

Causal Associations of Hypothyroidism with Prostate Cancer, Colorectal Cancer and Lung Cancer: A Mendelian Randomization Study

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Abstract: Previous observational studies have suggested that hypothyroidism may affect the risk of cancer. However, the causal effects were still unclear and controversial. Therefore, a two-sample Mendelian randomization (MR) analysis was conducted to evaluate the association between hypothyroidism and six types of cancer, including colorectum, pancreas, prostate, lung, bladder and stomach cancer. The inverse variance weighted (IVW), MR-Egger and Weighted Media were carried out to estimate the relation in the MR analysis. Different approaches such as leave-one-out analysis and MR-PRESSO for sensitivity analyses were used to assess the stability of the results. The MR results showed that hypothyroidism was causally associated with decreased risk of lung cancer (OR [odds ratio] = 0.19, P= 0.007), colorectal cancer (OR = 0.27, P= 0.016) and prostate cancer (OR = 0.18, P= 7.44e-05). Significant association between hypothyroidism and the risk of bladder cancer, stomach cancer and pancreas were not found with IVW P>0.05. These results indicated that hypothyroidism can decrease the risk of lung cancer, colorectal cancer and prostate cancer. But evidence of the association between hypothyroidism and other three types of cancer was not found in our study. Further research is necessary to clarify the relation.

1. Introduction

Hypothyroidism is considered to be one of the most common clinical hormone deficiencies across the world. A study shows that the prevalence of it in China after universal salt iodization can reach 13.95% in adults, with the higher prevalence among the elderly and women in a section, or to continue after an extract^[1, 2]. The occurrence of hypothyroidism is related to genetic, environmental and other elements. Iodine deficiency and Hashimoto's thyroiditis are thought to be the main causes^[2, 3]. Thyroid hormones are essential to the metabolism of almost all cells and tissues in human body. And hypothyroidism has a variety of non-specific symptoms, which is estimated to affect cancer incidence^[4]. On one hand, patients with hypothyroidism are supposed to have an upregulation of ApoD, which is related to decreased oxidative stress and inflammation^[5]. On the other hand,

hypothyroidism is well documented to be related to lipid abnormalities, which could both affect cancer risk^[6, 7]. But past studies have mainly focused on the link between hypothyroidism and several types of cancers^[6, 7, 8, 9]. And their conclusions are also controversial. Exactly speaking, take colorectal cancer for example, Guifang Mu and colleagues carried out a case–control study which indicated that the prevalence of CRC in patients with subclinical hypothyroidism (SCH) was higher compared to patients with euthyroid subjects^[10]. And another study from Ben Boursi showed that untreated hypothyroidism was associated with increased risk of CRC^[11]. This result conflicted with an observatory study in Spain by Juan J. Diez, which indicated that hypothyroid patients aged 65 or over had a decreased risk of colorectal cancer^[12].

Although previous observational studies have been helpful and meaningful, they could be influenced by residual confounding and reverse causality. Therefore, randomized controlled trials are necessary to test these links.

Mendel Randomization (MR) is an effective method to study the causal relationship between exposure and results of cross-sectional studies while controlling for uncertain mixing. Conceptually speaking, it is similar to a randomized controlled experiment (RCT)^[13, 14]. Instrumental variables (IVs) are randomly classified as "cases" or "control groups" at birth and remain unchanged for life according to Mendel's second Law^[14]. MR can prove a causal relationship between exposure and outcome by analyzing the relationship between the study's instrumental variables and the outcome. In order to guarantee the robustness of causal predictions, MR Designs rely on the following three essential assumptions. Firstly, Single Nucleotide Polymorphisms (SNPs) are supposed to be strongly linked to exposure. Secondly, SNPs must be independent of possible confounding factors. Thirdly, the association between SNPs and outcome is only mediated by exposure and not through any other pathway^[15].

In the present study, we investigated the causal relationship between hypothyroidism and Non-Small Cell lung Cancer, prostate cancer, colorectal cancer, bladder cancer, stomach cancer and pancreas cancer by conducting two-sample MR analysis. By applying the MR approach, we can explore whether hypothyroidism casually affects the risk of cancer. On this basis, we try to further find out the role of hypothyroidism in cancer development, and ultimately help patients make a comprehensive diagnosis and choose appropriate treatment strategies^[16].

2. Materials and methods

2.1. Exposure Data

The IEU Open GWAS database (<https://gwas.mrcieu.ac.uk/>) provided the summary-level GWAS data correlated with hypothyroidism (GWAS ID: ukb-b-19732). The GWAS data included 22,687 patients and 440, 246 controls.

2.2. Outcome Data

GWAS data related to the six types was obtained In the FinnGen website database R8 edition including bladder cancer (2380 cases), stomach cancer (1227 cases), colorectal cancer (5458 cases), pancreatic cancer (1249 cases), prostate cancer (11590 cases), non-small cell lung cancer (3865 cases). Diagnose cancer according to the International Classification of Diseases Code (edition 8, 9, 10)^[17]. Details of data source are shown in Table 1.

Table 1: Source of data for the analysis.

Phenotype	Data Source	Cases(n)	Controls(n)	Population
Hypothyroidism	MRC-IEU	22,687	440,246	European
Bladder cancer	The FinnGen consortium	2,380	259,583	European
Pancreas cancer	The FinnGen consortium	1,249	259,583	European
Colorectal cancer	The FinnGen consortium	5,458	259,583	European
Stomach cancer	The FinnGen consortium	1,227	259,583	European
Prostate cancer	The FinnGen consortium	11,590	110,189	European
Non-small cell cancer	The FinnGen consortium	3,865	259,583	European

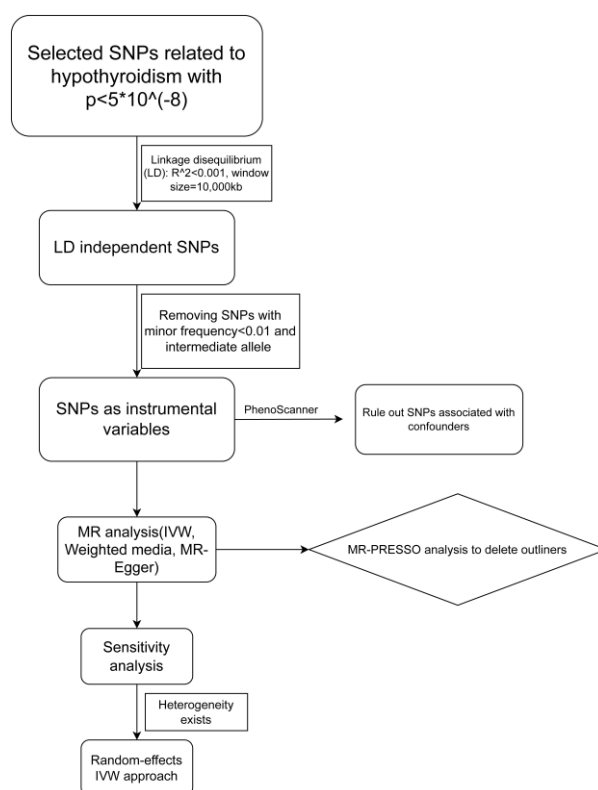
2.3. Method

We selected single nucleotide polymorphisms (SNPs) that have their p value $< 5 \times 10^{-8}$, which coincide with secondary particle frequency $> 1\%$ to prevent potential statistical bias from the original GWAS. After this, only SNPs with association disequilibria ($R^2 < 0.001$) at 10,000 kbp intervals across the genome was retained. Then we used the PhenoScanner database (<http://www.phenoscanter.medschl.cam.ac.uk/phenoscanter>) to examine the selected instruments variables associated with other phenotypes that may have effects on the outcome and made sure that the selected SNPs for exposure of hypothyroidism was not correlated with potential confounders and outcomes of genome-wide significance. In addition, F statistics was evacuated to prevent the disturbance from any weak instrumental variables ($F > 10$)^[18, 19, 20] and ensure the reliability of IVs. The detailed information of the selected SNPs is shown in the Supplementary File 1.

In the MR analysis, we used several approaches including inverse variance weighted (IVW), weighted median, and MR-Egger, to investigate the possible function of hyperthyroidism in different types of cancer after harmonization of effect alleles across the GWASs data. Diverse approaches were used because of their different fundamental assumptions about horizontal pleiotropy. IVW meta-analysis of Wald ratios for individual SNPs was considered to be the primary outcome, assuming that the instruments could influence the outcome only by interest exposure^[21]. Despite IVW, MR-Egger and Weighted Median methods were used to complete IVW outcome since these methods provided more reliable estimates in a broader range of scenarios but could have been more efficient^[22, 23].

Sensitivity analysis was performed to explore underlying pleiotropy and the heterogeneity in the MR study. We represented potential horizontal pleiotropy with heterogeneity markers from the IVW approach. And the MR-Egger regression was carried out to calculate the intercept indicating directional pleiotropy (P-value < 0.05 was considered to be the existence of directional multiplicity)^[24]. And the MR-PRESSO method was used to find out possible outliers while analyzing the relation between hypothyroidism and cancer^[25, 26, 27]. The Leave-One-Out (LOO) method was performed to assess the sensitivity of the results by sequentially removing one SNPs at a time to know whether the MR estimate was driven or biased by a single SNP^[27]. A flow chart of the MR analysis was shown in Figure 1.

The MR analysis was implemented by the package TwoSampleMR (version 0.5.7) in R (version 4.3.0).



Abbreviations: IVW, inverse variance weighted; MR, Mendel randomization.

Figure 1: Flow chart of the MR analysis design.

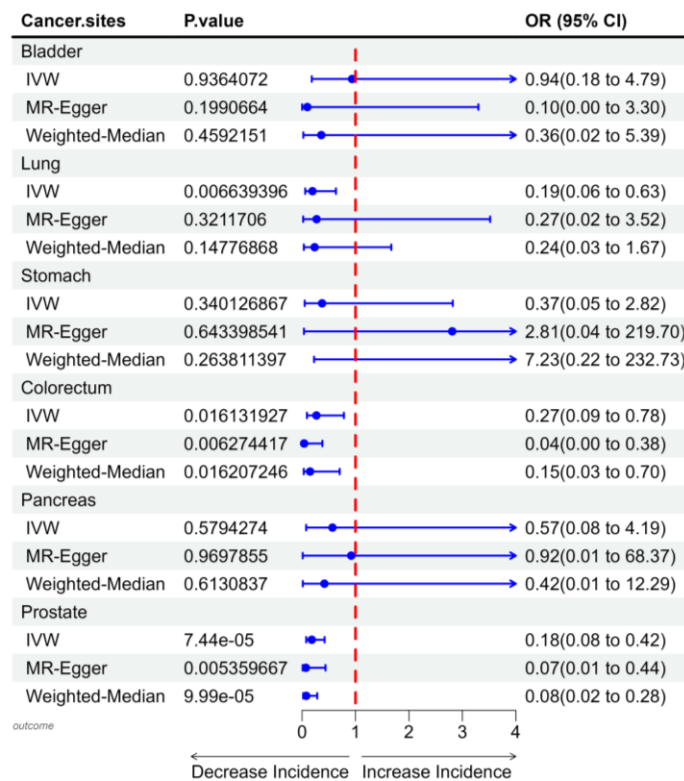
3. Results

Following the rigorous selection criteria and 116 SNPs were identified to genetically predict Hypothyroidism. All of the genetic tools used in the study were of high quality and reliability with F statistic values >10 . Three outliers (rs12271161, rs174599, rs61776678) were found out by the MR-PRESSO method while analyzing the relation of colorectal cancer. And two outliers (rs7705526, rs897586) while analyzing the relation between prostate cancer and hypothyroidism. After deleting these values and applying MR Analysis, the results of estimated causal associations between hypothyroidism and six types of cancers were estimated and shown in Figure 2.

The results indicated that hypothyroidism was associated with a falling risk of occurrence of Colorectal cancer (odds ratio (OR)_{IVW} = 0.27, $P = 0.016$; OR_{MR-Egger} = 0.04, $P = 0.006$; OR_{weighted median} = 0.15, $P = 0.016$). What's more, a significant association of prostate cancer was found (OR_{IVW} = 0.18, $P = 7.44e-05$; OR_{MR-Egger} = 0.07, $P = 0.005$; OR_{weighted median} = 0.08, $P = 9.99e-05$). Additionally, IVW showed that hypothyroidism had a decreased influence on non-small cell lung cancer with OR_{IVW} = 0.19, $P = 0.007$. But the relation was not found in MR-Egger and Weighted Median with $P > 0.05$. The reason why three analysis methods resulted in inconsistent outcome was that they were based on different assumption. However, assuming that the instruments could influence the outcome only by interest exposure, IVW analysis was considered to be the most accurate method. Additionally, the consistency of their directions improved the results' persuasiveness as well.

IVW showed that hypothyroidism had a significant causal relationship with colorectal cancer, prostate cancer and non-small cell lung cancer (IVW: $P < 0.05$), but no significant causal relationship with bladder cancer, stomach cancer, and pancreatic cancer (IVW : $P > 0.05$). The MR results are shown in Figure 2.

To measure the stability and possible biases of our outcome, we conducted a sensitivity analysis using multiple methods, including the Cochran Q statistic, the funnel plot, the scatter plot, the MR-Egger intercept, and leave-one-out (LOO) analyses. Heterogeneity was found in the analysis of prostate cancer. After deleting two outliers by MR-PRESSO, heterogeneity was still apparent ($P=0.0389$, derived from Cochran q validation). Therefore, we used the random-effects IVW approach to test the former result [24, 28, 29]. With the random-effects IVW $P=7.44e-05$, the relation was proved. In addition, no evidence of heterogeneity was found in other MR Analyses with $p > 0.05$ derived from the Cochran Q test. And horizontal pleiotropy ($p > 0.05$ induced by MR-Egger slice) were not found in all MR analysis. Scatter plots did not present any apparent intercepts, and funnel plots were symmetrical, indicating that there was no heterogeneity or pleiotropy. The results of the leave-one-out sensitivity analyses showed that no single influential SNP affected the overall causal link and the robustness of our conclusion [27]. All scatter plots, funnel plots, and LOO plots were displayed in Supplementary File 2.



Abbreviations: OR, odds ratio; CI, confidence interval; IVW, inverse variance weighted.

Figure 2: Associations of hypothyroidism with risk of cancer.

4. Discussion

In this study, a two-sample MR analysis was performed using the instrumental variables of large-scale GWAS to assess the causal relationship between Hypothyroidism and cancers using genetic data from populations of European descent. In our MR analysis, genetic predisposition to Hypothyroidism was associated with a lower risk of lung cancer (OR=0.19, 95% CI = 0.06-0.63), colorectal cancer (OR=0.27, 95% CI = 0.09-0.78) and prostate cancer (OR=0.18, 95% CI = 0.08-0.42). And no strong evidence was found to support associations between hypothyroidism and the risk of bladder, stomach and pancreas cancer. These results might have implications for public health interventions and decrease cancer risk.

Despite the contradictory conclusions, a growing number of observational studies have provided the evidence of the link between hypothyroidism and other cancers. For instance, L'Heureux conducted a case-control study in Taiwan including 69,713 CRC patients and 69,713 controls. It showed a 22% lower risk of CRC in people with hypothyroidism with an adjusted OR of 0.78 [30, 31]. Another study by Mondul et al. observed that people with hypothyroidism were at decreased risk of prostate cancer compared to men with normal thyroid function [32, 33]. However, there were studies that conflicted with the association [10, 30, 34].

In this MR analysis, we didn't observe significant associations of genetic predisposition to hypothyroidism with bladder cancer, stomach cancer and pancreas cancer with $P > 0.05$. According to an observational study in Spain, hypothyroidism increased risk of all the specific neoplasms studied, except for bladder cancer but the conclusion was totally different in the hypothyroid patients aged 65 or over [12]. And another study in Danish indicated that there was no causal association between hypothyroidism and long-term risk of gastrointestinal cancer [35].

The present MR study observed little evidence of the association of hypothyroidism with pancreas, stomach and bladder cancers. Possible explanations behind the discrepancy in results across studies may be residual confounding or reverse causality in the observational studies. The definitive causal relationship requires more in-depth mechanism studies and RCT studies in the future [36].

Mendelian randomization can avoid the risk of bias with a large size of independent datasets used to select genetic instruments [37, 38]. This study tried to avoid some confounding factors and reverse causation, but some limitations still existed. First of all, with the data from people of European descent to investigate the causal relationship, it may not be possible to be generalized to other populations. And the absence of sex-stratified summary information should also be considered. Moreover, types of hypothyroidism such as overt and subclinical hypothyroidism are not distinguished, which might have different effects on the risk of disease. And like other MR studies, horizontal pleiotropy is difficult to totally avoid. Although some MR methods like the leave-one-out method and MR-Egger were used to test the possible pleiotropy, the possibility of bias could not be prevented.

5. Conclusion

In a nutshell, hypothyroidism can decrease the risk of lung cancer, colorectal cancer and prostate cancer. But evidence of the association between hypothyroidism and bladder cancer, stomach cancer and pancreas cancer were not found.

Although previous observational studies had shown an association between hypothyroidism and cancer, confounding factors are difficult to avoid in traditional observational study, which may lead to controversial outcome. More mechanism studies and RCT studies may be needed to clarify the relationship between hypothyroidism and important risk factors for cancer.

Data available statement

The GWAS data used in this study is available in the FinnGen Database and IEU Open GWAS database.

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Appendix

- Supplementary material 1 Instrumental SNPs from hypothyroidism GWASs.
- Supplementary material 2 Leave-one-out analysis, funnel plots and scatter plots