The influence of hypothyroidism, hyperthyroidism, autoimmune thyroiditis and thyroid cancer on the risk of polycystic ovary syndrome: a Mendelian Randomization study

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Research Article

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Abstract

Background

Observational studies have found a correlation between thyroid diseases and polycystic ovary syndrome (PCOS). However, their causal relationship remains uncertain. Our purpose was to investigate the causal relationship between four common thyroid diseases (hypothyroidism, hyperthyroidism, autoimmune thyroiditis, thyroid cancer) and PCOS risk.

Methods

In this study, using the Mendelian randomization (MR) approach, we obtained single nucleotide polymorphisms (SNPs) from the genome-wide association study (GWAS) database as instrumental variables (IVs) and used inverse variance weighting (IVW) to explore the causal relationship between four common thyroid diseases and PCOS.

Results

We found a significant causal association between hypothyroidism and increased risk of PCOS [OR = 34.90, 95% CI: (1.68, 724.53), P = 0.02]. However, hyperthyroidism, autoimmune thyroiditis, thyroid cancer has no significant causal association with PCOS.

Conclusion

Hypothyroidism may increase the risk of PCOS. Hyperthyroidism, autoimmune thyroiditis, thyroid cancer has no significant causal association with PCOS.

1. Introduction

PCOS is a common endocrine disorder in women of reproductive age. Its main clinical manifestations include hyperandrogenic syndrome and hirsutism, and imaging examinations often suggest polycystic ovarian changes [1]. In addition, patients with PCOS often have a combination of various endocrine metabolic problems, including hyperinsulinemia, endothelial dysfunction, and obesity [1]. Some studies have shown that PCOS is a risk factor for certain cardiovascular diseases and even cancer [2]. The thyroid gland is one of the important endocrine organs in the body, which regulates the Hypothalamus-Pituitary-Thyroid axis and affects the pituitary endocrinology of FSH and LH. In women, the cyclic changes of these hormones are closely related to the formation of the menstrual cycle and egg production. Currently, many observational studies have found a close association between thyroid diseases and PCOS. Glintborg, D. et al. found an increased risk of hypothyroidism, Graves' disease, goiter, and thyroiditis in the Danish population with PCOS [3]. Similar findings have been found in many studies [4–9]. A recent large sample meta-analysis study [10, 11] also revealed a strong association between thyroid disease and PCOS. Xing, Y. et al. showed that subclinical hypothyroidism and polycystic ovary syndrome frequently occur together and that patients with subclinical hypothyroidism combined with polycystic

ovary syndrome have poorer glucose and lipid metabolism [11]. However, there is no evidence to prove a causal relationship between thyroid diseases and PCOS. Moreover, due to the experimental design of previous observational studies, it could only conclude a correlation between hypothyroidism and PCOS without being able to determine a causal relationship between the two, and its results may be influenced by some potentially confounding factors.

MR analysis is an epidemiological method [12] that can be used to assess the causal relationship between exposure and outcome, which is unidirectional. In addition, this analytical approach is better able to circumvent the influence of confounding factors on the relationship between exposure and outcome, since genetic variants follow a strict random distribution at the time of conception and they usually act independently of environmental risk factors on the relationship between exposure and outcome [12]. Meanwhile, two-sample MR analyses can efficiently investigate disease causality based on data from open-access genome-wide association studies (GWASs). MR methods have been successful in explaining the causal relationship between exposure and outcome in many studies. For example, Shizheng Qiu et al found that hypothyroidism can increase the risk of NAFLD [13], and Yaokai Wen et al found that patients with PCOS have a higher risk of breast cancer [14]. However, there are no published MR studies between thyroid diseases and the risk of PCOS.

In our study, genetic variants associated with four common thyroid diseases (hypothyroidism, hyperthyroidism, autoimmune thyroiditis, thyroid cancer) were introduced as instrumental variables (IVs). By calculating the ratio of the effect of single nucleotide polymorphisms (SNPs) on the probability of PCOS occurrence to the effect of SNPs on four thyroid diseases, we attempted to explore the causal relationship between four thyroid diseases and PCOS.

2. Materials And Methods

2.1. Overall study design

Meeting three key assumptions is the basis of MR analysis. As shown in Fig. 1, in order to provide a valid interpretation of the MR analysis, the following three assumptions must hold [15]. (1) IVs are significantly associated with hypothyroidism ($p < 5 \times 10^{-8}$); (2) IVs affect PCOS only through their effect on hypothyroidism. (3) IVs are independent of any confounders of the hypothyroidism-PCOS relationship. All SNPs and their associated information in this study were obtained from the GWAS (https://gwas.mrcieu.ac.uk/) website, which is a publicly available database. The latter analysis and graphing were achieved by R (version 4.1.1).

2.2. Data sources

The relevant information on the four thyroid diseases included in the study and their corresponding GWAS database are shown in Table 1. All the data is publicly available and the original research was approved by the Institute's institutional ethics approval committee. SNPs selected as IVs in our study needed to meet several conditions: $p < 5 \times 10^{-8}$, long physical distance in DNA (more than 10,000 kb), and no locus exceeding the limit in linkage disequilibrium (LD) analysis ($R^2 < 0.001$). This was to comply with the principles associated with Mendelian randomization analysis. In addition, these SNPs were required to exclude duplicate and echo SNPs. The PCOS dataset was obtained from the study on GWAS (Dataset ID: finn-b-E4_POCS), which included

European populations and contained 118870 samples and 16379676 SNPs. The data is publicly available and the original research was approved by the Institute's institutional ethics approval committee.

Table 1
The relevant information of the four thyroid diseases (hypothyroidism, hyperthyroidism, autoimmune thyroiditis, thyroid cancer) included in the study. SNP, single-nucleotide polymorphism.

Disease	GWAS ID	Population	Sample size	Number of SNPs	Author	Consortium
hypothyroidism	ukb-a-77	European	337,159	10,894,596	Neale	Neale Lab
hyperthyroidism	ukb-a-76	European	337,159	10,894,596	Neale	Neale Lab
Autoimmune thyroiditis	finn-b-E4_THYROIDITAUTOIM	European	187928	16,380,358	NA	NA
Thyroid cancer	ieu-a-1082	European	1,080	572,028	Kohler A	NA

2.3. Mendelian randomization analysis

Statistical tests for MR analysis were performed using the “TwoSampleMR” (version 0.5.6) package in R (version 4.1.1). In the MR analysis, the inverse variance weighted (IVW) method was used as the main analysis to access each IV’s combined causal effects, supplemented by the weighted median, simple mode, weight mode and MR-Egger methods. The IVW approach was used to explore the causal relationship between genetically determined exposure factors ($\beta_{hypothyroidism}$, $\beta_{hyperthyroidism}$, $\beta_{autoimmune\ thyroiditis}$, $\beta_{thyroid\ cancer}$) and the outcome (β_{PCOS}). $p < 0.05$ was considered statistically significant. The Wald ratio (β_{MR}) of hypothyroidism to PCOS through specified genetic variants is calculated as follows:

$$\beta_{MR} = \beta_{outcome}/\beta_{exposure}$$

2.4. Sensitivity analysis and pluripotency analysis

We used the “TwoSampleMR” (version 0.5.6) package in R (version 4.1.1) for sensitivity analysis and pluripotency analysis. Sensitivity analyses included weighted medians, and MR- egger. The weighted median is more robust to pooled effect sizes when more than 50% of the instrumental variables are valid (majority validity assumption). The MR-Egger method gives causal estimates in terms of regression slopes, while the MR-Egger intercept also provides an assessment of unbalanced horizontal polymorphism for all variants. In addition, we used a leave-one-out cross-validation [15]. Assuming that the set of genetic variants has k samples, we choose one genetic variant as the test set and k-1 genetic variants as the training set. It is possible to assess whether the overall results are driven by a genetic variant with a high degree of pluripotency. The fluctuation of the results before and after genetic variant removal reflects the sensitivity of the genetic variant. If the estimated value changes significantly when one of the genetic variants is discarded, it can be determined that this genetic variant is an outlier or sensitive value.

3. Results

Our final selection of SNPs met the basic conditions for Mendelian randomization analysis [$p < 5 \times 10^{-8}$, physically distant in DNA (more than 10,000 kb), and none of the loci exceeded the limiting value in linkage

disequilibrium (LD) analysis ($R^2 < 0.001$)]. For hypothyroidism, we selected 79 eligible SNPs as IVs. In addition, we selected 4 SNPs, 2 SNPs, 339 SNPs as IVs for hyperthyroidism, autoimmune thyroiditis and thyroid cancer [16]. Their information, including chromosome number, location, beta-value, P-value, is shown in Supplementary Table 1–4.

3.1. Hypothyroidism increases the risk of PCOS

IVW analysis found that hypothyroidism significantly increased the risk of PCOS [OR = 34.90, 95% CI: (1.68, 724.53), P = 0.02] (Fig. 2, Table 2). Since there was no significant heterogeneity between genetic variants (P = 0.17), a fixed model was preferred for MR analysis.

Table 2
The result of MR analysis. Causal effects of four thyroid diseases (hypothyroidism, hyperthyroidism, autoimmune thyroiditis, thyroid cancer) on PCOS. SNP, single-nucleotide polymorphism.

Exposure	Methods	nSNP	OR (95%CI)	*P*
Hypothyroidism				
	MR Egger	79	10.69498 (0.013281473, 8612.1885)	0.48966886
	Weighted median	79	21.19550 (0.210461176, 2134.5951)	0.1943816
	Inverse variance weighted	**79**	**34.90300 (1.681390116, 724.5313)**	**0.02168748**
	Simple mode	79	57.59131 (0.006404258, 517898.9654)	0.3855407
	Weighted mode	79	29.78323 (0.180173424, 4923.2606)	0.19662826
Hyperthyroidism				
	MR Egger	4	2.812321*10^-7 (3.813993*10^-95, 2.073719*10^81)	0.8972199
	Weighted median	4	9.226651*10^5 (2.701528*10^-10, 3.151220*10^21)	0.4516507
	Inverse variance weighted	4	5.881278*10^6 (4.629958*10^-7, 7.470787*10^19)	0.3112819
	Simple mode	4	20.02731 (7.876961*10^-20, 5.091977*10^21)	0.9084108
	Weighted mode	4	43.42831 (1.572994*10^-18, 1.198999*10^21)	0.8793515
Autoimmune thyroiditis				
	MR Egger	NA	NA	NA
	Weighted median	NA	NA	NA
	Inverse variance weighted	2	1.198641 (0.9706917, 1.480119)	0.09225952
	Simple mode	NA	NA	NA
	Weighted mode	NA	NA	NA
Thyroid cancer				
	MR Egger	339	1.0029354 (0.9939187, 1.012034)	0.5251146
	Weighted median	339	1.0001839 (0.9926931, 1.007731)	0.9617634
	Inverse variance weighted	339	0.9999478 (0.9947547, 1.005168)	0.9843229
	Simple mode	339	0.9992148 (0.9844690, 1.014182)	0.9175889

Exposure	Methods	nSNP	OR (95%CI)	*P*
	Weighted mode	339	0.9992148 (0.9893031, 1.009226)	0.8773647

3.2. The causal effect of hyperthyroidism, autoimmune thyroiditis and thyroid cancer on PCOS was not statistically significant

We found that the causal effect of hyperthyroidism, autoimmune thyroiditis and thyroid cancer on PCOS was not statistically significant. IVW analysis suggested the causal effect of hyperthyroidism on PCOS [OR = 5.881278e + 06, 95%CI: (4.629958e-07, 7.470787e + 19), P = 0.3112819], Table 2; autoimmune thyroiditis on PCOS [OR = 1.198641, 95%CI: (0.9706917, 1.480119), P = 0.09225952], Table 2; thyroid cancer on PCOS [OR = 0.9999478, 95%CI: (0.9947547, 1.005168), P = 0.09225952], Table 2. Next, we performed further sensitivity analysis and pluripotency analysis of the causal effect of hypothyroidism on PCOS.

3.3. Sensitivity analysis

By the leave-one-out method, we performed a sensitivity analysis of the causal effect of hypothyroidism on PCOS. We calculated the MR results after removing the remaining veins one by one. The results showed that the beta value was greater than zero regardless of which gene variant was removed. Thus, the conclusion that hypothyroidism increases the risk of PCOS is definitive (Fig. 3). There was also no significant difference in the overall results (P < 0.01). All results indicated that all genetic variants were insensitive. These analysis tools were derived from the "TwoSampleMR" package in R (version 4.1.1).

3.4. Pluripotency analysis

We used five methods, including MR Egger, Weighted median, Inverse variance weighted, Simple mode, and Weighted mode, to evaluate the causal effect of hypothyroidism on PCOS (Fig. 4). Among them, MR- egger method was used to measure the pleiotropy of IVs. we found no statistical difference between the zero intercept of IVW and the intercept of MR-Egger (p > 0.05). These analysis tools were obtained from the "TwoSampleMR" package in R (version 4.1.1).

4. Discussion

In our study, the causal relationship between four common thyroid diseases (hypothyroidism, hyperthyroidism, autoimmune thyroiditis, thyroid cancer) and PCOS was explored using a two-sample MR. We found that hypothyroidism can significantly increase the risk of PCOS [OR = 34.90, 95% CI: (1.68, 724.53), P = 0.02]. However, the causal effect of hyperthyroidism, autoimmune thyroiditis and thyroid cancer on PCOS was not statistically significant.

Many studies have investigated the relationship between thyroid diseases and PCOS from different perspectives. Singh, J. et al found multiple causes of hypothyroidism leading to the development of PCOS, among which, there was a significant relationship between the incidence of polycystic ovary syndrome and thyroiditis [17]. A study by Nayak, P. K. et al found that patients with PCOS were more often associated with subclinical hypothyroidism [7]. Raj, D found similar findings in an Asian population where SCH was more

prevalent in patients with PCOS (43.5% vs. 20.5%; P < 0.001) [18]. In addition, subclinical hypothyroidism can exacerbate insulin resistance [5].

There is a lack of clarity regarding the interrelationship between hypothyroidism and PCOS [19]. Hypothyroidism is characterized by decreased T3 and T4 levels and increased TSH levels. In contrast, PCOS is characterized by increased pituitary sensitivity to GnRH and excessive LH secretion leading to excessive androgen production in the ovarian mesenchyme. This suggests a possible interaction of the hypothalamic-pituitary-thyroid (ovarian) axis in the development of both diseases. In addition, it has been found that the angiopoietin-like protein 8 (ANGPTL8) plays an important role in the pathogenesis of both hypothyroidism [20, 21] and PCOS [22].

Our study has several advantages. First, to the best of our knowledge, this study is the first MR study on the causal relationship between thyroid diseases and PCOS. Second, we included the PCOS-related SNP dataset from the largest GWAS available, which contains 118,870 samples and 16,379,676 SNPs, and the thyroid diseases datasets were obtained from the largest GWAS datasets, which can increase the statistical power of our study. Moreover, the MR approach allows us to obtain a simple causal relationship between thyroid diseases and PCOS and to reduce the influence of confounding factors on the relationship between hypothyroidism and PCOS. This is because MR is based on the Mendelian correlation law of inheritance, where randomization among SNPs in different individuals is done at the time of gamete generation, and this randomization is similar to the randomized grouping in randomized controlled studies. It is also because individual SNP randomization is completed before an individual is born that the use of SNP as an instrumental variable to study exposure and disease causation is less likely to be confounded by acquired confounding factors.

However, some limitations of our study should not be overlooked. First, because of the small number of SNP datasets currently available for study, the samples included in this study were from European populations, so it is unknown whether our findings can be generalized to other ethnic groups. In addition, because PCOS is a heterogeneous disease that may vary greatly among patients, and because there are no GWAS datasets available on PCOS subgroups, we did not subgroup the PCOS population in our two-sample MR study. Besides, due to the limitation of the GWAS database, we only found four common thyroid disease-related data sets and included them in our study.

In conclusion, the results of our MR study suggest a significant causal relationship between hypothyroidism and PCOS development in a sample of European ancestry. The causal effect of hyperthyroidism, autoimmune thyroiditis and thyroid cancer on PCOS was not statistically significant. More studies about the causality between PCOS and thyroid diseases are needed in the future to explore the relationship between these two diseases.

Declarations

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Data Availability Statement: All data in this study are public and available through GWAS (https://gwas.mrcieu.ac.uk/)

Conflicts of Interest: The authors declare to have no competing interests.

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Figures

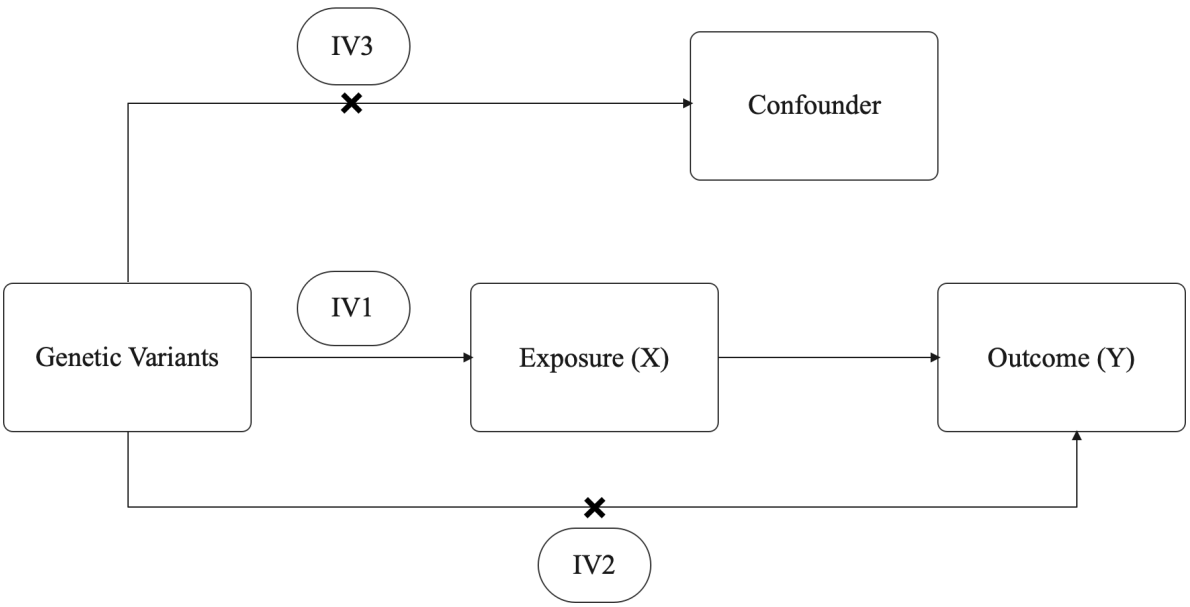

Figure 1

Three key assumptions of MR analysis. (1) IVs are significantly associated with exposure ($p < 5 \times 10^{-8}$); (2) IVs affect the outcome only through their effect on exposure. (3) IVs are independent of any confounders of the exposure-outcome relationship. MR, Mendelian randomization

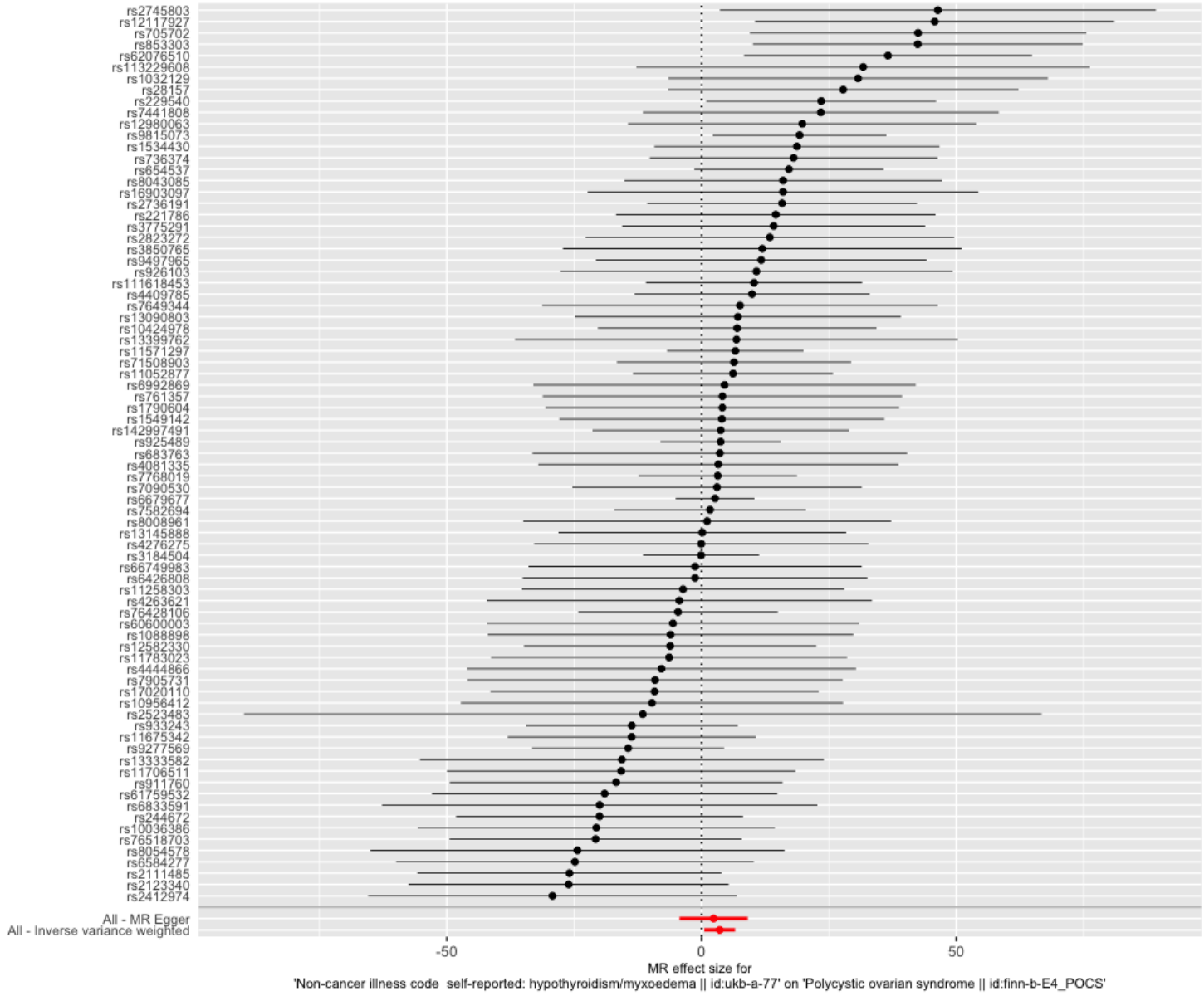

Figure 2

Forest plot of SNPs associated with hypothyroidism and PCOS risk. Each black point represents the log odds ratio (OR) for hypothyroidism levels in PCOS, produced by 79 hypothyroidism-related SNPs as IVs. SNP, single-nucleotide polymorphism.

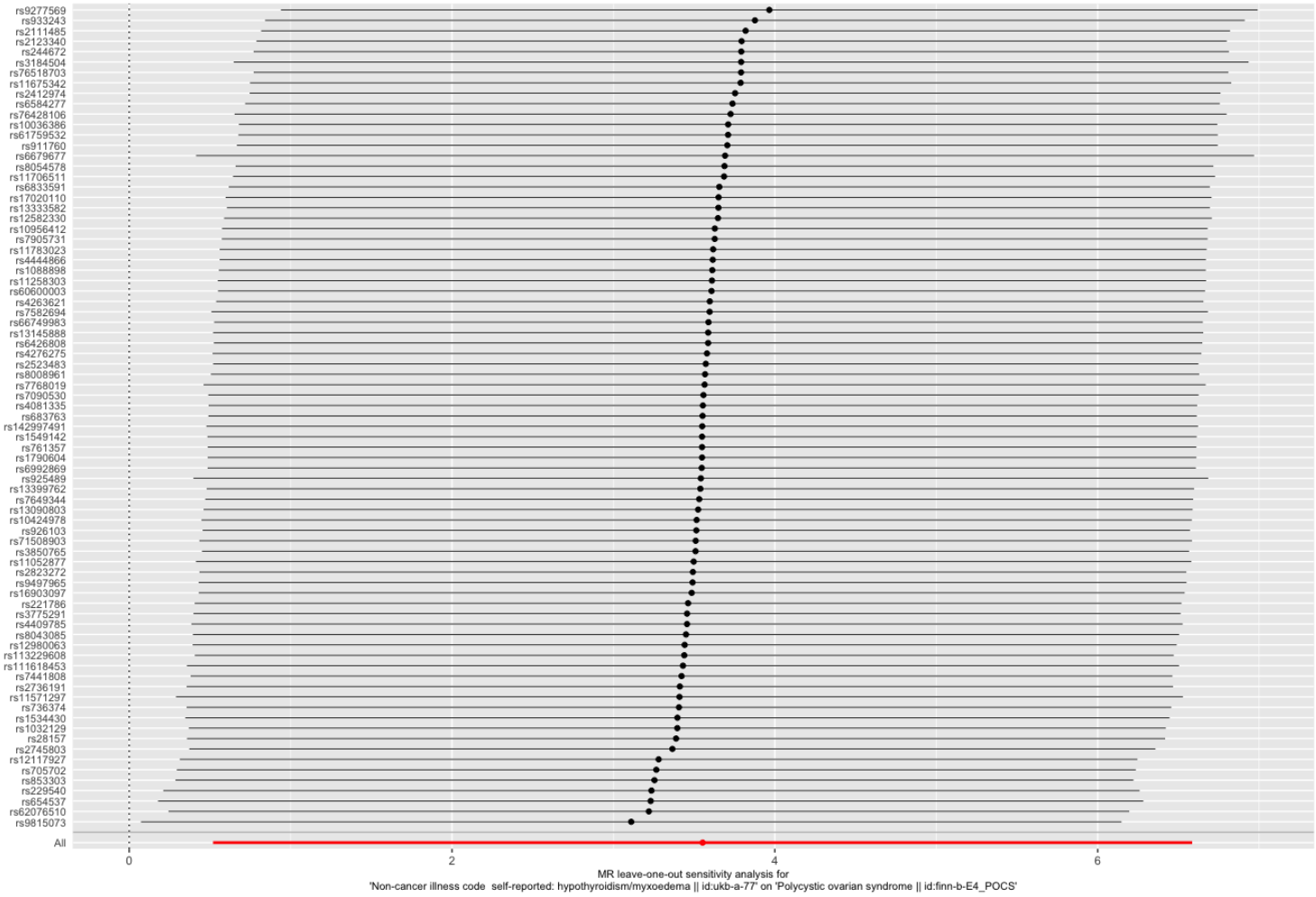

Figure 3

Leave-one-out cross-validation. The red line is the average of all β values after leave-one-out.

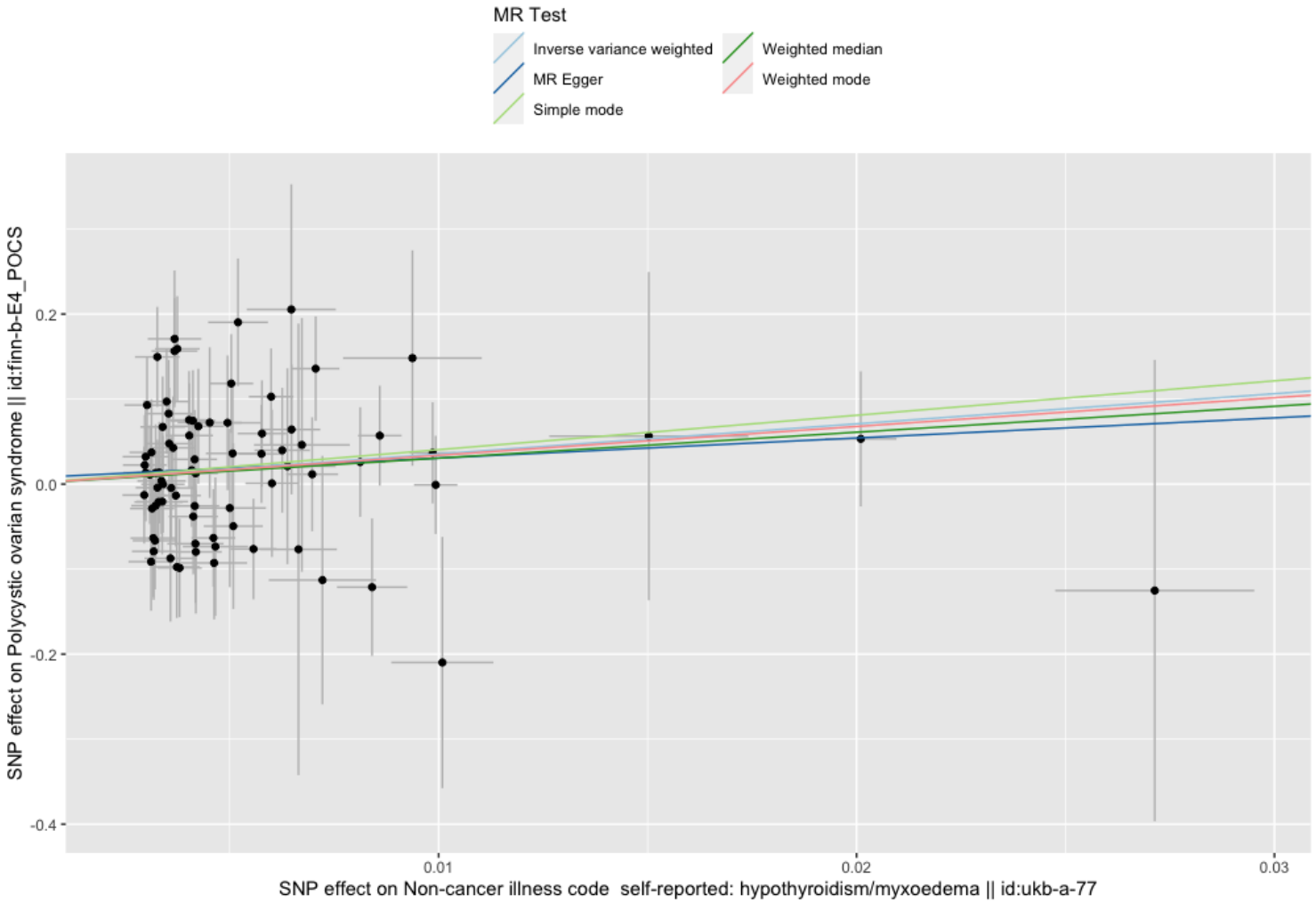

Figure 4

Pluripotency analysis. The estimate of intercept can be interpreted as an estimate of the average pleiotropy of all SNPs, and the slope coefficient provides an estimate of the bias of the causal effect. MR, Mendelian randomization; SNP, single-nucleotide polymorphism.

Supplementary Files

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- supplementtable1.xlsx
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