, Cambridge, UK

6 MRC Biostatistics Unit, University of Cambridge, Cambridge, UK

\*Corresponding author

Susanna C. Larsson, PhD, Associate professor

Institute of Environmental Medicine, Karolinska Institutet, Nobelsväg 13, Stockholm, 17177, Sweden;

Department of Surgical Sciences, Uppsala University, Dag Hammarskjölds Väg 14B, Uppsala, 75185, Sweden.

Tel: 46-852486059; E-mail: susanna.larsson@ki.se

**Abstract word count: 240**

**Main text word count: 3414**

**Number of Tables: 1**

**Number of Figures: 4**

**Number of supplementary tables: 2**

**Number of supplementary tables: 1**

**Abbreviation**

BCAC Breast Cancer Association Consortium

CI confidence interval

ER estrogen-receptor

GWAS genome-wide association study

MR Mendelian randomization

MR-PRESSO MR-pleiotropy residual sum and outlier

OR odds ratio

SD standard deviation

SNPs single-nucleotide polymorphisms

TSH thyroid-stimulating hormone

**Novelty & Impact**

We found evidence of a causal inverse association between thyroid-stimulating hormone levels and overall cancer. Increased thyroid-stimulating hormone levels and hypothyroidism were associated with decreased risk of breast (mainly ER positive tumors) and thyroid cancer, whereas hyperthyroidism and increased free thyroxine levels were associated with a higher risk of breast cancer (mainly ER positive tumors). It is suggested that treatment of subclinical and diagnosed hyperthyroidism may be an efficient cancer prevention strategy.

**Abstract**

Whether thyroid dysfunction plays a causal role in the development of cancer remains inconclusive. We conducted a two-sample Mendelian randomization study to investigate the associations between genetic predisposition to thyroid dysfunction and 22 site-specific cancers. Single-nucleotide polymorphisms associated with four traits of thyroid function were selected from a genome-wide association meta-analysis with up to 72 167 European-descent individuals. Summary-level data for breast cancer and 21 other cancers were extracted from the Breast Cancer Association Consortium (122 977 breast cancer cases and 105 974 controls) and UK Biobank (367 643 individuals). For breast cancer, a meta-analysis was performed using data from both sources. Genetically predicted thyroid dysfunction was associated with breast cancer, with similar patterns of associations in the Breast Cancer Association Consortium and UK Biobank. The combined odds ratios of breast cancer were 0.94 (0.91-0.98; *p*=0.007) per genetically predicted one standard deviation increase in TSH levels, 0.96 (0.91-1.00; *p*=0.053) for genetic predisposition to hypothyroidism, 1.04 (1.01-1.07; *p*=0.005) for genetic predisposition to hyperthyroidism, and 1.07 (1.02-1.12; *p*=0.003) per genetically predicted one standard deviation increase in free thyroxine levels. Genetically predicted TSH levels and hypothyroidism were inversely with thyroid cancer; the odds ratios were 0.47 (0.30-0.73; *p*=0.001) and 0.70 (0.51-0.98; *p*=0.038), respectively. This study provides evidence of a causal association between thyroid dysfunction and breast cancer (mainly ER positive tumors) risk. The role of TSH and hypothyroidism for thyroid cancer and the associations between thyroid dysfunction and other cancers need further exploration.

**Keywords:** cancer; hyperthyroidism; hypothyroidism; Mendelian randomization; thyroid-stimulating hormone; thyroxine

**Introduction**

Subclinical thyroid dysfunction is defined as abnormal serum thyroid-stimulating hormone (TSH) levels with physiologically normal free thyroxine levels in asymptomatic patients 1 and is a common disorder among adults 2, particularly in older women 2. Considering the important role of thyroid hormones in cell proliferation and differentiation, thyroid dysfunction has been proposed as a potential and preventable risk factor for cancer, such as thyroid 3, 4 and breast cancer 5, 6.

Observational data on the associations between thyroid dysfunction and risk of cancer are conflicting. Low TSH levels were associated with an increased risk of thyroid carcinoma in one study 3, while it was stated that higher TSH levels were associated with a higher frequency and more advanced stage of thyroid cancer in another study 7. With regard to breast cancer, a meta-analysis of 13 case-control studies published until June 2016 found no association between hypo- or hyperthyroidism and breast cancer risk 8. However, results from a nationwide population-based cohort study in Denmark showed that women diagnosed with hypothyroidism and hyperthyroidism had respectively lower and higher risk of breast cancer compared with the general population 9. Similarly, hyperthyroidism was associated with a significant increased risk of breast cancer mortality in a prospective cohort study of 75 076 US women 10. A limitation of available evidence is that observational findings are prone to be influenced by residual confounding and reverse causality, thereby impeding the causal inference on association between thyroid dysfunction and cancer risk.

Genetic variants explicitly associated with a potential risk factor (e.g., TSH levels) can be used as unbiased proxies for the risk factor to determine causality. This approach, named as Mendelian randomization (MR), is a genetic method that can strengthen the inference on the causal nature of exposure-outcome associations by diminishing the likelihood of confounding and eliminating reverse causality in conventional observational studies 11. This is because the genetic alleles associated with the exposure are randomly assorted at conception and thus unrelated to self-selected lifestyle and environmental factors and are not modified by disease. Given the controversy and uncertainty of the role of thyroid dysfunction for cancer, we conducted a two-sample Mendelian randomization study to explore the causal associations of four indicators of thyroid function and dysfunction, including circulating TSH levels, hypothyroidism, hyperthyroidism and free thyroxine levels, with overall cancer and 22 site-specific cancers.

**Materials and Methods**

**Assumptions of MR study and study design overview**

There are three key assumptions for MR analysis: 1) the genetic variants used as instrumental variables should be robustly associated with the risk factor of interest (Relevance assumption); 2) the used genetic variants should not be associated with potential confounders (Independent assumption); and 3) the genetic variants should affect the risk of the outcome only through the risk factor, not via alternative pathways (Exclusion restriction assumption). The present two-sample MR study was based on summary-level data from the ThyroidOmics Consortium 12, the Breast Cancer Association Consortium (BCAC) 13 and the UK Biobank 14 (**Supplementary Table 1)**. Assumptions of MR study and study design is shown in **Figure 1**. The original genome-wide association studies (GWASs) had been approved by corresponding ethical committee and all participants provided informed consent. The present study was approved by the Swedish Ethical Review Authority.

**Instrumental variable selection**

Single-nucleotide polymorphisms (SNPs) associated with TSH (n=61), hypothyroidism (n=8), hyperthyroidism (n=8) and free thyroxine levels (n=31) at the genome-wide significance level (*p*<5×10-8) were identified from a meta-analysis of GWASs of thyroid function and dysfunction with up to 72 167 individuals of European ancestry in both discovery and replication stages 12 (**Supplementary Table 1**).One TSH-related SNP located in the *ABO* locus was excluded due to pleiotropic effects (blood group is associated with many cancers 15, 16, through effects independent of TSH), leaving 60 SNPs as instrumental variables for TSH levels. An SNP in the *GLIS1* gene showed genome-wide significant association with both TSH and free thyroxine, and SNPs in *FOXE1*, *PDE8B* and *PDE10A* locus were associated with both hyper- and hypothyroidism. In addition, the original GWAS verified the associations of TSH and free thyroxine level with hyper- and hypothyroidism using a genetic risk score 12. Proxy SNPs were chosen at R2>0.9 among CEU population by searching in the dataset of Division of Cancer Epidemiology and Genetics, National Cancer Institute 17. We harmonized all instrumental variables for each trait so that the effect alleles reflected the allele associated with an increased probability or level of the exposure. All used SNPs were uncorrelated (*R*2<0.01) and details of the included SNPs are displayed in **Supplementary Table 2.**

**Outcome data sources**

Summary-level data for the associations of the thyroid-associated SNPs with breast cancer were obtained from BCAC, including 228 951 individuals of European ancestry (122 977 breast cancer cases and 105 974 controls) 13, and UK Biobank 14 with 13 666 breast cancer cases. The GWAS based on the BCAC used 1000 Genomes Project (Phase 3) reference panel in imputation stage and adjusted for genetic principal components and country. From UK Biobank, summary-level data for the SNP-cancer associations were also derived for overall cancer and additionally 21 site-specific cancers using logistic regression models adjusted for age, sex, and ten genetic principal components. The analyses of UK Biobank were based on 367 643 participants after exclusion of related individuals (third-degree relatives or closer), low call rate, and excess heterozygosity (3 or more standard deviations from the mean). Follow-up was until March 31, 2017 or death, and in total 75 037 cancer cases were included.

**Statistical analysis**

The Wald method was used to estimate the ratio between the SNP-outcome and SNP-exposure estimates for each SNP. The ratio estimates for every used SNPs for one trait were combined by using the fixed-effects or multiplicative random-effects inverse-variance weighted meta-analysis method. The inverse-variance weighted method can provide the most precise estimates but could be influenced by invalid instrumental variables and pleiotropic effects. Thus, for overall cancer as well as associations reaching the conventional significance level (*p*<0.05), we further conducted three sensitivity analyses based on the weighted median, MR-Egger, and MR-pleiotropy residual sum and outlier (MR-PRESSO) methods to examine and correct for possible pleiotropy. The weighted median method gives accurate estimates if at least 50% of the instrumental variables are valid 18. The MR-Egger regression can detect and adjust for pleiotropy albeit with low power 19. The MR-PRESSO test can detect possible outliers and estimations obtained from the MR-PRESSO analysis are corrected for horizontal pleiotropy via outlier removal 20. To increase the power for the analysis of breast cancer, a meta-analysis with fixed effects was performed to combine the data from the BCAC and UK Biobank. Odds ratios (ORs) of cancer risk were scaled to one standard deviation (SD) increase in genetically predicted TSH and free thyroxine levels and one-unit increase in the log OR of hypothyroidism and hyperthyroidism in all analyses. All statistical analyses were two-sided and performed in Stata/SE 15.0 and R 3.6.0 software. We did not use *P* values strictly to define statistical significance but interpreted the results based on the patterns of associations across the thyroid-related traits and the strengths of the associations 21.

**Data availability**

Data for thyroid function can be obtained from the GWAS (accession code phs000930 in dbGaP, http://locuszoom.sph.umich.edu/genform.php)12. Summary-level data from BCAC 13 are publicly available (http://bcac.ccge.medschl.cam.ac.uk/). UK Biobank 14 data are available through application (https://www.ukbiobank.ac.uk/). Summary-level data for the used SNPs in the present study are available upon a reasonable request to the corresponding author.

**Results**

Seven of the thyroid-associated SNPs were unavailable in the UK Biobank dataset. Proxy SNPs were found for four SNPs, resulting in 58 SNPs in the analyses of TSH, 7 SNPs in the analyses of hypothyroidism, 8 SNPs in the analyses of hyperthyroidism, and 31 SNPs in the analyses of free thyroxine. One SNP for TSH was not available in the dataset of the BCAC and no suitable proxy was found.

Genetically predicted TSH levels showed a consistent association with overall cancer across the different MR methods (**Figure 2**). The OR was 0.93 (95% CI, 0.91-0.96; *p*=2.28×10-6) per one standard deviation increase in TSH levels in the inverse variance weighted analysis. Results for genetic liability to hypo- and hyperthyroidism were directionally consistent with those for TSH levels but less precise; in particular, the results from the MR-Egger analyses were very imprecise, indicative of very low power of this method.

Genetically predicted TSH and free thyroxine levels as well as genetic predisposition to thyroid dysfunction were associated with breast cancer, with similar patterns of associations in the BCAC and UK Biobank (**Table 1** and **Figure 3**). In the meta-analysis combining the results from the BCAC and UK Biobank (136 643 breast cancer cases and 459 951 non-cases), the combined ORs of breast cancer were 0.94 (95% confidence interval (CI) 0.91-0.98; *p*=0.007) per genetically predicted one SD increase in TSH levels, 0.96 (95% CI, 0.91-1.00; *p*=0.053) per one-unit increase in log odds of hypothyroidism, 1.04 (95% CI, 1.01-1.07; *p*=0.005) per one-unit increase in log odds of hyperthyroidism, and 1.07 (95% CI, 1.02-1.12; *p*=0.003) per genetically predicted one SD increase in free thyroxine levels (**Figure 3**). The OR estimates were similar but less precise in the sensitivity analyses (**Supplementary** **Figure 1**). There was suggestive evidence that thyroid dysfunction, in particular hyperthyroidism and free thyroxine levels, was associated with estrogen-receptor (ER) positive but not negative tumors (**Table 1**).

Genetically higher TSH levels and liability to hypothyroidism were associated with lower odds of thyroid cancer (**Table 1 and Figure 4**). For one SD increase of TSH levels and one-unit increment of the log odds of hypothyroidism, the ORs of thyroid cancer were 0.47 (0.30-0.73; *p*=0.001) and 0.70 (0.51-0.98; *p*=0.038), respectively. Significant heterogeneity (I2=39; *p*=0.002) among estimates of individual SNPs was detected in the analysis of TSH. After removal of two outliers, the magnitude and the significance of the association between TSH and thyroid cancer remained in the MR-PRESSO analysis (OR=0.48, 0.32-0.77; *p*=0.001) (**Figure 4**). The associations were similar in sensitivity analyses using the weighted median and MR-Egger methods (**Figure 4**).

There was no clear pattern of associations of genetically predicted thyroid dysfunction with the other 20 cancers studied (**Table 1**). Nevertheless, there was suggestive evidence of inverse associations of genetically predicted TSH levels with uterine and prostate cancer; an inverse association between genetic liability to hyperthyroidism and brain cancer; and inverse associations between free thyroxine levels and ovarian and bladder cancer and melanoma.

**Discussion**

In the present study, we found evidence of a causal inverse association between TSH levels and overall cancer. Furthermore, increased TSH levels and hypothyroidism were associated with a decreased odds of breast cancer (mainly ER positive tumors) and thyroid cancer, whereas hyperthyroidism and increased free thyroxine levels were associated with a higher odds of breast cancer (mainly ER positive tumors). We found limited evidence supporting causal associations of thyroid dysfunction with 20 other cancers.

Observational studies have found that thyroid disorder and thyroid hormone levels were related to risk of overall cancer 10, 22-24. Consistent with our findings, a 9-year cohort study of 29 691 individuals without previously known thyroid disease found that participants with low TSH levels had increased cancer risk compared with the euthyroid reference group after adjustment of age, sex, and smoking status 24. Another cohort study of 75 076 US women and 30-year follow-up period showed that women with hyperthyroidism had an elevated risk of cancer, especially breast and ovarian cancer 10. In contrast, a cohort study of 115 746 Asians found that subclinical hypothyroidism was associated with increased cancer mortality over a 10-year follow-up period 25.

In line with our MR results, most observational prospective studies have found that hypothyroidism is associated with a lower risk of breast cancer 9, 26, whereas hyperthyroidism 9, 10 and high free thyroxine levels 5, 27, 28 are associated with an increased breast cancer risk, especially among overweight 27 and postmenopausal women 28. TSH levels were inversely associated with breast cancer risk in one study 28 but not in two other 5, 27. On the contrary, two meta-analyses based on data from 12 or 13 case-control studies showed no association of hypo- or hyperthyroidism with odds of breast cancer 8, 29. The inconsistency among studies may be attributable to reverse causation bias in the case-control studies, measurement bias (causing dilution of the effect), or inadequate power. In this MR study, results for thyroid function in relation to breast cancer were in the same direction in the BCAC and UK Biobank albeit less precise in UK Biobank. When combining the results from the two data sources, thereby increasing the sample size, all associations became statistically significant. In the present study, based on data from the BCAC, the associations of thyroid function and dysfunction with breast cancer risk were mainly observed for ER positive tumors, though a suggestive association was also observed between hypothyroidism and ER negative tumors. Considering that ER positive tumors make up around 70% of total breast cancers in all populations, the observed association with overall breast cancer may reflect the association between thyroid dysfunction and ER positive breast cancer. However, as data for ER status of the breast tumors were not available in UK Biobank, this difference could not be replicated in an independent population and needs confirmation.

The results of the present study are in line with most findings supporting a protective role of high TSH levels in thyroid cancer. An individual-matched nested case-control study with 1482 individuals found an inverse association between physiologically high TSH levels and thyroid cancer 30. Similarly, another small case-control study showed that low levels of TSH might predispose to thyroid cancer 3. The possible mechanism behind the association may be TSH-specific mediated effect on thyroid tissue through cellular proliferation 31. However, a retrospective study including 3791 patients with thyroidectomy found that increased serum TSH levels were related to higher odds of papillary thyroid cancer 32. Among patients with thyroid nodule, several studies have shown that high serum TSH levels increase thyroid cancer risk 33, 34. In addition, TSH suppression therapy has been suggested as an efficient treatment for patients with high-risk thyroid cancer or recurrent tumor. However, in the present study, we observed an inverse association between higher TSH levels and thyroid cancer risk, which is in line with previous population-based epidemiological and genetic studies 35, 36. Discrepancy may be explained by the differences of response to TSH in normal and cancerous tissues. A nationwide cohort study found no association of hypothyroidism with thyroid cancer among 63 143 patients with hypothyroidism compared with the general population but observed that benign thyroid disease was associated with higher standardized incidence ratio of thyroid cancer 37. A possible reason for the null association between hypothyroidism and thyroid cancer risk may be that once diagnosed with hypothyroidism, most patients are treated for the disorder and no longer hypothyroid, leading to altered cancer risk. This MR study found a possible inverse association between genetic liability to hypothyroidism and thyroid cancer, which needs verification due to a small number of thyroid cancer cases. In addition, even though there was no causal association between hyperthyroidism and thyroid cancer in our study, a systematic review documented that pathological hyperthyroid caused by Graves’ disease was associated with an increased risk of thyroid cancer 38.

Some observational studies have reported associations of thyroid-related traits with other cancers, such as colorectal, prostate and lung cancer. Elevated TSH levels, hypothyroidism and decreased free thyroxine levels have been reported to associate with a higher risk of prostate cancer 39, 40. A large-scale nested case-control study showed that hyperthyroidism and untreated hypothyroidism were associated with a modestly elevated risk of colorectal cancer 41. However, results for lung cancer have been conflicting with a positive 5 or null 39 association found between free thyroxine levels and lung cancer risk. The present MR study showed little evidence in support for an association of thyroid dysfunction with colorectal, prostate and lung cancers, except for an inverse association between TSH levels and prostate cancer. Possible explanations behind the discrepancy in results across studies may be residual confounding or reverse causality in the observational studies or an inadequate power in the present MR study owing to a small number of cases for these site-specific cancers.

Thyroid hormones are involved in physiological processes vital to normal metabolism, development, and growth, and hypothyroidism is a known cause of growth retardation. Hence, not unexpectedly, genetic risk scores for elevated TSH and decreased free thyroxine levels are associated with decreased body height 12. Adult height is positively associated with risk of breast 42 and thyroid cancer 43. This suggests that height (through more cells) might mediate the associations of thyroid dysfunction with breast and thyroid cancer, or that growth processes related to thyroid hormones are driving the positive association between height and cancer risk. Nevertheless, we did not detect any association of the thyroid hormones with other site-specific cancers that are also associated with height 44, 45. Hence, it is reasonable to conclude that height is not biasing the results through vertical pleiotropy but that other dominating mechanisms explain the associations of thyroid dysfunction with breast and thyroid cancer. Previous studies indicated that body mass index might act as a mediator in the pathway from thyroid dysfunction to breast cancer 46. Nevertheless, genetically predicted TSH and free thyroxine levels were not associated with BMI using the genetic risk score analysis 12 and we detected no such association of the liability to hypothyroidism and hyperthyroidism with BMI either (not shown).

The exact mechanisms linking thyroid dysfunction to breast and thyroid cancer have not been clarified. There are several potential hypotheses, such as uptake and oxidation of iodine 6 and a proliferative effect of triiodothyronine 9. Moreover, it has been shown that thyroxine is a proliferative factor *in vitro* for breast cancer cells and that thyroxine can promote nuclear estrogen receptor alpha-dependent proliferation of breast cancer cells bearing this receptor 47. Genetic studies have established a link connecting circulating TSH levels and thyroid cancer and found that *DIRC3*, *MBIP* and *NRG1* (encoding the signaling protein neuregulin 1) genes may play vital roles in this association 36. More studies focusing on the downstream of certain gene regions, such as key enzymes, metabolites and signal, transport and receptor proteins, are warranted for prevention strategy and therapy development.

This is the first MR study comprehensively assessing the associations of four thyroid function indicators with overall and 22 site-specific cancers. The major advantage of this study is the two-sample MR study design, which diminishes unobserved confounding and reverse causality potentially distorting the results of observational studies. The results were less likely to be biased by population stratification since we only used data from European populations, but this confined the transferability of our findings to other populations.

A major limitation of this study is that the number of cases was few for several cancers, leading to low precision of the estimates. Even though our results showed limited evidence supporting a causal association between thyroid dysfunction and site-specific cancers except breast and thyroid cancer, we cannot exclude that we may have missed weak associations owing to few cases. However, the precision was high in the analysis of breast cancer by combining the results from the BCAC and UK Biobank. Furthermore, the thyroid-related traits showed similar patterns of associations in both BCAC and UK Biobank, indicating that a false positive finding is unlikely. The SNPs used as instrumental variables for the thyroid-function related traits have been reported to be associated with other factors, such as height, age at menarche, obesity-related traits, serum lipids, blood metabolite levels and serum urate 12. However, considering the biological roles of thyroid hormones, these factors are more likely to act as mediators (known as the vertical pleiotropy) in the pathway from thyroid function to cancer, which will not bias our findings. In addition, the consistency in results across sensitivity analyses and no detectable directional pleiotropy suggest a negligible distortion of the results by potential horizontal pleiotropy. Nevertheless, several thyroid-function related SNPs were directly associated with thyroid cancer. Thus, we cannot rule out that there are other direct pathways causing this cancer and consequently decreases TSH levels.

In summary, the present two-sample MR study strengthened the evidence of causal associations of thyroid function and dysfunction with risk of overall cancer and breast cancer (mainly ER positive tumors). The observed inverse associations of circulating TSH levels and hypothyroidism with thyroid cancer need verification in other MR studies with larger number of cases. Along with the benefits of thyroid dysfunction treatment on cancer survival 48 and cardiovascular diseases 49, it is suggested that treatment of subclinical and diagnosed hyperthyroidism may be an efficient cancer prevention strategy.

**Additional information**

**Acknowledgments**

Summary-level data for SNPs associated with thyroid function-related traits were extracted from The ThyroidOmics Consortium. Summary-level data for genetic associations with the cancers have been contributed by the Breast Cancer Association Consortium. The authors thank all investigators for sharing these data. The analyses of UK Biobank data were conducted under application 29202.

**Author contributions**

S.Y. analyzed and interpreted data and wrote and reviewed the manuscript. S.K., M.V. and P.C. reviewed the manuscript. A.M.M. and S.B. prepared the data and reviewed the manuscript. S.C.L. designed the research, analyzed and interpreted the data and reviewed the manuscript. All authors read and approved the final manuscript.

**Sources of Funding**

Funding for this study came from the Swedish Research Council for Health, Working Life and Welfare (Forte) and the Swedish Research Council (Vetenskapsrådet). Stephen Burgess is supported by a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (Grant Number 204623/Z/16/Z). Siddhartha Kar is supported by a Cancer Research UK programme grant, the Integrative Cancer Epidemiology Programme (C18281/A19169), and a Junior Research Fellowship from Homerton College, Cambridge.

**Conflict of interest**

The authors declare no conflicts of interest.

**References**

1. Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. *Endocr Rev* 2008;29: 76-131.

2. Taylor PN, Albrecht D, Scholz A, et al. Global epidemiology of hyperthyroidism and hypothyroidism. *Nat Rev Endocrinol* 2018;14: 301-16.

3. Rinaldi S, Plummer M, Biessy C, et al. Thyroid-stimulating hormone, thyroglobulin, and thyroid hormones and risk of differentiated thyroid carcinoma: the EPIC study. *J Natl Cancer Inst* 2014;106: dju097.

4. Balasubramaniam S, Ron E, Gridley G, Schneider AB, Brenner AV. Association between benign thyroid and endocrine disorders and subsequent risk of thyroid cancer among 4.5 million U.S. male veterans. *J Clin Endocrinol Metab* 2012;97: 2661-9.

5. Khan SR, Chaker L, Ruiter R, et al. Thyroid Function and Cancer Risk: The Rotterdam Study. *J Clin Endocrinol Metab* 2016;101: 5030-6.

6. Hardefeldt PJ, Eslick GD, Edirimanne S. Benign thyroid disease is associated with breast cancer: a meta-analysis. *Breast Cancer Res Treat* 2012;133: 1169-77.

7. Fiore E, Vitti P. Serum TSH and risk of papillary thyroid cancer in nodular thyroid disease. *J Clin Endocrinol Metab* 2012;97: 1134-45.

8. Fang Y, Yao L, Sun J, et al. Does thyroid dysfunction increase the risk of breast cancer? A systematic review and meta-analysis. *J Endocrinol Invest* 2017;40: 1035-47.

9. Sogaard M, Farkas DK, Ehrenstein V, et al. Hypothyroidism and hyperthyroidism and breast cancer risk: a nationwide cohort study. *Eur J Endocrinol* 2016;174: 409-14.

10. Journy NMY, Bernier MO, Doody MM, et al. Hyperthyroidism, Hypothyroidism, and Cause-Specific Mortality in a Large Cohort of Women. *Thyroid* 2017;27: 1001-10.

11. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003;32: 1-22.

12. Teumer A, Chaker L, Groeneweg S, et al. Genome-wide analyses identify a role for SLC17A4 and AADAT in thyroid hormone regulation. *Nat Commun* 2018;9: 4455.

13. Michailidou K, Lindstrom S, Dennis J, et al. Association analysis identifies 65 new breast cancer risk loci. *Nature* 2017;551: 92-4.

14. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* 2015;12: e1001779.

15. Iodice S, Maisonneuve P, Botteri E, et al. ABO blood group and cancer. *Eur J Cancer* 2010;46: 3345-50.

16. Zhang BL, He N, Huang YB, et al. ABO blood groups and risk of cancer: a systematic review and meta-analysis. *Asian Pac J Cancer Prev* 2014;15: 4643-50.

17. Machiela MJ, Chanock SJ. LDassoc: an online tool for interactively exploring genome-wide association study results and prioritizing variants for functional investigation. *Bioinformatics* 2018;34: 887-9.

18. Bowden J, Davey Smith G, Haycock PC, et al. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016;40: 304-14.

19. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol* 2017;32: 377-89.

20. Verbanck M, Chen CY, Neale B, et al. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet* 2018;50: 693-8.

21. Sterne JA, Davey Smith G. Sifting the evidence-what's wrong with significance tests? *Bmj* 2001;322: 226-31.

22. Mellemgaard A, From G, Jorgensen T, et al. Cancer risk in individuals with benign thyroid disorders. *Thyroid* 1998;8: 751-4.

23. Hercbergs AH, Ashur-Fabian O, Garfield D. Thyroid hormones and cancer: clinical studies of hypothyroidism in oncology. *Curr Opin Endocrinol Diabetes Obes* 2010;17: 432-6.

24. Hellevik AI, Asvold BO, Bjoro T, et al. Thyroid function and cancer risk: a prospective population study. *Cancer Epidemiol Biomarkers Prev* 2009;18: 570-4.

25. Tseng FY, Lin WY, Li CI, et al. Subclinical hypothyroidism is associated with increased risk for cancer mortality in adult Taiwanese-a 10 years population-based cohort. *PLoS One* 2015;10: e0122955.

26. Cristofanilli M, Yamamura Y, Kau SW, et al. Thyroid hormone and breast carcinoma. Primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma. *Cancer* 2005;103: 1122-8.

27. Tosovic A, Becker C, Bondeson AG, et al. Prospectively measured thyroid hormones and thyroid peroxidase antibodies in relation to breast cancer risk. *Int J Cancer* 2012;131: 2126-33.

28. Kim EY, Chang Y, Lee KH, et al. Serum concentration of thyroid hormones in abnormal and euthyroid ranges and breast cancer risk: A cohort study. *Int J Cancer* 2019.

29. Angelousi AG, Anagnostou VK, Stamatakos MK, et al. Mechanisms in endocrinology: primary HT and risk for breast cancer: a systematic review and meta-analysis. *Eur J Endocrinol* 2012;166: 373-81.

30. Huang H, Rusiecki J, Zhao N, et al. Thyroid-Stimulating Hormone, Thyroid Hormones, and Risk of Papillary Thyroid Cancer: A Nested Case-Control Study. *Cancer Epidemiol Biomarkers Prev* 2017;26: 1209-18.

31. Cantara S, D'Angeli F, Toti P, et al. Expression of the ring ligase PRAJA2 in thyroid cancer. *J Clin Endocrinol Metab* 2012;97: 4253-9.

32. Sohn SY, Kim HJ, Jang HW, et al. Lack of association between high serum thyroid-stimulating hormone level and risk of papillary thyroid microcarcinomas. *Head Neck* 2014;36: 43-6.

33. Haymart MR, Repplinger DJ, Leverson GE, et al. Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. *J Clin Endocrinol Metab* 2008;93: 809-14.

34. Azizi G, Keller JM, Lewis M, et al. Association of Hashimoto's thyroiditis with thyroid cancer. *Endocr Relat Cancer* 2014;21: 845-52.

35. Biondi B, Filetti S, Schlumberger M. Thyroid-hormone therapy and thyroid cancer: a reassessment. *Nat Clin Pract Endocrinol Metab* 2005;1: 32-40.

36. Gudmundsson J, Sulem P, Gudbjartsson DF, et al. Discovery of common variants associated with low TSH levels and thyroid cancer risk. *Nat Genet* 2012;44: 319-22.

37. Kitahara CM, D KRF, Jorgensen JOL, et al. Benign Thyroid Diseases and Risk of Thyroid Cancer: A Nationwide Cohort Study. *J Clin Endocrinol Metab* 2018;103: 2216-24.

38. Pazaitou-Panayiotou K, Michalakis K, Paschke R. Thyroid cancer in patients with hyperthyroidism. *Horm Metab Res* 2012;44: 255-62.

39. Chan YX, Knuiman MW, Divitini ML, et al. Lower TSH and higher free thyroxine predict incidence of prostate but not breast, colorectal or lung cancer. *Eur J Endocrinol* 2017;177: 297-308.

40. Mondul AM, Weinstein SJ, Bosworth T, et al. Circulating thyroxine, thyroid-stimulating hormone, and hypothyroid status and the risk of prostate cancer. *PLoS One* 2012;7: e47730.

41. Boursi B, Haynes K, Mamtani R, et al. Thyroid dysfunction, thyroid hormone replacement and colorectal cancer risk. *J Natl Cancer Inst* 2015;107: djv084.

42. Zhang B, Shu XO, Delahanty RJ, et al. Height and Breast Cancer Risk: Evidence From Prospective Studies and Mendelian Randomization. *J Natl Cancer Inst* 2015;107.

43. Jing Z, Hou X, Liu Y, et al. Association between height and thyroid cancer risk: a meta-analysis of prospective cohort studies. *Int J Cancer* 2015;137: 1484-90.

44. Khankari NK, Shu XO, Wen W, et al. Association between Adult Height and Risk of Colorectal, Lung, and Prostate Cancer: Results from Meta-analyses of Prospective Studies and Mendelian Randomization Analyses. *PLoS Med* 2016;13: e1002118.

45. Lai FY, Nath M, Hamby SE, et al. Adult height and risk of 50 diseases: a combined epidemiological and genetic analysis. *BMC Med* 2018;16: 187.

46. Ortega-Olvera C, Ulloa-Aguirre A, Angeles-Llerenas A, et al. Thyroid hormones and breast cancer association according to menopausal status and body mass index. *Breast Cancer Res* 2018;20: 94.

47. Tang HY, Lin HY, Zhang S, et al. Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor. *Endocrinology* 2004;145: 3265-72.

48. Brandt J, Borgquist S, Almquist M, et al. Thyroid function and survival following breast cancer. *Br J Surg* 2016;103: 1649-57.

49. Larsson SC, Allara E, Mason AM, et al S. Thyroid Function and Dysfunction in Relation to 16 Cardiovascular Diseases. *Circ Genom Precis Med* 2019;12: e002468.

**Figure 1. Assumptions of MR study and study design overview**

**Figure 2. Associations of genetically predicted TSH and free thyroxine levels, hypothyroidism and hyperthyroidism with overall cancer in UK Biobank with 75 037 cancer cases at any site.** CI indicates confidence interval; MR-PRESSO, Mendelian randomization-pleiotropy residual sum and outlier; TSH, thyroid-stimulating hormone; IVW, inverse-variance weighted; OR, odds ratio; Heterogeneity was observed in the analysis of hypothyroidism and hyperthyroidism. There was no detected pleiotropy in all MR-Egger analyses. Two and one outliers were detected and corrected in the MR-PRESSO analysis of hypothyroidism and hyperthyroidism, respectively. No outlier was detected in the analysis of TSH and free thyroxine.

**Figure 3. Meta-analysis of the associations of genetically** **predicted TSH and** **free thyroxine levels, hypothyroidism and hyperthyroidism with breast cancer**. BCAC indicates breast cancer association consortium; CI; confidence interval; TSH, thyroid-stimulating hormone; OR, odds ratio; UKBB, UK Biobank.

**Figure 4. Associations of genetically predicted TSH and free thyroxine levels, hypothyroidism and hyperthyroidism with thyroid cancer.** CI indicates confidence interval; MR-PRESSO, Mendelian randomization-pleiotropy residual sum and outlier; TSH, thyroid-stimulating hormone; IVW, inverse-variance weighted; OR, odds ratio; Heterogeneity was observed in the analysis of TSH, hyperthyroidism and free thyroxine. There was no detected pleiotropy in all MR-Egger analyses. Two, one and two outliers were detected and corrected in the MR-PRESSO analysis of TSH, hyperthyroidism and thyroxine, respectively.

**Table 1. Associations of genetically predicted TSH and free thyroxine levels, hypothyroidism and hyperthyroidism with 22 cancers in random effects inverse-variance weighted model**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cancer site or cancer** | **Cases** | **TSH** | **Hypothyroidism** | **Hyperthyroidism** | **Free thyroxine** |
| **OR (95% CI)** | **P** | **OR (95% CI)** | **P** | **OR (95% CI)** | **P** | **OR (95% CI)** | **P** |
| **Sex-specific** |  |  |  |  |  |  |  |  |  |
| Breast (BCAC) | 122 977 | 0.95 (0.90, 1.00)  | 0.064 | 0.97 (0.92, 1.03) | 0.303 | 1.03 (1.00, 1.06) | 0.053 | 1.07 (1.02, 1.13) | 0.006 |
| Breast ER+ (BCAC) | 69 501 | 0.95 (0.90, 1.00) | 0.066 | 0.98 (0.93, 1.04) | 0.553 | 1.04 (1.00, 1.07) | 0.026 | 1.08 (1.02, 1.14) | 0.005 |
| Breast ER- (BCAC) | 21 468 | 1.02 (0.95, 1.09) | 0.585 | 0.95 (0.91, 1.00) | 0.067 | 0.99 (0.96, 1.03) | 0.670 | 1.04 (0.96, 1.13) | 0.297 |
| Breast (UKBB) | 13 666 | 0.93 (0.86, 1.00) | 0.045 | 0.92 (0.84, 1.00) | 0.045 | 1.08 (1.03, 1.12) | 0.001 | 1.05 (0.96, 1.16) | 0.269 |
| Uterus | 1931 | 0.83 (0.70, 0.98) | 0.025 | 0.93 (0.80, 1.08) | 0.331 | 1.10 (0.96, 1.26) | 0.177 | 1.06 (0.85, 1.34) | 0.591 |
| Cervix | 1928 | 1.00 (0.84, 1.17) | 0.953 | 0.94 (0.81, 1.09) | 0.432 | 1.09 (0.97, 1.22) | 0.156 | 1.17 (0.93, 1.48) | 0.169 |
| Ovary | 1520 | 1.05 (0.89, 1.25) | 0.552 | 0.96 (0.82, 1.14) | 0.650 | 1.07 (0.91, 1.25) | 0.408 | 0.79 (0.63, 1.00) | 0.049 |
| Prostate | 7872 | 0.91 (0.84, 0.99) | 0.026 | 0.96 (0.89, 1.04) | 0.305 | 0.99 (0.93, 1.06) | 0.820 | 1.06 (0.93, 1.20) | 0.400 |
| Testis | 735 | 0.89 (0.69, 1.16) | 0.388 | 0.92 (0.71, 1.19) | 0.520 | 1.01 (0.85, 1.20) | 0.883 | 0.97 (0.70, 1.35) | 0.859 |
| **Gastrointestinal tract** |  |  |  |  |  |  |  |  |  |
| Oesophagus | 843 | 0.80 (0.64, 1.01) | 0.061 | 0.91 (0.72, 1.14) | 0.393 | 1.08 (0.89, 1.31) | 0.463 | 1.28 (0.94, 1.75) | 0.122 |
| Stomach | 736 | 0.97 (0.76, 1.23) | 0.788 | 0.91 (0.72, 1.15) | 0.440 | 1.06 (0.89, 1.25) | 0.529 | 1.12 (0.81, 1.56) | 0.487 |
| Colorectum | 5486 | 1.00 (0.91, 1.09) | 0.915 | 0.94 (0.80, 1.10) | 0.454 | 0.97 (0.91, 1.04) | 0.390 | 1.02 (0.89, 1.18) | 0.743 |
| Pancreas | 1264 | 0.92 (0.76, 1.11) | 0.366 | 1.04 (0.86, 1.24) | 0.698 | 1.10 (0.96, 1.25) | 0.166 | 0.99 (0.77, 1.28) | 0.959 |
| Liver | 324 | 0.82 (0.55, 1.23) | 0.336 | 0.76 (0.45, 1.30) | 0.322 | 1.14 (0.78, 1.65) | 0.496 | 0.82 (0.46, 1.46) | 0.509 |
| Biliary tract | 387 | 1.18 (0.84, 1.66) | 0.329 | 1.04 (0.75, 1.44) | 0.814 | 0.93 (0.73, 1.18) | 0.552 | 1.33 (0.84, 2.08) | 0.223 |
| **Urinary tract** |  |  |  |  |  |  |  |  |  |
| Bladder | 2588 | 1.14 (0.97, 1.33) | 0.116 | 0.99 (0.87, 1.13) | 0.929 | 0.93 (0.79, 1.09) | 0.379 | 0.82 (0.68, 0.99) | 0.036 |
| Kidney | 1310 | 0.90 (0.74, 1.08) | 0.257 | 0.91 (0.74, 1.13) | 0.402 | 1.09 (0.95, 1.24) | 0.212 | 0.97 (0.76, 1.25) | 0.835 |
| **Blood/bone marrow/lymph** |  |  |  |  |  |  |  |  |  |
| Leukemia | 1403 | 0.90 (0.76, 1.08) | 0.254 | 0.96 (0.81, 1.14) | 0.639 | 1.01 (0.89, 1.14) | 0.926 | 0.98 (0.78, 1.25) | 0.921 |
| Non-Hodgkin lymphoma | 2296 | 1.03 (0.90, 1.18) | 0.687 | 0.87 (0.74, 1.01) | 0.075 | 1.02 (0.93, 1.12) | 0.663 | 0.88 (0.73, 1.06) | 0.190 |
| Multiple myeloma | 656 | 0.99 (0.75, 1.30) | 0.917 | 1.13 (0.88, 1.46) | 0.328 | 0.95 (0.79, 1.14) | 0.582 | 0.97 (0.68, 1.37) | 0.853 |
| **Other**  |  |  |  |  |  |  |  |  |  |
| Brain | 810 | 0.92 (0.72, 1.17) | 0.497 | 0.82 (0.61, 1.09) | 0.168 | 1.24 (1.05, 1.46) | 0.010 | 0.82 (0.59, 1.12) | 0.207 |
| Head and neck | 1615 | 0.95 (0.80, 1.12) | 0.528 | 0.98 (0.76, 1.26) | 0.878 | 0.95 (0.85, 1.07) | 0.398 | 1.13 (0.89, 1.43) | 0.308 |
| Lung | 2838 | 1.00 (0.88, 1.14) | 0.964 | 1.03 (0.91, 1.16) | 0.648 | 1.07 (0.96, 1.18) | 0.224 | 0.98 (0.79, 1.23) | 0.880 |
| Melanoma | 4869 | 1.03 (0.93, 1.14) | 0.614 | 1.00 (0.88, 1.13) | 0.997 | 1.04 (0.96, 1.12) | 0.356 | 0.85 (0.73, 0.99) | 0.040 |
| Thyroid | 375 | 0.47 (0.30, 0.73) | 0.001 | 0.70 (0.51, 0.98) | 0.038 | 0.95 (0.46, 1.96) | 0.897 | 0.60 (0.26, 1.38) | 0.228 |

BCAC indicates Breast Cancer Association Consortium; CI, confidence interval; ER, estrogen receptor; TSH, thyroid-stimulating hormone; OR, odds ratio; UKBB, UK Biobank.