ISSN 2618-1584 Vol. 7, Issue 2: 118-124, DOI: 10.25236/FMSR.2025.070217

Causal Relationship between Hypothyroidism and Adverse Pregnancy Outcomes: A Multivariate Mendelian Randomization Study

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Abstract: *Hypothyroidism has been associated with adverse pregnancy outcomes in observational studies, but these associations may be influenced by residual confounding or reverse causation. We conducted a pioneering two-sample Mendelian randomization (MR) investigation using aggregated data from a large-scale genome-wide association study to examine the correlation between hypothyroidism and preeclampsia, gestational diabetes, miscarriage, placental abruption, and preterm labor. Inverse variance weighting served as the primary analytical approach, complemented by MR Egger regression, weighted median, simple modal, and weighted methods. Sensitivity analyses, including MR-PRESSO, MR-Egger regression, and leave-one-out analysis, were performed to test the robustness of the findings. Multivariable MR (MVMR), adjusting for smoking and body mass index, was further conducted to validate the MR results. The IVW analysis indicated that hypothyroidism may increase the risk of miscarriage and preeclampsia (OR = 1.004, 95% CI = 1.000–1.008, p = 0.039; OR = 1.588, 95% CI = 1.027–2.456, p = 0.038). MVMR demonstrated that the causal effect of hypothyroidism on miscarriage and preeclampsia persisted after adjusting for body mass index (OR = 0.016, 95% CI = 1.005–0.995, p = 0.005; OR = 1.7169, 95% CI = 1.065–2.767, p = 0.0264). These findings furnish compelling evidence supporting a causal association between hypothyroidism and miscarriage, as well as preeclampsia.*

Keywords: *hypothyroidism, adverse pregnancy outcome, Mendelian randomization*

1. Introduction

Hypothyroidism is a prevalent endocrine disorder characterized by inadequate secretion of thyroid hormones (TH) or a deficiency in their physiological effects. This condition encompasses both subclinical hypothyroidism (SCH) and overt hypothyroidism (OH) [1]. Research indicates that the prevalence of thyroid dysfunction in non-pregnant women of reproductive age is approximately 17.2% [2]. A meta-analysis of 97 studies estimates that the prevalence of hypothyroidism during pregnancy ranges from 0.4% to 13.1%, whereas subclinical hypothyroidism ranges from 3.3% to 42.9% [3]. Elevating the risk of fetal developmental complications and adverse pregnancy outcomes [4]. Numerous observational studies have demonstrated that maternal hypothyroidism is linked to a heightened risk of adverse pregnancy outcomes (APOs), such as miscarriage [5], preeclampsia, gestational diabetes, preterm birth, placental abruption, and detrimental neurodevelopmental effects on offspring. However, these conclusions remain controversial. Presently, the body of existing research is predominantly observational and encompasses only a limited segment of the population. Therefore, there is an urgent need for more robust evidence to establish a definitive causal relationship between hypothyroidism and adverse pregnancy outcomes. Accurate identification of women at risk for adverse outcomes, coupled with early intervention in pregnant women with hypothyroidism, can significantly enhance maternal and neonatal prognoses.

MR analysis is an effective tool for causal inference, which can be employed to examine the potential causal relationships between exposure and outcomes of interest [6]. Compared to randomized controlled trials, the MR approach is advantageous because the genetic variations individuals possess are randomly assigned at conception and remain unchanged throughout their lives. Consequently, this method is not subject to the biases of confounding factors and reverse causation [7]. This study aims to employ two-sample MR analysis using aggregated data from large-scale, publicly available GWAS to investigate the causal relationships between hypothyroidism and outcomes such as miscarriage, preeclampsia,

ISSN 2618-1584 Vol. 7, Issue 2: 118-124, DOI: 10.25236/FMSR.2025.070217

gestational diabetes, placental abruption, and preterm birth.

2. Methods and Materials

2.1 Study Design

This study employs a two-sample MR approach to evaluate the causal relationship between hypothyroidism and APOs. Our study adhered rigorously to the MR guidelines and the STROBE-MR study report, thereby ensuring the robustness of the MR investigation. Instrumental variable analysis was employed to emulate randomized controlled trials (RCTs) by simulating the random allocation of progeny single nucleotide polymorphisms (SNPs). SNPs were selected as instrumental variables (IVs) from existing datasets derived from GWAS meta-analysis studies or the Integrated Epidemiology Unit's Open GWAS project. SNPs were chosen to satisfy the following three assumptions (Figure 1).

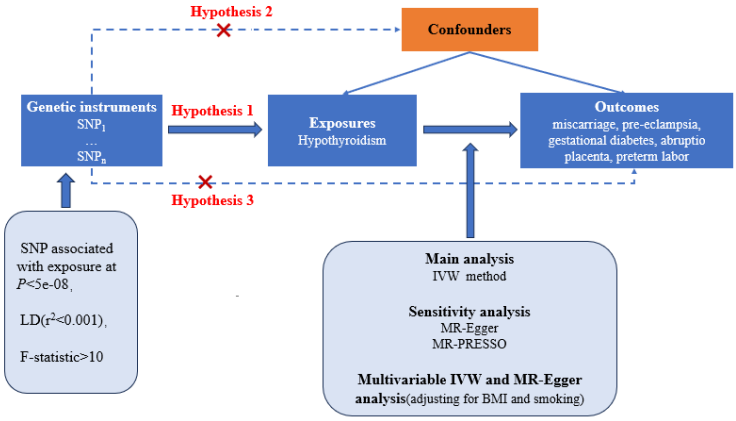

Figure 1: Schematic of the two-sample Mendelian randomization analysis

2.2 Data Sources

Publicly accessible summary statistics pertaining to hypothyroidism were acquired from the IEU OpenGWAS project website (comprising a sample size of 410,141 cases). GWAS data concerning miscarriage, preeclampsia, gestational diabetes mellitus, placental abruption, and preterm labor encompassed 247,180, 114,818, 123,579, 104,541, and 64,923 subjects, respectively, sourced from the FinnGen database. (Table 1). All GWAS data utilized in this study were derived from cohorts of European ancestry. All data underwent approval from the respective ethical review boards, and participants provided informed consent. Consequently, no supplementary ethical review board approval was necessary.

Table 1 Overview of study data.

Disease	***Source***	***Cases***	***Controls***	***Sample size***	***Population***
hypothyroidism	*MRC-IEU*	*30,155*	*379,986*	*410,141*	*European*
miscarriage	*MRC-IEU*	*79,047*	*168,133*	*247,180*	*European*
pre-eclampsia	*FinnGen*	*83*	*114,735*	*114,818*	*European*
gestational diabetes	*FinnGen*	*5,687*	*117,892*	*123,579*	*European*
abruptio placenta	*FinnGen*	*294*	*104,247*	*104,541*	*European*
preterm birth	*EGG Consortium*	*4,775*	*60,148*	*64,923*	*European*

2.3 Single Nucleotide Polymorphisms Selection

The accuracy of inferring a causal effect of hypothyroidism on APOs was ensured through the following quality control step, applied to the section on genetic predictors associated with hypothyroidism. To satisfy the three hypotheses of the MR analysis, we initially incorporated SNPs

ISSN 2618-1584 Vol. 7, Issue 2: 118-124, DOI: 10.25236/FMSR.2025.070217

meeting the genome-wide significance threshold of P<5e-8 in this study, ensuring strong associations between the selected SNPs as IV and hypothyroidism. Subsequently, SNPs with corresponding linkage disequilibrium (LD) status were evaluated, identified, and then excluded (r^2<0.001, kb=10000). Additionally, to avoid weak instrument bias, the F-statistic is calculated using the formula $F = R^2 \times (n - k - 1) / [(1 - R^2) \times k]$, where n is the sample size for the exposure, k is the number of SNPs, and R^2 is the proportion of variance explained by the exposure variable. The R^2 is computed as $R^2 = 2 \times MAF \times (1 - MAF) \times beta^2$, where MAF is the minor allele frequency and beta represents the estimated effect of the variant on the exposure. where an F-statistic <10 indicates potential weak instrumental bias, leading to the exclusion of the corresponding SNPs [8].

2.4 Statistical Analysis

This study primarily employed inverse variance weighting (IVW) to evaluate the causal relationship between hypothyroidism and APOs, supplemented by MR Egger regression, weighted median, simple modal, and weighted models as complementary approaches. We conducted three sensitivity analyses to evaluate the robustness of the MR results, comprising the heterogeneity test, the multiple validity test, and the leave-one-out sensitivity analysis. Heterogeneity was assessed using Cochran's Q test, where P>0.05 indicates the absence of heterogeneity [9]. In the presence of heterogeneity, IVW multiplicative random-effects models should be employed [10]. Horizontal pleiotropy was assessed using the intercept of the MR-Egger analysis results, where P>0.05 suggests the absence of horizontal pleiotropy [11]. Furthermore, the MR PRESSO method is capable of simultaneously identifying outliers and detecting horizontal pleiotropy [12]. Additionally, we conducted leave-one-out analyses to assess whether causal associations were driven by a single SNP, employing MR iteratively to exclude various SNPs with the "mr leave one out plot" tool. Scatter plots, forest plots, and funnel plots were employed to visualize the results and assess the stability of the MR. All analyses were conducted using the "Two-Sample-MR" and "MR-PRESSO" packages in R software (version 4.2.3).

3. Results

In this study, 66, 68, 67, 68, and 59 SNPs were ultimately identified as IVs for hypothyroidism to investigate the relationship between hypothyroidism and miscarriage, preeclampsia, gestational diabetes mellitus, placenta previa, and preterm labor, respectively. The F-statistics for all of these genetic variants exceeded 10, suggesting a low likelihood of weak instrumental bias.

IVW analysis indicated that hypothyroidism was linked to a heightened risk of miscarriage (odds ratio (OR) = 1.004, 95%CI = 1.000-1.008, p = 0.039) and preeclampsia (OR = 1.588, 95%CI = 1.027-2.456, p = 0.038), both with statistically significant associations. MR-Egger analysis yielded similar findings for pre-eclampsia (OR = 2.984, 95%CI = 1.180-7.543, p = 0.024), suggesting that hypothyroidism serves as a risk factor for both miscarriage and pre-eclampsia, thereby elevating the risk for these conditions in patients with hypothyroidism. However, no evidence of an association between hypothyroidism and gestational diabetes mellitus (OR = 0.988, 95%CI = 0.926-1.055, p = 0.723) or placental abruption (OR = 0.963, 95%CI = 0.758-1.224, p = 0.759) was found. In MVMR analyses, after adjusting for BMI, a significant high-risk causal association was observed between hypothyroidism and miscarriage (OR = 0.016, 95%CI = 1.005-0.995, p = 0.005), as well as smoking (OR = 0.134, 95%CI = 1.075-1.010, p = 0.032). Additionally, a causal relationship was identified between hypothyroidism and a significantly higher risk of pre-eclampsia (OR = 1.7169, 95%CI = 1.065-2.767, p = 0.0264). This was consistent with the findings of the two-sample MR analysis. Nevertheless, no causal association was observed between hypothyroidism and gestational diabetes mellitus, placental abruption, or preterm labor in MVMR.

Sensitivity analyses for all diseases revealed consistent findings, with both MR-Egger and MR-PRESSO indicating no evidence of horizontal pleiotropy and p-values exceeding 0.05 (Table 2). Additionally, scatter plots and funnel plots comparing hypothyroidism with APOs yielded similar outcomes, and once more, no evidence of horizontal pleiotropy was observed in the forest plot. The leave-one-out plot indicates that individual SNPs do not significantly impact the overall estimate. (Figure 2-3).

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Table 2 Outcomes of heterogeneity and multiplicity analysis

Outcome	Heterogeneity		Pleiotropy test		MR-PRESSO
	Q	*P* value	Egger Intercept	*P* value	*P* value
miscarriage	80.8266	0.0891	-0.0002	0.6727	0.092
pre-eclampsia	74.2569	0.2538	-0.0742	0,1367	0.275
gestational diabetes	88.8895	0.0317	-0.0055	0.4601	0.174
abruptio placenta	79.6182	0.1389	-0.0210	0.4463	0.139
preterm birth	55.1749	0.5810	-0.0108	0.1815	0.531

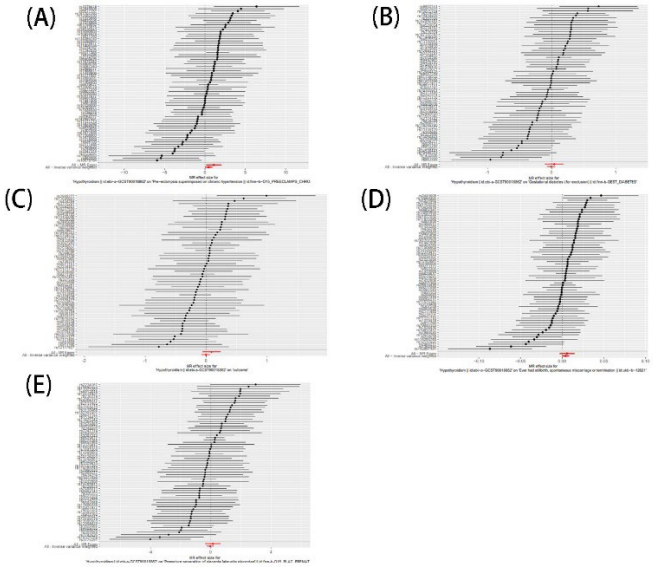

Figure 2: Forset plot of two-sample Mendelian randomization analysis

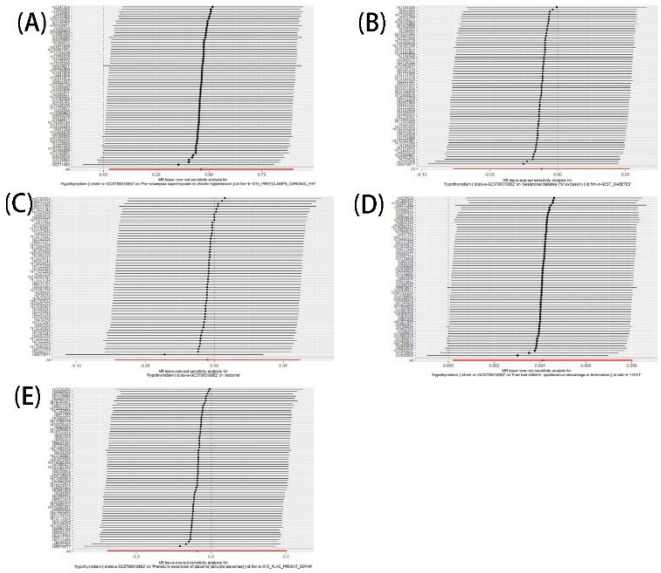

Figure 3: Result of "leave-one-out" sensitivity analysis

4. Discussion

This study represents the first attempt to investigate the causal relationship between hypothyroidism and adverse pregnancy outcomes using MR analysis with GWAS data. In this study, we employed two-

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sample MR to investigate the association between hypothyroidism and APO and conducted a comprehensive assessment of causality. The findings revealed a positive association between hypothyroidism and miscarriage and preeclampsia; however, supportive evidence of hypothyroidism was not observed for gestational diabetes mellitus, placental abruption, or preterm labor. Furthermore, the association between hypothyroidism and miscarriage, as well as preeclampsia, persisted in MVMR models following adjustment for confounders such as BMI and smoking. These findings offer novel insights and may be valuable in educating pregnant women about the relationship between hypothyroidism and adverse pregnancy outcomes to offer informed guidance.

In this study, MR analysis and multivariable regression demonstrated a significant association between the presence of hypothyroidism and miscarriage (OR = 1.004, 95% CI = 1.000-1.008, p = 0.039). This finding is consistent with numerous previous studies. A large retrospective analysis conducted using the Danish National Database on 14,744 pregnancies reported that women with TSH levels >10 μIU/mL have a significantly increased risk of spontaneous abortion or stillbirth [13]. A similar analysis from a unified cohort study based on 4,739,421 pregnancies found an association between elevated TSH levels and an increased risk of miscarriage [14]. A nested case-control study indicated that higher TSH levels are associated with early pregnancy loss [15], For every one-unit increase in the standard deviation of TSH concentration, the rate of spontaneous miscarriage increases by 78%. However, the underlying mechanisms and etiologies remain unclear.

The epidemiology and clinical characteristics of thyroid diseases are contingent upon iodine supply, as iodine is a fundamental element in thyroid hormone synthesis. Studies have found that higher miscarriage rates are associated with TPO antibody-positive cases [16]. In 1990, Stagnaro-Green et al. [17] reported an association between thyroid autoimmunity, defined as positive titers of thyroid peroxidase antibodies (TPOAb) and/or thyroglobulin antibodies, and miscarriage. Thyroid peroxidase antibodies (TPOAb) impair the function of the TPO enzyme and are the most common anti-thyroid autoantibodies [18]. The only autoantibodies that increase miscarriage rates are anti-thyroid microsomal antibodies and anti-nuclear antibodies. TPOAb can catalyze thyroid hormone and induce thyroid damage by activating complement, mediating cytotoxicity, and sensitizing T cells. A systematic review involving 12,000 women found that the presence of thyroid autoantibodies significantly increases the miscarriage rate in women compared to those without autoantibodies [19]. In mice induced with thyroid peroxidase antibodies, the rate of miscarriage increases [20]. Additionally, thyroid hormones may influence trophoblast invasion. Epidermal growth factor (EGF) and T3 may synergistically regulate the proliferation and differentiation functions of human trophoblasts. By regulating the maturation and function of dendritic cells, they influence implantation and placentation [21]. The α-subunit of HCG is similar to that of TSH. A reduction in HCG levels may lead to spontaneous miscarriage.

Pre-eclampsia (PE) is characterized by a series of disorders occurring after 20 weeks of gestation, presenting with normal blood pressure and the absence of prior proteinuria in the woman [22]. Regarding the association between hypothyroidism and preeclampsia, our study identified a robust correlation between the two (OR = 1.588, 95%CI = 1.027-2.456, p = 0.038). This finding is consistent with findings from multiple observational studies [23] and a meta-analysis. A systematic evaluation study observed that pregnant women with both the lowest and highest TSH concentrations had an elevated risk of developing pre-eclampsia. Iodine deficiency can induce preeclampsia, while iodine diminishes the antioxidant capacity of the placenta, an organ where the sodium iodide symporter protein maintains high iodine concentrations. One of the functions of the sodium iodide symporter is to mitigate oxidative stress and lipid peroxide formation [24]. Persistent iodine deficiency predisposes to elevated TSH levels, which, by interacting with endothelial receptors, have been demonstrated to decrease endothelial nitric oxide and prostacyclin production and increase endothelin production, resulting in endothelial dysfunction and systemic vasoconstriction [25]. Moreover, maternal hypothyroidism contributes to placental dysfunction [26]. Failure of trophoblast migration hinders the remodeling of uterine spiral arteries, consequently impacting the oxygenated blood supply at the maternal-fetal interface [27].

Our study did not find any evidence supporting a causal relationship between genetically predicted hypothyroidism and gestational diabetes mellitus, placental abruption, or preterm labor. The discrepancy in findings across studies may be attributed to limitations in sample size and susceptibility to residual confounding factors. Larger randomized controlled trials are warranted in the future to elucidate the underlying reasons for this discrepancy.

Our study has several notable strengths. It is the first to employ an MR framework to investigate the association between hypothyroidism and adverse pregnancy outcomes. Furthermore, extensive sensitivity analyses were conducted to assess the plausibility of core Mendelian randomization

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assumptions. Additionally, we conducted a confounder-adjusted multivariate regression analysis for hypothyroidism with a larger sample size than previous studies, thereby enhancing statistical power and furnishing robust evidence for the existence of an association. Nonetheless, it is crucial to acknowledge the limitations of our research; both MR and multivariate regression analyses may be vulnerable to selection bias response. Despite accounting for body mass index and smoking in our multivariate Mendelian randomization analysis, we did not consider other variables such as environmental and lifestyle factors. Future research endeavors ought to endeavor to incorporate these variables into their investigations, thereby augmenting a more holistic comprehension of the causal pathways linking hypothyroidism-associated traits and adverse pregnancy consequences. Moreover, the GWAS pooled data utilized in this study predominantly originated from European populations, which, while mitigating population stratification bias, restricts the generalizability of our findings to other demographic groups.

5. Conclusion

Our findings indicate a genetic association between genetically predicted hypothyroidism and miscarriage as well as preeclampsia. Therefore, we advocate for heightened emphasis on prenatal care and early intervention among pregnant women with hypothyroidism to mitigate the risk of adverse pregnancy outcomes.

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